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PATHOPHYSIOLOGY OF THE GH / IGF-1 AXIS:

Long-term consequences on joints and bone

Kim M.J.A. Claessen

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Appelboompjes

Voller wordend met de dagen,
vastgegroeid in 't ogenblik,
bestemd, mijn zustertjes, – als ik –
te wortelen, rijpen en vrucht te dragen.

M. Vasalis (1909-1998)

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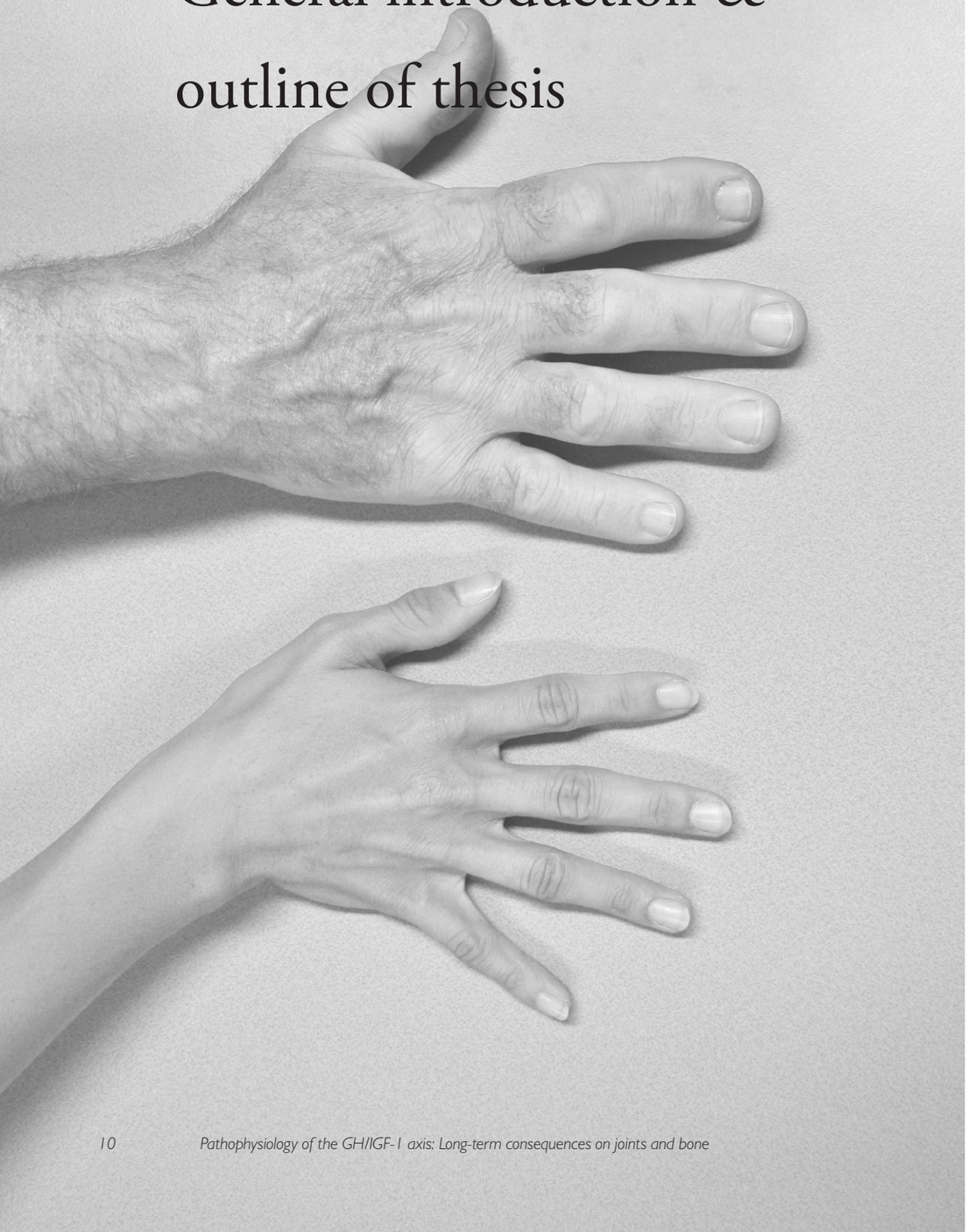
General introduction & outline of thesis



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).

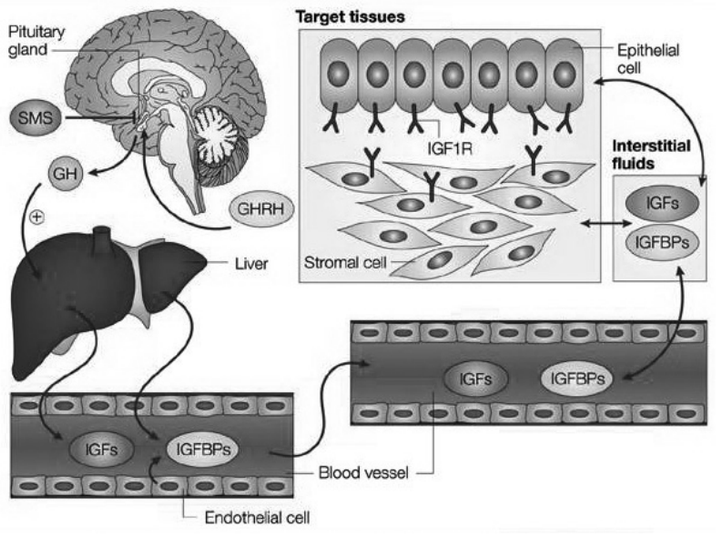


Figure 1. Regulation of the GH / IGF-1 axis *(Adapted from Pollak et al. Nat Rev Cancer 2004; 4(7): 505-518)*

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).

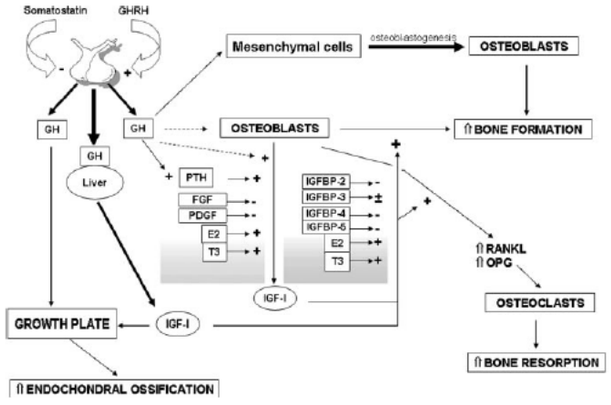


Figure 2. The effects of GH and IGF-1 on bone *(Adapted from Giustina et al. End Rev 2008 (5):535-559)*

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.

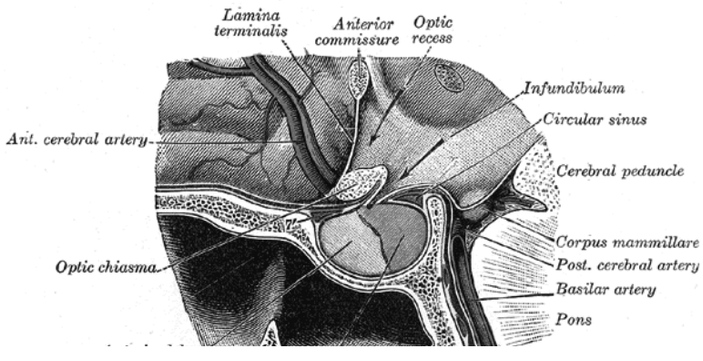


Figure 3. Localization of the pituitary gland (Adapted from Gray's Anatomy of the Human Body)

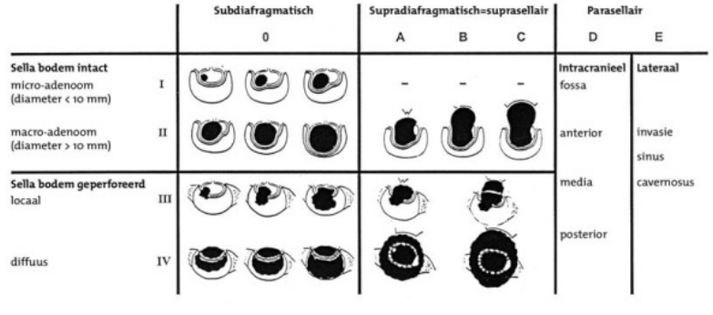


Figure 4. Hardy-Wilson classification of pituitary tumors (Adapted from Schutte et al. NTvNN 2003(104);99-104)

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.

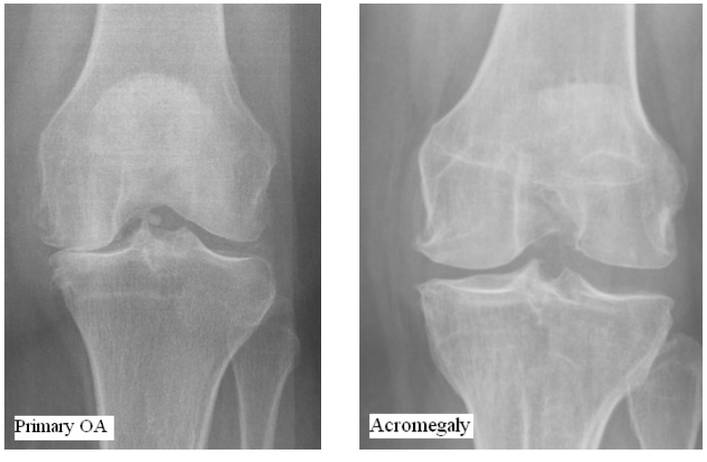


Figure 5. Radiographic features of osteoarthritis in the knee joint in a characteristic patient with primary OA (left) and acromegaly (right)

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.

REFERENCE LIST

1. Giustina A, Veldhuis JD. Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human. Endocr Rev 1998; 19(6):717-797.
2. Kopchick JJ, Parkinson C, Stevens EC, Trainer PJ. Growth hormone receptor antagonists: discovery, development, and use in patients with acromegaly. Endocr Rev 2002; 23(5):623-646.
3. Leung KC, Doyle N, Ballesteros M et al. Estrogen inhibits GH signaling by suppressing GH-induced JAK2 phosphorylation, an effect mediated by SOCS-2. Proc Natl Acad Sci U S A 2003; 100(3):1016-1021.
4. Ho KY, Evans WS, Blizzard RM et al. Effects of sex and age on the 24-hour profile of growth hormone secretion in man: importance of endogenous estradiol concentrations. J Clin Endocrinol Metab 1987; 64(1):51-58.
5. Van den Berg G, Veldhuis JD, Frolich M, Roelfsema F. An amplitude-specific divergence in the pulsatile mode of growth hormone (GH) secretion underlies the gender difference in mean GH concentrations in men and premenopausal women. J Clin Endocrinol Metab 1996; 81(7):2460-2467.
6. Le Roith D, Bondy C, Yakar S, Liu JL, Butler A. The somatomedin hypothesis: 2001. Endocr Rev 2001; 22(1):53-74.
7. LeRoith D, Clemmons D, Nissley P, Rechler MM. NIH conference. Insulin-like growth factors in health and disease. Ann Intern Med 1992; 116(10):854-862.
8. Copeland KC, Colletti RB, Devlin JT, McAuliffe TL. The relationship between insulin-like growth factor-I, adiposity, and aging. Metabolism 1990; 39(6):584-587.
9. Juul A. Serum levels of insulin-like growth factor I and its binding proteins in health and disease. Growth Horm IGF Res 2003; 13(4):113-170.
10. Urbanek M, Russell JE, Cooke NE, Liebhaber SA. Functional characterization of the alternatively spliced, placental human growth hormone receptor. J Biol Chem 1993; 268(25):19025-19032.
11. Kratzsch J, Wu Z, Kiess W et al. The exon 3-retaining and the exon 3-deleted forms of the growth hormone-binding protein (GHBP) in human serum are regulated differently. Clin Endocrinol (Oxf) 2001; 54(1):61-68.
12. Dos Santos C, Essioux L, Teinturier C, Tauber M, Goffin V, Bougneres P. A common polymorphism of the growth hormone receptor is associated with increased responsiveness to growth hormone. Nat Genet 2004; 36(7):720-724.
13. Pantel J, Grulich-Henn J, Bettendorf M, Strasburger CJ, Heinrich U, Amselem S. Heterozygous nonsense mutation in exon 3 of the growth hormone receptor (GHR) in severe GH insensitivity (Laron syndrome) and the issue of the origin and function of the GHRd3 isoform. J Clin Endocrinol Metab 2003; 88(4):1705-1710.
14. Binder G, Baur F, Schweizer R, Ranke MB. The d3-growth hormone (GH) receptor polymorphism is associated with increased responsiveness to GH in Turner syndrome and short small-for-gestational-age children. J Clin Endocrinol Metab 2006; 91(2):659-664.
15. Jorge AA, Marchisotti FG, Montenegro LR, Carvalho LR, Mendonca BB, Arnhold IJ. Growth hormone (GH) pharmacogenetics: influence of GH receptor exon 3 retention or deletion on first-year growth response and final height in patients with severe GH deficiency. J Clin Endocrinol Metab 2006; 91(3):1076-1080.
16. Mercado M, Gonzalez B, Sandoval C et al. Clinical and biochemical impact of the d3 growth hormone receptor genotype in acromegaly. J Clin Endocrinol Metab 2008; 93(9):3411-3415.
17. Giustina A, Mazziotti G, Canalis E. Growth hormone, insulin-like growth factors, and the skeleton. Endocr Rev 2008; 29(5):535-559.
18. Ohlsson C, Nilsson A, Isaksson O, Lindahl A. Growth hormone induces multiplication of the slowly cycling germinal cells of the rat tibial growth plate. Proc Natl Acad Sci U S A 1992; 89(20):9826-9830.
19. Nilsson O, Marino R, De LF, Phillip M, Baron J. Endocrine regulation of the growth plate. Horm Res 2005; 64(4):157-165.
20. DiGirolamo DJ, Mukherjee A, Fulzele K et al. Mode of growth hormone action in osteoblasts. J Biol Chem 2007; 282(43):31666-31674.
21. Kassem M, Blum W, Ristelli J, Mosekilde L, Eriksen EF. Growth hormone stimulates proliferation and differentiation of normal human osteoblast-like cells in vitro. Calcif Tissue Int 1993; 52(3):222-226.

22. Canalis E, Economides AN, Gazzerro E. Bone morphogenetic proteins, their antagonists, and the skeleton. Endocr Rev 2003; 24(2):218-235.
23. Canalis E. The fate of circulating osteoblasts. N Engl J Med 2005; 352(19):2014-2016.
24. Parfitt AM. The bone remodeling compartment: a circulatory function for bone lining cells. J Bone Miner Res 2001; 16(9):1583-1585.
25. Lacey DL, Timms E, Tan HL et al. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 1998; 93(2):165-176.
26. Simonet WS, Lacey DL, Dunstan CR et al. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 1997; 89(2):309-319.
27. Mochizuki H, Hakeda Y, Wakatsuki N et al. Insulin-like growth factor-I supports formation and activation of osteoclasts. Endocrinology 1992; 131(3):1075-1080.
28. Niu T, Rosen CJ. The insulin-like growth factor-I gene and osteoporosis: a critical appraisal. Gene 2005; 361:38-56.
29. Rubin J, Ackert-Bicknell CL, Zhu L et al. IGF-I regulates osteoprotegerin (OPG) and receptor activator of nuclear factor-kappaB ligand in vitro and OPG in vivo. J Clin Endocrinol Metab 2002; 87(9):4273-4279.
30. Matkovic V, Jelic T, Wardlaw GM et al. Timing of peak bone mass in Caucasian females and its implication for the prevention of osteoporosis. Inference from a cross-sectional model. J Clin Invest 1994; 93(2):799-808.
31. Theintz G, Buchs B, Rizzoli R et al. Longitudinal monitoring of bone mass accumulation in healthy adolescents: evidence for a marked reduction after 16 years of age at the levels of lumbar spine and femoral neck in female subjects. J Clin Endocrinol Metab 1992; 75(4):1060-1065.
32. Cushing H. III. Partial Hypophysectomy for Acromegaly: With Remarks on the Function of the Hypophysis. Ann Surg 1909; 50(6):1002-1017.
33. Marie P. Sur deux cas d'acromegalie: hypertrophie singuliere, non congenitale, des extremites superieures, inferieures et cephalique. Rev Med Liege 1886; 6:297-333.
34. Alexander L, Appleton D, Hall R, Ross WM, Wilkinson R. Epidemiology of acromegaly in the Newcastle region. Clin Endocrinol (Oxf) 1980; 12(1):71-79.
35. Ritchie CM, Atkinson AB, Kennedy AL et al. Ascertainment and natural history of treated acromegaly in Northern Ireland. Ulster Med J 1990; 59(1):55-62.
36. Melmed S. Medical progress: Acromegaly. N Engl J Med 2006; 355(24):2558-2573.
37. Molitch ME. Clinical manifestations of acromegaly. Endocrinol Metab Clin North Am 1992; 21(3):597-614.
38. Nabarro JD. Acromegaly. Clin Endocrinol (Oxf) 1987; 26(4):481-512.
39. Scarpa R, De BD, Pivonello R et al. Acromegalic axial arthropathy: a clinical case-control study. J Clin Endocrinol Metab 2004; 89(2):598-603.
40. Mazziotti G, Bianchi A, Bonadonna S et al. Prevalence of vertebral fractures in men with acromegaly. J Clin Endocrinol Metab 2008; 93(12):4649-4655.
41. Padova G, Borzi G, Incorvaia L et al. Prevalence of osteoporosis and vertebral fractures in acromegalic patients. Clin Cases Miner Bone Metab 2011; 8(3):37-43.
42. Wassenaar MJ, Biermasz NR, Hamdy NA et al. High prevalence of vertebral fractures despite normal bone mineral density in patients with long-term controlled acromegaly. Eur J Endocrinol 2011; 164(4):475-483.
43. Colao A, Spinelli L, Marzullo P et al. High prevalence of cardiac valve disease in acromegaly: an observational, analytical, case-control study. J Clin Endocrinol Metab 2003; 88(7):3196-3201.
44. Lie JT. Pathology of the heart in acromegaly: anatomic findings in 27 autopsied patients. Am Heart J 1980; 100(1):41-52.
45. Minniti G, Moroni C, Jaffrain-Rea ML et al. Prevalence of hypertension in acromegalic patients: clinical measurement versus 24-hour ambulatory blood pressure monitoring. Clin Endocrinol (Oxf) 1998; 48(2):149-152.
46. Pietrobelli DJ, Akopian M, Olivieri AO et al. Altered circadian blood pressure profile in patients with active acromegaly. Relationship with left ventricular mass and hormonal values. J Hum Hypertens 2001; 15(9):601-605.
47. Beauregard C, Truong U, Hardy J, Serri O. Long-term outcome and mortality after transsphenoidal adenomectomy for acromegaly. Clin Endocrinol (Oxf) 2003; 58(1):86-91.
48. Bengtsson BA, Eden S, Ernest I, Oden A, Sjogren B. Epidemiology and long-term survival in acromegaly. A study of 166 cases diagnosed between 1955 and 1984. Acta Med Scand 1988; 223(4):327-335.
49. Orme SM, McNally RJ, Cartwright RA, Belchetz PE. Mortality and cancer incidence in acromegaly: a retrospective cohort study. United Kingdom Acromegaly Study Group. J Clin Endocrinol Metab 1998; 83(8):2730-2734.
50. Swearingen B, Barker FG, Katznelson L et al. Long-term mortality after transsphenoidal surgery and adjunctive therapy for acromegaly. J Clin Endocrinol Metab 1998; 83(10):3419-3426.
51. Wright AD, Hill DM, Lowy C, Fraser TR. Mortality in acromegaly. Q J Med 1970; 39(153):1-16.
52. Barkan AL, Stred SE, Reno K et al. Increased growth hormone pulse frequency in acromegaly. J Clin Endocrinol Metab 1989; 69(6):1225-1233.
53. Eskildsen PC, Kruse A, Kirkegaard C. The pituitary-thyroid axis in acromegaly. Horm Metab Res 1988; 20(12):755-757.
54. Greenman Y, Tordjman K, Kisch E, Razon N, Ouaknine G, Stern N. Relative sparing of anterior pituitary function in patients with growth hormone-secreting macroadenomas: comparison with nonfunctioning macroadenomas. J Clin Endocrinol Metab 1995; 80(5):1577-1583.
55. Kaltsas GA, Mukherjee JJ, Jenkins PJ et al. Menstrual irregularity in women with acromegaly. J Clin Endocrinol Metab 1999; 84(8):2731-2735.
56. Melmed S, Casanueva F, Cavagnini F et al. Consensus statement: medical management of acromegaly. Eur J Endocrinol 2005; 153(6):737-740.
57. Biermasz NR, Romijn JA, Pereira AM, Roelfsema F. Current pharmacotherapy for acromegaly: a review. Expert Opin Pharmacother 2005; 6(14):2393-2405.
58. Biermasz NR, Pereira AM, Frolich M, Romijn JA, Veldhuis JD, Roelfsema F. Octreotide represses secretory-burst mass and nonpulsatile secretion but does not restore event frequency or orderly GH secretion in acromegaly. Am J Physiol Endocrinol Metab 2004; 286(1):E25-E30.
59. Peacey SR, Shalet SM. Growth hormone pulsatility in acromegaly following radiotherapy. Pituitary 1999; 2(1):63-69.
60. Peacey SR, Toogood AA, Veldhuis JD, Thorner MO, Shalet SM. The relationship between 24-hour growth hormone secretion and insulin-like growth factor I in patients with successfully treated acromegaly: impact of surgery or radiotherapy. J Clin Endocrinol Metab 2001; 86(1):259-266.
61. Van den Berg G, Frolich M, Veldhuis JD, Roelfsema F. Growth hormone secretion in recently operated acromegalic patients. J Clin Endocrinol Metab 1994; 79(6):1706-1715.
62. Van Thiel SW, Romijn JA, Biermasz NR et al. Octreotide long-acting repeatable and lanreotide Autogel are equally effective in controlling growth hormone secretion in acromegalic patients. Eur J Endocrinol 2004; 150(4):489-495.
63. Ahmed S, Elsheikh M, Stratton IM, Page RC, Adams CB, Wass JA. Outcome of transphenoidal surgery for acromegaly and its relationship to surgical experience. Clin Endocrinol (Oxf) 1999; 50(5):561-567.
64. Long H, Beauregard H, Somma M, Comtois R, Serri O, Hardy J. Surgical outcome after repeated transsphenoidal surgery in acromegaly. J Neurosurg 1996; 85(2):239-247.
65. Biermasz NR, van DH, Roelfsema F. Ten-year follow-up results of transsphenoidal microsurgery in acromegaly. J Clin Endocrinol Metab 2000; 85(12):4596-4602.
66. Barkan AL. Radiotherapy in acromegaly: the argument against. Clin Endocrinol (Oxf) 2003; 58(2):132-135.
67. Biermasz NR, Dulken HV, Roelfsema F. Postoperative radiotherapy in acromegaly is effective in reducing GH concentration to safe levels. Clin Endocrinol (Oxf) 2000; 53(3):321-327.
68. Biermasz NR, van DH, Roelfsema F. Long-term follow-up results of postoperative radiotherapy in 36 patients with acromegaly. J Clin Endocrinol Metab 2000; 85(7):2476-2482.
69. Powell JS, Wardlaw SL, Post KD, Freda PU. Outcome of radiotherapy for acromegaly using normalization of insulin-like growth factor I to define cure. J Clin Endocrinol Metab 2000; 85(5):2068-2071.
70. Thorner MO. Controversy: radiotherapy for acromegaly. Clin Endocrinol (Oxf) 2003; 58(2):136-137.
71. Wass JA. Radiotherapy in acromegaly: a protagonists viewpoint. Clin Endocrinol (Oxf) 2003; 58(2):128-131.
72. Littley MD, Shalet SM, Beardwell CG, Robinson EL, Sutton ML. Radiation-induced hypopituitarism is dose-dependent. Clin Endocrinol (Oxf) 1989; 31(3):363-373.
73. Brada M, Ford D, Ashley S et al. Risk of second brain tumour after conservative surgery and radiotherapy for pituitary adenoma. BMJ 1992; 304(6838):1343-1346.
74. Mahmoud-Ahmed AS, Suh JH, Mayberg MR. Gamma knife radiosurgery in the management of patients with acromegaly: a review. Pituitary 2001; 4(4):223-230.
75. Brada M, Ajithkumar TV, Minniti G. Radiosurgery for pituitary adenomas. Clin Endocrinol (Oxf) 2004; 61(5):531-543.

76. Attanasio R, Epaminonda P, Motti E et al. Gamma-knife radiosurgery in acromegaly: a 4-year follow-up study. J Clin Endocrinol Metab 2003; 88(7):3105-3112.
77. Landolt AM, Haller D, Lomax N et al. Stereotactic radiosurgery for recurrent surgically treated acromegaly: comparison with fractionated radiotherapy. J Neurosurg 1998; 88(6):1002-1008.
78. Lamberts SW, van der Lely AJ, de Herder WW, Hofland LJ. Octreotide. N Engl J Med 1996; 334(4):246-254.
79. Hofland LJ, Lamberts SW. The pathophysiological consequences of somatostatin receptor internalization and resistance. Endocr Rev 2003; 24(1):28-47.
80. Freda PU. Somatostatin analogs in acromegaly. J Clin Endocrinol Metab 2002; 87(7):3013-3018.
81. Ben-Shlomo A, Melmed S. Clinical review 154: The role of pharmacotherapy in perioperative management of patients with acromegaly. J Clin Endocrinol Metab 2003; 88(3):963-968.
82. Biermasz NR, van DH, Roelfsema F. Direct postoperative and follow-up results of transsphenoidal surgery in 19 acromegalic patients pretreated with octreotide compared to those in untreated matched controls. J Clin Endocrinol Metab 1999; 84(10):3551-3555.
83. Bonapart IE, van DR, ten Have SM et al. The 'bio-assay' quality of life might be a better marker of disease activity in acromegalic patients than serum total IGF-I concentrations. Eur J Endocrinol 2005; 152(2):217-224.
84. Van Thiel SW, Bax JJ, Biermasz NR et al. Persistent diastolic dysfunction despite successful long-term octreotide treatment in acromegaly. Eur J Endocrinol 2005; 153(2):231-238.
85. Van der Lely AJ, Hutson RK, Trainer PJ et al. Long-term treatment of acromegaly with pegvisomant, a growth hormone receptor antagonist. Lancet 2001; 358(9295):1754-1759.
86. Jaffe CA, Barkan AL. Treatment of acromegaly with dopamine agonists. Endocrinol Metab Clin North Am 1992; 21(3):713-735.
87. Roelfsema F, Goslings BM, Frolich M, Moolenaar AJ, Seters AP, Van SH. The influence of bromocriptine on serum levels of growth hormone and other pituitary hormones and its metabolic effects in active acromegaly. Clin Endocrinol (Oxf) 1979; 11(2):235-244.
88. Cozzi R, Attanasio R, Lodrini S, Lasio G. Cabergoline addition to depot somatostatin analogues in resistant acromegalic patients: efficacy and lack of predictive value of prolactin status. Clin Endocrinol (Oxf) 2004; 61(2):209-215.
89. Clemmons DR. IGF-I assays: current assay methodologies and their limitations. Pituitary 2007; 10(2):121-128.
90. Freda PU, Nuruzzaman AT, Reyes CM, Sundeen RE, Post KD. Significance of "abnormal" nadir growth hormone levels after oral glucose in postoperative patients with acromegaly in remission with normal insulin-like growth factor-I levels. J Clin Endocrinol Metab 2004; 89(2):495-500.
91. Biermasz NR, Dekker FW, Pereira AM et al. Determinants of survival in treated acromegaly in a single center: predictive value of serial insulin-like growth factor I measurements. J Clin Endocrinol Metab 2004; 89(6):2789-2796.
92. Colao A, Ferone D, Marzullo P, Lombardi G. Systemic complications of acromegaly: epidemiology, pathogenesis, and management. Endocr Rev 2004; 25(1):102-152.
93. Pereira AM, van Thiel SW, Lindner JR et al. Increased prevalence of regurgitant valvular heart disease in acromegaly. J Clin Endocrinol Metab 2004; 89(1):71-75.
94. Wassenaar MJ, Cazemier M, Biermasz NR et al. Acromegaly is associated with an increased prevalence of colonic diverticula: a case-control study. J Clin Endocrinol Metab 2010; 95(5):2073-2079.
95. Wassenaar MJ, Biermasz NR, van DN et al. High prevalence of arthropathy, according to the definitions of radiological and clinical osteoarthritis, in patients with long-term cure of acromegaly: a case-control study. Eur J Endocrinol 2009; 160(3):357-365.
96. Wassenaar MJ, Biermasz NR, Bijsterbosch J et al. Arthropathy in long-term cured acromegaly is characterised by osteophytes without joint space narrowing: a comparison with generalised osteoarthritis. Ann Rheum Dis 2011; 70(2):320-325.
97. Dekkers OM, Biermasz NR, Pereira AM, Romijn JA, Vandenbroucke JP. Mortality in acromegaly: a metaanalysis. J Clin Endocrinol Metab 2008; 93(1):61-67.
98. Wassenaar MJ, Biermasz NR, Kloppenburg M et al. Clinical osteoarthritis predicts physical and psychological QoL in acromegaly patients. Growth Horm IGF Res 2010; 20(3):226-233.
99. Bijlsma JW, Berenbaum F, Lafeber FP. Osteoarthritis: an update with relevance for clinical practice. Lancet 2011; 377(9783):2115-2126.
100. Holderbaum D, Haqqi TM, Moskowitz RW. Genetics and osteoarthritis: exposing the iceberg. Arthritis Rheum 1999; 42(3):397-405.
101. Murray CJ, Vos T, Lozano R et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012; 380(9859):2197-2223.
102. Lawrence RC, Helmick CG, Arnett FC et al. Estimates of the prevalence of arthritis and selected musculoskeletal disorders in the United States. Arthritis Rheum 1998; 41(5):778-799.
103. Bijkerk C, Houwing-Duistermaat JJ, Valkenburg HA et al. Heritabilities of radiologic osteoarthritis in peripheral joints and of disc degeneration of the spine. Arthritis Rheum 1999; 42(8):1729-1735.
104. Yusuf E, Kortekaas MC, Watt I, Huizinga TW, Kloppenburg M. Do knee abnormalities visualised on MRI explain knee pain in knee osteoarthritis? A systematic review. Ann Rheum Dis 2011; 70(1):60-67.
105. Lohmander LS, Felson DT. Defining the role of molecular markers to monitor disease, intervention, and cartilage breakdown in osteoarthritis. J Rheumatol 1997; 24(4):782-785.
106. Kellgren JH. Epidemiology of chronic rheumatism. Philadelphia F A Davis 1963.
107. Altman RD, Hochberg M, Murphy WA, Jr., Wolfe F, Lequesne M. Atlas of individual radiographic features in osteoarthritis. Osteoarthritis Cartilage 1995; 3 Suppl A:3-70.
108. Kellgren JH, LAWRENCE JS. Radiological assessment of osteo-arthrosis. Ann Rheum Dis 1957; 16(4):494-502.
109. Bellamy N, Buchanan WW, Goldsmith CH, Campbell J, Stitt LW. Validation study of WOMAC: a health status instrument for measuring clinically important patient relevant outcomes to antirheumatic drug therapy in patients with osteoarthritis of the hip or knee. J Rheumatol 1988; 15(12):1833-1840.
110. Bellamy N, Campbell J, Haraoui B et al. Clinimetric properties of the AUSCAN Osteoarthritis Hand Index: an evaluation of reliability, validity and responsiveness. Osteoarthritis Cartilage 2002; 10(11):863-869.
111. Dieppe PA. Recommended methodology for assessing the progression of osteoarthritis of the hip and knee joints. Osteoarthritis Cartilage 1995; 3(2):73-77.
112. Lane NE, Nevitt MC, Genant HK, Hochberg MC. Reliability of new indices of radiographic osteoarthritis of the hand and hip and lumbar disc degeneration. J Rheumatol 1993; 20(11):1911-1918.
113. Bruynesteyn K, Boers M, Kostense P, van der LS, van der HD. Deciding on progression of joint damage in paired films of individual patients: smallest detectable difference or change. Ann Rheum Dis 2005; 64(2):179-182.
114. Botha-Scheepers S, Watt I, Breedveld FC, Kloppenburg M. Reading radiographs in pairs or in chronological order influences radiological progression in osteoarthritis. Rheumatology (Oxford) 2005; 44(11):1452-1455.
115. Barkan AL. Acromegalic arthropathy. Pituitary 2001; 4(4):263-264.
116. Biermasz NR, van Thiel SW, Pereira AM et al. Decreased quality of life in patients with acromegaly despite long-term cure of growth hormone excess. J Clin Endocrinol Metab 2004; 89(11):5369-5376.
117. Biermasz NR, Wassenaar MJ, van der Klaauw AA et al. Pretreatment insulin-like growth factor-I concentrations predict radiographic osteoarthritis in acromegalic patients with long-term cured disease. J Clin Endocrinol Metab 2009; 94(7):2374-2379.
118. Wassenaar MJ, Biermasz NR, Pereira AM et al. The exon-3 deleted growth hormone receptor polymorphism predisposes to long-term complications of acromegaly. J Clin Endocrinol Metab 2009; 94(12):4671-4678.
119. Colao A, Marzullo P, Vallone G et al. Reversibility of joint thickening in acromegalic patients: an ultrasonography study. J Clin Endocrinol Metab 1998; 83(6):2121-2125.
120. Colao A, Cannavo S, Marzullo P et al. Twelve months of treatment with octreotide-LAR reduces joint thickness in acromegaly. Eur J Endocrinol 2003; 148(1):31-38.
121. McQuillan DJ, Handley CJ, Campbell MA, Bolis S, Milway VE, Herington AC. Stimulation of proteoglycan biosynthesis by serum and insulin-like growth factor-I in cultured bovine articular cartilage. Biochem J 1986; 240(2):423-430.
122. Okazaki K, Jingushi S, Ikenoue T et al. Expression of insulin-like growth factor I messenger ribonucleic acid in developing osteophytes in murine experimental osteoarthritis and in rats inoculated with growth hormone-secreting tumor. Endocrinology 1999; 140(10):4821-4830.
123. Meulenbelt I, Bijkerk C, Miedema HS et al. A genetic association study of the

IGF-1 gene and radiological osteoarthritis in a population-based cohort study (the Rotterdam Study). Ann Rheum Dis 1998; 57(6):371-374.
124. Urano T, Narusawa K, Shiraki M et al. Association of a single nucleotide polymorphism in the insulin-like growth factor-1 receptor gene with spinal disc degeneration in postmenopausal Japanese women. Spine (Phila Pa 1976) 2008; 33(11):1256-1261.
125. Zhai G, Rivadeneira F, Houwing-Duistermaat JJ et al. Insulin-like growth factor I gene promoter polymorphism, collagen type II alpha1 (COL2A1) gene, and the prevalence of radiographic osteoarthritis: the Rotterdam Study. Ann Rheum Dis 2004; 63(5):544-548.
126. Bos SD, Slagboom PE, Meulenbelt I. New insights into osteoarthritis: early developmental features of an ageing-related disease. Curr Opin Rheumatol 2008; 20(5):553-559.
127. Mackie EJ, Tatarczuch L, Mirams M. The skeleton: a multi-functional complex organ: the growth plate chondrocyte and endochondral ossification. J Endocrinol 2011; 211(2):109-121.
128. Pass C, MacRae VE, Ahmed SF, Farquharson C. Inflammatory cytokines and the GH/IGF-1 axis: novel actions on bone growth. Cell Biochem Funct 2009; 27(3):119-127.
129. Carroll PV, Christ ER, Bengtsson BA et al. Growth hormone deficiency in adulthood and the effects of growth hormone replacement: a review. Growth Hormone Research Society Scientific Committee. J Clin Endocrinol Metab 1998; 83(2):382-395.
130. De Boer H, Blok GJ, Van d, V. Clinical aspects of growth hormone deficiency in adults. Endocr Rev 1995; 16(1):63-86.
131. Rosen T, Bengtsson BA. Premature mortality due to cardiovascular disease in hypopituitarism. Lancet 1990; 336(8710):285-288.
132. Tomlinson JW, Holden N, Hills RK et al. Association between premature mortality and hypopituitarism. West Midlands Prospective Hypopituitary Study Group. Lancet 2001; 357(9254):425-431.
133. Bjork S, Jonsson B, Westphal O, Levin JE. Quality of life of adults with growth hormone deficiency: a controlled study. Acta Paediatr Scand Suppl 1989; 356:55-59.
134. Gilchrist FJ, Murray RD, Shalet SM. The effect of long-term untreated growth hormone deficiency (GHD) and 9 years of GH replacement on the quality of life (QoL) of GH-deficient adults. Clin Endocrinol (Oxf) 2002; 57(3):363-370.
135. McGauley GA, Cuneo RC, Salomon F, Sonksen PH. Psychological well-being before and after growth hormone treatment in adults with growth hormone deficiency. Horm Res 1990; 33 Suppl 4:52-54.
136. Rosen T, Wiren L, Wilhelmsen L, Wiklund I, Bengtsson BA. Decreased psychological well-being in adult patients with growth hormone deficiency. Clin Endocrinol (Oxf) 1994; 40(1):111-116.
137. Webb SM, Badia X. Quality of life in growth hormone deficiency and acromegaly. Endocrinol Metab Clin North Am 2007; 36(1):221-232.
138. Wiren L, Bengtsson BA, Johannsson G. Beneficial effects of long-term GH replacement therapy on quality of life in adults with GH deficiency. Clin Endocrinol (Oxf) 1998; 48(5):613-620.
139. Hoffman DM, O'Sullivan AJ, Baxter RC, Ho KK. Diagnosis of growth-hormone deficiency in adults. Lancet 1994; 343(8905):1064-1068.
140. Ghigo E, Aimaretti G, Corneli G. Diagnosis of adult GH deficiency. Growth Horm IGF Res 2008; 18(1):1-16.
141. Van der Klaauw AA, Biermasz NR, Feskens EJ et al. The prevalence of the metabolic syndrome is increased in patients with GH deficiency, irrespective of long-term substitution with recombinant human GH. Eur J Endocrinol 2007; 156(4):455-462.
142. Maison P, Griffin S, Nicoue-Beglah M, Haddad N, Balkau B, Chanson P. Impact of growth hormone (GH) treatment on cardiovascular risk factors in GH-deficient adults: a Metaanalysis of Blinded, Randomized, Placebo-Controlled Trials. J Clin Endocrinol Metab 2004; 89(5):2192-2199.
143. Maison P, Chanson P. Cardiac effects of growth hormone in adults with growth hormone deficiency: a meta-analysis. Circulation 2003; 108(21):2648-2652.
144. Gibney J, Wallace JD, Spinks T et al. The effects of 10 years of recombinant human growth hormone (GH) in adult GH-deficient patients. J Clin Endocrinol Metab 1999; 84(8):2596-2602.
145. Stouthart PJ, Deijen JB, Roffel M, Delemarre-van de Waal HA. Quality of life of growth hormone (GH) deficient young adults during discontinuation and restart of GH therapy. Psychoneuroendocrinology 2003; 28(5):612-626.
146. Deijen JB, Arwert LI, Witlox J, Drent ML. Differential effect sizes of growth hormone replacement on Quality of Life, well-being and health status in growth hormone deficient patients: a meta-analysis. Health Qual Life Outcomes 2005; 3:63.
147. Gotherstrom G, Bengtsson BA, Bosaeus I, Johannsson G, Svensson J. A 10-year, prospective study of the metabolic effects of growth hormone replacement in adults. J Clin Endocrinol Metab 2007; 92(4):1442-1445.
148. Van der Klaauw AA, Romijn JA, Biermasz NR et al. Sustained effects of recombinant GH replacement after 7 years of treatment in adults with GH deficiency. Eur J Endocrinol 2006; 155(5):701-708.
149. Biermasz NR, Hamdy NA, Pereira AM, Romijn JA, Roelfsema F. Long-term skeletal effects of recombinant human growth hormone (rhGH) alone and rhGH combined with alendronate in GH-deficient adults: a seven-year follow-up study. Clin Endocrinol (Oxf) 2004; 60(5):568-575.
150. Gotherstrom G, Svensson J, Koranyi J et al. A prospective study of 5 years of GH replacement therapy in GH-deficient adults: sustained effects on body composition, bone mass, and metabolic indices. J Clin Endocrinol Metab 2001; 86(10):4657-4665.
151. Gotherstrom G, Bengtsson BA, Bosaeus I, Johannsson G, Svensson J. Ten-year GH replacement increases bone mineral density in hypopituitary patients with adult onset GH deficiency. Eur J Endocrinol 2007; 156(1):55-64.
152. van Bunderen CC, van N, I, Arwert LI et al. Does growth hormone replacement therapy reduce mortality in adults with growth hormone deficiency? Data from the Dutch National Registry of Growth Hormone Treatment in adults. J Clin Endocrinol Metab 2011; 96(10):3151-3159.
153. Carel JC, Ecosse E, Landier F et al. Long-term mortality after recombinant growth hormone treatment for isolated growth hormone deficiency or childhood short stature: preliminary report of the French SAGhE study. J Clin Endocrinol Metab 2012; 97(2):416-425.
154. Kreitschmann-Andermahr I, Siegel S, Francis F et al. Variation of the baseline characteristics and treatment parameters over time: an analysis of 15 years of growth hormone replacement in adults in the German KIMS database. Pituitary 2012.

Part A

Long-term effects of acromegaly on joints and bone

II.

Progression of acromegalic arthropathy despite long-term biochemical control: a prospective, radiological study

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ABSTRACT

OBJECTIVE: Arthropathy is an invalidating complication of acromegaly, of which the prognosis and determinants are currently unknown in treated acromegaly. Therefore, the objective of the present study was to investigate radiographic progression of arthropathy over a mean follow-up period of 2.6 years and determinants of outcome in patients with long-term well-controlled acromegaly.

DESIGN: Prospective follow-up study

METHODS: In a prospective cohort study we studied 58 patients (mean age 62 years, women 41%) with controlled acromegaly for a mean of 17.6 years. Radiographic progression of joint disease was defined by the Osteoarthritis Research Society International (OARSI) classification as a 1-point increase in joint space narrowing (JSN) or osteophyte scores on radiographs of the hands, knees, and hips obtained at the first study visit and after 2.6 years. Potential risk factors for progression were assessed.

RESULTS: Progression of osteophytes and JSN was observed in 72% and 74% of patients, respectively. Higher age predisposed for osteophyte progression. Patients with biochemical control by somatostatin (SMS) analogs had more progression of osteophytosis than surgically cured patients (OR=18.9, p=0.025), independently of age, sex, BMI, baseline insulin-like growth factor-1 (IGF-1) SDS and exon 3 deletion of the GHR. This was also evident for JSN progression, as were higher age and higher baseline IGF-1 SDS.

CONCLUSIONS: Acromegalic patients have progressive JSN and osteophytosis, despite long-term biochemical control. Parameters reflecting GH/IGF-1 activity were associated with progressive joint disease. Remarkably, biochemical control by SMS analogs was associated with more progression than surgical cure. Although the present study is not a randomized controlled trial, this may indicate insufficient GH control according to current criteria and the need of more aggressive therapy.

INTRODUCTION

Acromegaly is a chronic, progressive disease, caused by a growth hormone (GH)-producing pituitary adenoma, resulting in elevated GH and insulin-like growth factor-1 (IGF-1) concentrations. Available treatments are transsphenoidal surgery (TPS), radiotherapy and medical therapy with somatostatin (SMS) analogs and Pegvisomant. In acromegaly, the risk to develop secondary osteoarthritis (OA) is increased. However, little is known on the pathophysiology of acromegalic joint disease or the role of the GH/IGF-I system in primary OA (1).

In a well-characterized cohort patients with long-term disease control, we recently observed a 4- to 12-fold increased prevalence of arthropathy at young ages, leading to limited physical functioning and psychological well-being (2;3). Interestingly, the distribution of radiological abnormalities, such as osteophytes and joint space narrowing (JSN), differed from primary OA. In acromegaly, GH hypersecretion results in a characteristic radiographic OA phenotype with severe osteophytosis, but wide joint spaces (4), indicating that cartilage hypertrophy is maintained despite long-term remission. This observation indicates that transient GH/IGF-1 excess is mainly involved in bone formation resulting in osteophytosis, but may protect against cartilage loss (4). We recently documented a predictive role for pre-treatment IGF-1 levels on radiographic appearance of OA in acromegaly, in a dose-dependent manner (5). In addition, we found that patients with a common GH receptor polymorphism, exon 3 deletion (d3-GHR) which results in enhanced GH responsiveness, had an increased prevalence of irreversible complications of acromegaly, such as radiographic OA, dolichocolon and adenomatous colonic polyps (6;7).

These observations were obtained in a cross-sectional study. At present, the disease course of OA during prolonged follow-up in patients with long-term biochemical control of acromegaly is unknown. It is unclear whether cartilage hypertrophy is permanent and stable in these patients or whether deterioration occurs in hypertrophied cartilage. Therefore, we designed a prospective follow-up study during 2.6 years to assess the course of acromegalic arthropathy, and to identify potential risk factors.

MATERIALS AND METHODS

Study design and patient selection

PATIENTS: All consecutive patients with acromegaly, who were referred to the Leiden University Medical Center, are collected in a database. In the baseline study (2007), 89 patients in long-term biochemical remission were included (2). All 89 patients were invited for a follow-up study visit (2010), of which 58 consented to participate. Thirty-one (35%) declined to consent, with not OA-related health problems (N=16), travel distance (N=6), and lack of time (N=4) as most frequent reasons. Demographic and disease characteristics did not statistically differ between included and non-included patients (data not shown), except for a higher number of females among non-consenters (p=0.025) (2;3).

Detailed yearly follow-up was performed from the onset of acromegaly treatment. The first treatment option in the majority of patients was TPS 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.

Disease activity was assessed yearly by oral glucose tolerance tests (except in medically treated patients), fasting serum GH and IGF-1 levels. Remission of acromegaly was defined as a normal glucose-suppressed serum GH <1.25 (RIA assay until 1992) or 0.38µg/l (immunofluorometric assay (IFMA) from 1992 onwards), serum GH levels of <1.9µg/l (all years), and normal IGF-1 levels for age (from 1986 onwards) (8-10). Patients not meeting these criteria were offered additional treatment.

Hypopituitarism was supplemented with thyroxine, hydrocortisone, testosterone, and estrogens (only in pre-menopausal women) according to the following definitions (11). Estrogen deficiency in women was present in case of LH/FSH deficiency in premenopausal women with prolonged amenorrea >1 year without adequate replacement therapy or by a low serum oestradiol concentration of <70nmol/l and all postmenopausal women. In men, LH/FSH deficiency was defined as testosterone level below the reference range (8.0nmol/l). TSH deficiency was defined as a free thyroxine level below the reference range (<10pmol/l). ACTH deficiency was defined as an insufficient increase of cortisol (peak <0.55µmol/l) after corticotrophin releasing hormone test or insulin tolerance test. GH deficiency was not routinely assessed.

The Medical Ethics Committee approved the study protocol, and all subjects gave written consent.

PROTOCOL: Fifty-eight patients were seen at the outpatient clinic for two study visits with a 2.6-year interval. The baseline assessment was performed between September and December 2007, the follow-up visit between March and September 2010. At baseline, patients had a mean duration of remission of 15.0 years. All patients completed a standardized questionnaire concerning demographic data and medical history. Treatment and patient characteristics were derived from patient records. At both time points, conventional radiographs were obtained, according to a standardized protocol (see below). Blood samples were taken in the post-absorptive states to assess actual GH and IGF-1 concentrations.

Study parameters

PARAMETERS OF ACROMEGALIC DISEASE: Duration of active disease was estimated using the start of symptoms and signs to the date of normalization of serum IGF-1 concentration after treatment. Duration of remission was calculated from the date of biochemical remission until the start of the present study. Cure of acromegaly was defined by normal glucose-suppressed GH levels and IGF-1 levels for age after surgery and/or irradiation. Biochemical control of acromegaly was defined by normal serum IGF-1 levels for age during SMS analog treatment. Both cured and biochemically controlled patients were referred to as 'in remission'.

ASSAYS: Serum GH was measured with a sensitive IFMA (Wallac, Turku, Finland), specific for the 22 kDA GH protein (detection limit: 0.01µg/l, interassay coefficient of variation (CV): 1.6-8.4% of 0.01-15.38µg/l) from 1992 onwards. For the conversion of µg/l to mU/l, multiply by 2.6. Before 1992, GH was measured by RIA (Biolab, Serona, Coissins, Switzerland), detection limit: 0.5mU/l, with an interassay CV <5%; for the conversion of µg/l to mU/l, multiply by 2.

From 1986 to 2005, serum IGF-1 concentrations were determined by RIA (Incstar, Stillwater, MN) with a detection limit of 1.5 nmol/L and an interassay CV less than 11%. IGF-1 is expressed as SD score for age- and gender-related normal levels determined in the same laboratory (12). From 2005, serum IGF-1 concentrations (nmol/l) were measured using an immunometric technique on an Immulite 2500 system (Siemens Healthcare Diagnostics, Deerfield, IL, USA). The intra-assay variations at mean plasma levels of 8 and 75nmol/l were 5.0 and 7.5%, respectively. IGF-1 levels were expressed as SDS (normal range -2 to +2 SDS), using lambda-mu-sigma smoothed reference curves based on 906 controls (13;14).

DNA COLLECTION AND GENETIC ANALYSIS: DNA extraction was done 6-8 weeks after blood collection (8ml) at the baseline visit. DNA concentrations and purity (OD 260/280) were determined spectophotometrically using the nanodrop (Isogen, IJsselstein, The Netherlands). The d3-GHR polymorphism was detected as described previously (15). Both heterozygotes and homozygotes for the d3-GHR allele were referred to as d3 carriers.

RADIOGRAPHIC PROTOCOL: Conventional radiographs of the hands (dorsovolar), knees (posterior-anterior (PA), in weight-bearing/semi-flexed and lateral) and hips (PA, supine) were obtained from all participating patients, employing a standardized protocol with a fixed film-focus distance and fixed joint position. Knee radiographs were made in fixed-flexion (16). Radiographic examinations at both study visits were performed by a single experienced radiographer.

ASSESSMENT OF RADIOGRAPHIC OA PROGRESSION: For a semi-quantitative assessment of radiographic OA severity, radiographs were graded on a scale of 0-3 for JSN and osteophytes, using the Osteoarthritis Research Society (OARSI) atlas (17). In the hands, distal interphalangeal (DIP), proximal interphalangeal (PIP), metacarpophalangeal (MCP), first interphalangeal (IP), and first carpometacarpal (CMC1) joints were scored. All radiographs were scored by a single experienced reader (K.M.J.A. Claessen), blinded for patient characteristics. Radiographs of the same patient from both time points were assessed together in chronological order; this was previously demonstrated to be the most sensitive method to change, when assessing radiographic progression in primary OA (18).

THE JSN AND OSTEOPHYTE SCORES OF THE FOLLOWING JOINT GROUPS WERE ANALYZED IN COMBINATION: hands (DIPs, PIPs, MCPs, IPs, CMC1s), knees (medial and lateral tibiofemoral (TF) compartments) and hips. Total scores were calculated by adding left and right sites. The maximum total JSN score was 108 for a patient: 90 in the hands, 6 in the hips and 12 in the knees. The maximum total osteophyte score was 120 for a patient: 90 in the hands, 6 in the hips and 24 in the knees. In joint-specific analyses, left and right joints were analyzed independently.

The reproducibility for JSN and osteophytes, depicted by the intra-class correlation coefficient (ICC), was very good. ICCs for JSN and osteophytes were respectively 0.98 and 0.98 in the hands, 1.00 and 0.99 in

the knee, 0.98 and 1.00 in the hip. The reproducibility was based on the repeat reading of 15 randomly selected radiographs.

DEFINITION OF RADIOGRAPHIC OA PROGRESSION: Radiographic progression was defined both at patient level, including all hand, hip and knee joints, and at the specific joint level. Radiographic progression was only defined in patients and joints, respectively, with existing OA features (osteophytes and/or JSN) at baseline. Radiological progression was the change in JSN or osteophyte scores after 2.6 years above the smallest detectable change (SDC, 0.85 and 0.57 respectively), and was therefore defined by at least a 1-score increase in JSN or osteophyte total scores (19). Also at joint site level, SDC was used to assess radiographic change above measurement error (SDCs for osteophytes and JSN: knee, both 0.3; hip, both 0.4; hands, 0.8 and 0.4, resp.). Therefore, radiographic progression was defined as at least a 1-score increase in JSN or osteophyte scores at the specific joint.

Knees and hips without radiological end-stage disease (grade 3) in terms of JSN or osteophytes at the first study visit, which received hip or knee prosthesis during follow-up, were considered to have progressive JSN and osteophytosis in that particular joint. In addition, patients with end-stage OA or joint prostheses at baseline that were unable to further progress, were considered to have progressive disease in terms of JSN and osteophytes in their respective joints.

Statistical analysis

SPSS for Windows, version 17.0 (SPSS Inc.,Chicago,IL,USA), was used for data analysis. Data are presented as mean±SD, unless otherwise stated. To evaluate the magnitude of the changes observed during 2.6years, standardised response means (SRM) were calculated as the mean change between both study visits divided by the SD of change (20). Spearman rank correlations were used to correlate initial severity of radiographic OA with progression scores. The relationship between duration of SMS therapy and severity of arthropathy was studied with linear regression analysis with osteophyte/JSN scores as dependent variable, and duration of SMS treatment as independent variable, adjusted for age. Duration of SMS therapy was subdivided into 4 groups: (1) no SMS treatment, (2) <5years SMS treatment, (3) 5-10years SMS treatment, (4) >10years SMS treatment. Logistic regression analyses were performed to investigate risk factors for progression at patient level, with radiographic outcome as dependent variable. Crude and adjusted odds ratios (OR) were calculated, with adjustments for age, sex, BMI. Additional adjustments for baseline IGF1 SDS were performed when studying acromegaly cure *vs.* control. At joint site level, left and right joints were analyzed independently. Risk factor analysis was performed by generalized estimating equations (GEE) analysis to account for intra-patient effects, with corrections for age, sex, BMI, and baseline IGF1 SDS when appropriate.

RESULTS

Patient description

In total, 58 patients with long-term remission of acromegaly were studied in the present longitudinal study. Mean interval between both study visits was 2.6 years (range 2.3-2.9). Patient characteristics are shown in Table 1. Mean age was 61.8±10.9 years and 41% were women. The patients were in remission for a mean duration of 17.6±7.2 years (minimum 2 years) and mean actual IGF-1 SDS was 0.51±1.51. There were no recurrences during longitudinal follow-up. Remission was achieved by surgery, if necessary, followed by radiotherapy in 40 patients (69%). The other 18 patients (31%) were treated during the observation period with either primary and/or postoperative long-acting SMS analogs (mean duration 105 months, range 21-191). Only one patient was co-treated with Pegvisomant. At the first study visit, two patients had knee prostheses, and one patient had bilateral hip prostheses for end-stage OA.

Table 1. Clinical characteristics of 58 patients with acromegaly

Clinical characteristics	Patients (N=58)
Age (years)	61.8 (10.9)
Sex, female (n (%))	24 (41)
BMI (kg/m^2)	28.9 (4.5)
Tumor class (n %))	
Microadenoma	14 (24)
Macroadenoma	38 (66)
Unknown	6 (10)
Treatment (n (%))	
Surgery only	31 (53.4)
Surgery + RT	9 (15.5)
SMS analogues	
Primary	2 (3.4)
Following surgery *	13 (22.4)
Following RT	1 (1.7)
Following surgery + RT	2 (3.4)
Disease duration (years)	9.2 (8.1)
Duration of remission (years)	17.6 (7.2)
Pre-treatment GH (µg/L)	33.7 (45.4)
IGF-1 SD scores	
Pre-treatment	6.9 (3.6)
Actual	0.5 (1.5)
Hypopituitarism (n (%))	20 (34.5)
Corticotrope failure	15 (25.8)
Thyreotrope failure	10 (17.2)
Gonadotrope failure	
Males	8 (13.8)
Pre-menopausal	0 (0.0)
Post-menopausal	22 (37.9)
d3-GHR carrier (n (%))	21 (36.2)
ROA at baseline, (n (%))	
Patient level, n=58	
OP	58 (100.0)
JSN	49 (84.5)
OP and/or JSN	58 (100.0)
Knee, N=116	
OP	90 (77.6)
JSN	36 (31.0)
OP and/or JSN	97 (83.6)
Hip, N=116	
OP	83 (71.6)
JSN	16 (13.8)
OP and/or JSN	84 (72.4)
Hand, N=116	
OP	97 (83.6)
JSN	81 (69.8)
OP and/or JSN	104 (89.7)

Values are means (SD) unless stated otherwise.

*GH, growth hormone; IGF-1, insulin-like growth factor 1; BMI, body mass index; RT, radiotherapy; SMS, somatostatin (analogs); GHD, growth hormone deficiency; d3-GHR, exon 3 deletion of the GHR polymorphism; ROA, radiographic osteoarthritis, defined as Osteoarthritis Research Society International (OARSI) score ≥ 1; OP, osteophytes; JSN, joint space narrowing; N=number of joints. *, one patient is co-treated with Pegvisomant.*

Table 2. Values at baseline and follow-up, after additional 2.6 years of follow-up, and change scores in 58 patients (116 joints) with acromegaly

Joint site	Baseline	Follow-up	Change*	P value	SRM
Patient level					
OP, range 0-120	18.0 (12.9)	20.0 (13.5)	2.0 (1.9)	<0.001	1.0
JSN, range 0-108	5.4 (4.9)	7.1 (5.9)	1.7 (1.7)	<0.001	1.0
Knee					
OP, range 0-24	5.8 (5.5)	6.8 (6.2)	1.0 (1.7)	<0.001	0.6
JSN, range 0-12	1.2 (2.2)	1.6 (2.6)	0.4 (0.7)	<0.001	0.6
Hip					
OP, range 0-6	2.7 (2.0)	3.0 (2.0)	0.3 (0.6)	<0.01	0.5
JSN, range 0-6	0.5 (1.2)	0.7 (1.5)	0.2 (0.5)	<0.01	0.5
Hand					
OP, range 0-90	9.5 (7.9)	10.3 (8.2)	0.8 (1.1)	<0.001	0.7
JSN, range 0-90	3.7 (3.7)	4.8 (4.4)	1.1 (1.4)	<0.001	0.8

*Data are shown as mean (SD). *, mean change (SD) over 2.6 years.*

SRM, standardised response mean; OP, osteophytes; JSN, joint space narrowing.

Radiographic progression of osteophytes and JSN

Total scores of osteophytes and JSN deteriorated over time (Table 2), reflected in mean changes of total scores of 2.0±1.9 and 1.7±1.7, respectively. Radiographic progression of osteophytes and JSN at any joint site was present in 42 (72%) and 43 (74%) patients, respectively. Progression of osteophytosis was highest in the knee (31%), with slightly lower percentages in the hands (28%), and hip (26%). JSN progression occurred most often in the hands (40%), followed by the knee (23%), and hip (15%). During follow-up, two patients received unilateral knee prosthesis and one patient underwent unilateral hip replacement.

There was a distinct relationship between the severity of radiographic arthropathy features at baseline and the degree of increase in radiographic scoring over 2.6 years. The baseline severity of JSN correlated moderately with JSN progression over 2.6 years (Spearman rank correlation coefficient r=0.5, p<0.001); the baseline severity of osteophytosis correlated with both osteophyte and JSN progression (r=0.3, p<0.05 and r=0.5, p<0.001).

Risk factors for radiographic progression of arthropathy

PATIENT LEVEL: JSN progression was associated with higher age (p=0.01), but not with sex and baseline IGF-1 SDS. There was a difference between surgically cured patients and those controlled with SMS analogs (Table 3). Patients with biochemical control by SMS analogs had a 9.0-fold increased risk to develop osteophyte progression compared with patients cured by surgery or additional radiotherapy (OR=12.3, p=0.032, independently of age, sex, BMI and baseline IGF-1 SDS). This risk was even more increased after additional correction for d3-GHR polymorphism (OR=18.9, p=0.025). When comparing patient characteristics between SMS-treated and surgically cured patients, medically treated patients had a longer history of active disease (p=0.01) and higher IGF-1 SDS, both at baseline and follow-up (p=0.08 and p=0.07, respectively), albeit in the normal range (Table 4). Pre-treatment GH/IGF-1 levels, duration of remission and prevalence of hypopituitarism were not different between both groups.

Duration of SMS treatment was moderately correlated with the severity of arthropathy, especially osteophytosis (r=0.35, p<0.01), with highest correlation in the hip (r=0.5, p<0.001 for both osteophytosis and JSN). Upon further investigation, we found a clear dose-response relationship between duration of SMS treatment and severity of arthropathy, especially with osteophytosis (β=3.029, p=0.025, adjusted for age and baseline IGF-1 SD scores, Figure 1). After stratification for joints, the strongest relationship was found for the hip (β=1.235, p<0.001 and β=0.691, p<0.001 for osteophytes and JSN, respectively), followed by osteophytosis in the hand (β=0.708, p=0.014).

Table 3. Risk factors for radiographic progression of acromegalic arthropathy at patient level, in 58 patients

Risk factors	Radiographic OA progression	Crude OR (95%CI)	Adjusted OR (95%CI)
Age	Osteophytes	1.04 (0.99-1.10)	NA
	JSN	1.10 (1.03-1.17)*	
Female sex	Osteophytes	2.27 (0.6-8.3)	NA
	JSN	1.98 (0.5-7.3)	
BMI	Osteophytes	1.12 (0.96-1.30)	NA
	JSN	1.00 (0.87-1.14)	
Estimated disease duration	Osteophytes	1.09 (0.97-1.23)	1.09 (0.95-1.25)
	JSN	1.07 (0.96-1.19)	1.03 (0.90-1.16)
Baseline IGF-1 SDS	Osteophytes	0.95 (0.7-1.4)	0.92 (0.6-1.4)
	JSN	1.04 (0.7-1.5)	1.06 (0.7-1.6)
Medically controlled vs. cured disease	Osteophytes	8.62 (1.03-71.9)*	18.85 (1.4-247.2)*
	JSN	1.77 (0.4-7.4)	2.67 (0.4-16.6)
d3-GHR	Osteophytes	1.68 (0.5-6.2)	1.78 (0.4-7.3)
	JSN	0.48 (0.1-1.7)	0.42 (0.1-1.7)

Risk factors were analyzed with binary logistic regression analysis with OA progression as dependent variable. Baseline IGF-1 SDS were IGF-1 SD scores at the time of the first joint evaluation in 2007. Disease cure is defined as normal glucose-suppressed GH levels and IGF-1 levels for age after surgery and/or irradiation. Adjusted odds ratios were adjusted for age, sex and BMI. Additional adjustments were made for baseline IGF-1 SDS and d3-GHR polymorphism in the analysis on controlled vs. cured acromegaly disease.

*CI, confidence interval; JSN, joint space narrowing; BMI, body mass index; d3-GHR, exon 3 deletion of the GHR polymorphism. NA, not applicable. *, p<0.05.*

JOINT SITE LEVEL: Risk factors for progression of arthropathy were also studied for the specific joint sites, because their effect may differ between various sites (21) (Table 5, Figure 2). At the first study visit, 97 knees (84%), 84 hips (72%) and 104 (90%) hands showed radiographic OA features (Table 1), and were hence included in the present analysis. All analyses were adjusted for age, sex and BMI; analyses on acromegaly cure vs. medically control were also corrected for baseline IGF-1 SDS.

Knee: Higher baseline IGF-1 SDS was associated with JSN progression. Furthermore, SMS analog-treated patients had a 3.5-fold increased risk to develop JSN progression compared to surgically cured patients (p=0.02). d3-GHR polymorphism predisposed for osteophyte progression (OR=3.6, p=0.01). This could not be demonstrated for female sex or baseline IGF-1 SDS.

Hip: Risk factors for JSN progression were higher age (OR=1.1, p=0.047) and higher baseline IGF-1 SD levels. In addition, SMS analog treatment was associated with JSN progression (OR=5.6, p=0.016), irrespective of adjustment of IGF-1 SDS (OR=4.3, p=0.045); a trend was demonstrated for osteophyte progression (OR=2.9, p=0.06).

Hands: Both JSN and osteophyte progression were seen more frequently in older patients (p<0.001 and p=0.02, respectively). SMS-treated patients showed 4.2 times more osteophyte progression compared with surgically cured patients (p=0.01). In addition, longer duration of active disease was associated with osteophyte progression.

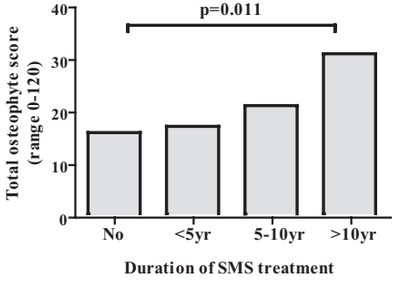


Figure 1: Dose-response relationship between duration of SMS therapy and severity of osteophytosis (at patient level), adjusted for age and baseline IGF-1 SD levels.

*Mean osteophyte and JSN scores (±SEM) of both study visits were shown for patients treated by TPS and/or RT versus patients treated by SMS analogs. The left symbol (circle) of each pair represents the baseline OP/JSN score; the right one (square) represents the score after 2.6 years of additional follow-up. TPS, transsphenoidal surgery; RT, radiotherapy; SMS, somatostatin analogs; *, p<0.001.*

Table 4. Characteristics of acromegalic patients with cured acromegaly (N=40) versus SMS-treated patients (N=18)

Clinical characteristics	**Disease cure (N=40)**	**Well-controlled disease (N=18)**	**P value**
Age (years)	62.0 (10.6)	62.0 (11.4)	0.99
Sex, female (%)	40%	41%	0.94
BMI (kg/m^2)	28.8 (4.6)	29.0 (4.2)	0.86
Tumor class (%)			
Microadenoma	30%	11%	0.16
Macroadenoma	63%	72%	
Unknown	7%	17%	
Estimated disease duration (years)	7.5 (7.6)	14.3 (7.5)	0.01*
Duration of remission (years)	19.0 (8.1)	17.5 (6.9)	0.56
GH (µg/L)			
Pre-treatment	32.6 (46.7)	36.5 (43.2)	0.78
Baseline	1.4 (1.8)	2.2 (1.4)	0.12
Actual	2.0 (4.0)	2.0 (1.2)	0.96
IGF-1 SD scores			
Pre-treatment	6.8 (3.2)	7.2 (4.3)	0.76
Baseline	0.5 (1.9)	1.4 (1.4)	0.08
Actual	0.2 (1.3)	1.1 (1.8)	0.07
Hypopituitarism (%)	38%	29%	0.56

*Values are means (SD) unless stated otherwise. Disease cure is defined as normal glucose-suppressed GH levels and IGF-1 levels for age after surgery and/or irradiation. Data are shown as mean (SD), unless mentioned otherwise. *, p<0.05.*

GH, growth hormone; IGF-1, insulin-like growth factor-1; BMI, body mass index.

Table 5. Risk factor analysis for progression of osteophytosis and JSN in joint-specific analyses of the knee, hip and hands in long-term controlled acromegaly patients (n=116 joints)

Joint site	Estimated disease duration	Baseline IGF-1 SDS	Medically controlled vs. cured disease	d3-GHR
Knee				
OP	0.98 (0.88-1.09)	1.19 (0.95-1.50)	1.20 (0.46-3.15)	3.64 (1.29-10.23)*
JSN	1.02 (0.94-1.10)	1.27 (1.00-1.62)*	3.53 (1.12-10.28)*	0.92 (0.27-3.08)
Hip				
OP	1.06 (0.95-1.19)	1.05 (0.76-1.44)	2.85 (0.94-8.58)	1.30 (0.39-4.31)
JSN	1.07 (0.94-1.23)	1.44 (1.05-1.98)*	4.29 (1.03-17.80)*	2.51 (0.54-11.60)
Hand				
OP	1.14 (1.03-1.26)*	0.73 (0.54-1.00)	4.19 (1.37-12.85)*	1.30 (0.44-3.84)
JSN	1.01 (0.95-1.08)	0.87 (0.71-1.07)	1.55 (0.67-3.59)	0.75 (0.28-2.04)

Data were presented as adjusted odds ratio with 95% confidence intervals (adjusted OR, 95% CI). Left and right joints were analyzed independently. Risk factors were analyzed by Generalized Estimating Equations (GEE) analysis to adjust for the intra-patient effect. Additional adjustments were made for age, sex, BMI, intra-patient effect and baseline IGF-1 SD scores, when appropriate.

*CI, confidence interval; OP, osteophytosis; JSN, joint space narrowing; BMI, body mass index; d3-GHR, exon 3 deletion of the GHR polymorphism. *, p<0.05.*

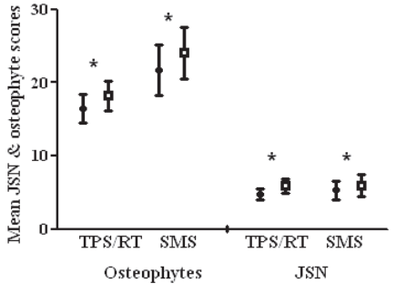


Figure 2: Mean osteophyte and JSN scores at baseline and follow-up, respectively, for acromegaly patients treated by surgery and/or radiotherapy versus patients treated by SMS analogs.

*Mean osteophyte and JSN scores (±SEM) of both study visits were shown for patients treated by TPS and/or RT versus patients treated by SMS analogs. The left symbol (circle) of each pair represents the baseline OP/JSN score; the right one (square) represents the score after 2.6 years of additional follow-up. TPS, transsphenoidal surgery; RT, radiotherapy; SMS, somatostatin analogs; *, p<0.001.*

DISCUSSION

This prospective study is the first to document that radiological features of acromegalic arthropathy progresses despite long-term biochemical remission of acromegaly, even in a relatively short follow-up period of 2.6 years and at all measured joint sites. Thus, it appears to be a progressive joint disease that is not merely halted or reversed by control of acromegaly. Remarkably, biochemical control by SMS analogs was associated with increased progression of radiological features of acromegalic arthropathy.

We previously demonstrated in a cross-sectional study that late effects of acromegaly on joints are striking, despite long-term disease control. The OA prevalence in these patients is much higher at all joint sites compared with the general population, and is associated with impaired quality of life (QoL) (2;3). Risk factors for radiographic OA in patients with long-term controlled acromegaly are high pre-treatment IGF-1 levels (5) and presence of d3-GHR polymorphism (6;7). In the present study, we observed progression of radiographic OA features in patients who were considered strictly controlled with medical therapy according to current guidelines. Several parameters reflecting GH/IGF-1 activity appear to predispose for progression. This finding may indicate that joints are a sensitive target organ to monitor GH/IGF-1 and could be used as biomarker to evaluate ongoing disease activity.

Several issues have to be considered with respect to the pathophysiology of progressive acromegalic osteoarthropathy. Progression of acromegalic arthropathy has probably a multifactorial pathophysiology, as known for primary OA. Traditionally, early-stage acromegalic arthropathy was considered to be driven by elevated IGF-1 levels, partially reversible after adequate treatment, and subsequently by mechanical changes. In later stages, acromegalic arthropathy was thought to act via one final common pathway with primary OA (1;22-24), indicating the same factors to be involved in progression. In accordance, we demonstrated that common risk factors for primary OA development, such as higher age, apply for patients with long-term controlled acromegaly (1;25-28). However, several parameters reflecting GH/IGF-1 activity appeared to influence progression of acromegalic arthropathy in the present study. Therefore, also the late stage of arthropathy might be mediated by the actual activity of the GH/IGF-I axis. First, IGF-1 SD concentrations measured at the baseline study visit were within the normal range in all patients, but, were associated with OA progression in knee and hip. Moreover, the functional d3-GHR polymorphism predicted osteophyte progression in the knee. In addition, medically well-controlled patients showed more radiographic progression

compared with surgically cured patients. In previous studies, GH secretion was found to be persistently abnormal during treatment with SMS analogs, despite appropriate biochemical control according to current criteria (29-32). Although disease history and treatment characteristics were not completely comparable between SMS-treated and surgically cured patients, the present study supports the hypothesis of suboptimal GH control in SMS-treated patients.

The classification of the radiographic changes of acromegalic arthropathy, which differs from those of primary OA, is subject to debate. Secondary OA in long-term controlled acromegaly presents with a characteristic phenotype of severe osteophytosis, frequently with extremely wide joint spaces, which is a well-known characteristic of active acromegalic disease (4). At present, no acromegalic-specific classification system for arthropathy exists, and primary OA scales are used, although with these scales joint space widening cannot be evaluated. Therefore, a main feature of acromegalic arthropathy is not taken into account. Because of the discrepancy between osteophyte severity and the lack of JSN, we preferred to use the OARSI atlas for grading radiographic OA with individual scores for osteophytes and JSN, and not a global OA scoring system such as Kellgren-Lawrence (33). However, it is difficult to define pathological JSN progression in acromegaly. To date, it is unknown whether joint space regression is a degenerative osteoarthritic feature or a reflection of ongoing normalization of hypertrophied cartilage after remission induction. Some short-term ultrasonography studies showed reduced joint space thickness after biochemical control (22;23). However, the fact that we also demonstrated osteophyte progression supports the hypothesis that in the present study joint space reduction is a pathological phenomenon. Future research, possibly with magnetic resonance imaging (MRI), is required on the regression of hypertrophied cartilage to normal thickness.

The most remarkable manifestation in our study is the increased progression of radiographic OA in medically treated patients, demonstrated in individual patients and in joint-specific analyses. In previous studies, differential effects on QoL and diastolic heart function were documented in patients with biochemical control by SMS analogs vs patients with surgical cure of acromegaly (29;34). This notion of inappropriate control of GH secretion by SMS analogs is supported by persistent abnormalities in GH secretion in these patients, despite clinically normal GH/IGF-1 levels (30). Recently, Neggers et al. hypothesized that in certain patients SMS analogs may normalize serum IGF-1 by a GH-independent factor that induces hepatic GH resistance,

which itself decreases hepatic IGF-1 production. Therefore, the reduction in circulating IGF-1 during SMS treatment does not necessarily imply disease control in peripheral tissues (extra-hepatic acromegaly) (35). Probably, SMS-treated patients might benefit from more aggressive disease control than obtained by applying current criteria. SMS analogs-Pegvisomant combination therapy was reported to have positive effects on QoL, especially on the physical dimension (31). Further studies have to confirm whether addition of Pegvisomant optimalizes disease control and therefore improves joint symptoms.

An alternative explanation for increased OA progression in SMS-treated patients may be a direct IGF-1-independent effect of SMS analogs on joint structure. There is evidence for direct local effects of SMS on cartilage, which are mostly inhibitive (36-39). In addition, SMS receptors were demonstrated in bone cells, which may mediate direct effects on the bone (40). Further studies are needed to confirm the physiological significance of SMS in chondrocyte and osteoblast growth regulation, with a view to articular effects of long-term SMS use in acromegaly. Another explanation is a generally less favourable previous course of acromegaly in SMS-treated patients (i.e. longer active disease duration or more severe acromegaly disease), which might result in more progressive disease. However, most previous studies failed to demonstrate a relationship between duration of active disease and arthropathy (8;22;41;42).

The degree of progression in primary OA varies considerably, depending on OA subtype, the radiographic protocol and the scoring method of progression. Therefore, the progression percentages observed in our study could not simply be compared with other studies investigating radiographic OA progression. A prospective study with the same radiographic protocol is the Genetics, ARthrosis and Progression (GARP) Study, involving patients with primary generalized OA (43). However, in this study progression was scored according to a different protocol and by another team of observers, resulting in unknown variation. The results from our study suggest more progression in acromegaly patients than in the GARP cohort (44;45). However, due to the different study design, no firm conclusions can be drawn.

Studies investigating OA progression over 2-3 years in the general population are scarce; most studies had longer follow-up. In several studies on hand OA, no significant change was demonstrated over 2 years (27;46-48). In the Rotterdam Study, radiographic OA progression was seen in 11.4% of the knees and 10.4% in the hips after 6.6 years follow-up, indicating lower percentages after 2-3 years (49). Although study designs are not fully comparable, the OA progression rate in acromegaly is

suggested to be much higher than in general population.

Some potential limitations of the present study have to be addressed. The first concerns the possibility of bias due to differences between consenters and non-consenters for follow-up. However, demographic and disease-specific characteristics did not differ, except for higher percentage females in non-consenters. We expect that this sex difference is a random finding and is, therefore, unlikely to affect the outcome. Second, paired scoring for progression with the films in chronological order may possibly have led to overestimation of progression when compared to paired scoring with films blinded for time sequence (18). However, because we were (a priori) especially interested in risk factors for progression, any misclassification of progression would have been non-differential, and, furthermore, radiographs of both cured and SMS-treated patients were scored in the same manner. Third, the maximal obtainable osteophyte/JSN scores in the hands were higher than in the hips or knees. It is very difficult to weight the responses in different joint sites and it is not clear if progression in one additional hand joint is as significant as one additional knee joint. Another limitation is the relatively short duration of additional follow-up after initial joint evaluation. Nonetheless, clear and multiple indications in different joint systems were obtained for progressive joint disease. In addition, in the present study we were especially interested in short-to-midterm follow-up, because we expected more differentiation in OA progression and, therefore, more possibilities to study risk factors.

In conclusion, our study indicates that many patients with long-term controlled acromegaly suffer from radiographic progression of acromegalic arthropathy, already within only 2.6 years of follow-up. Therefore, acromegalic arthropathy is a progressive joint disease that is not merely halted or reversed by biochemical disease control. Remarkably, biochemical control by SMS analogs was associated with increased radiographic progression of acromegalic arthropathy. However, since our study is not a randomized controlled trial, additional studies with longer follow-up duration are required to explore whether more aggressive treatment might be beneficial to improve the ultimate outcome of acromegalic arthropathy.

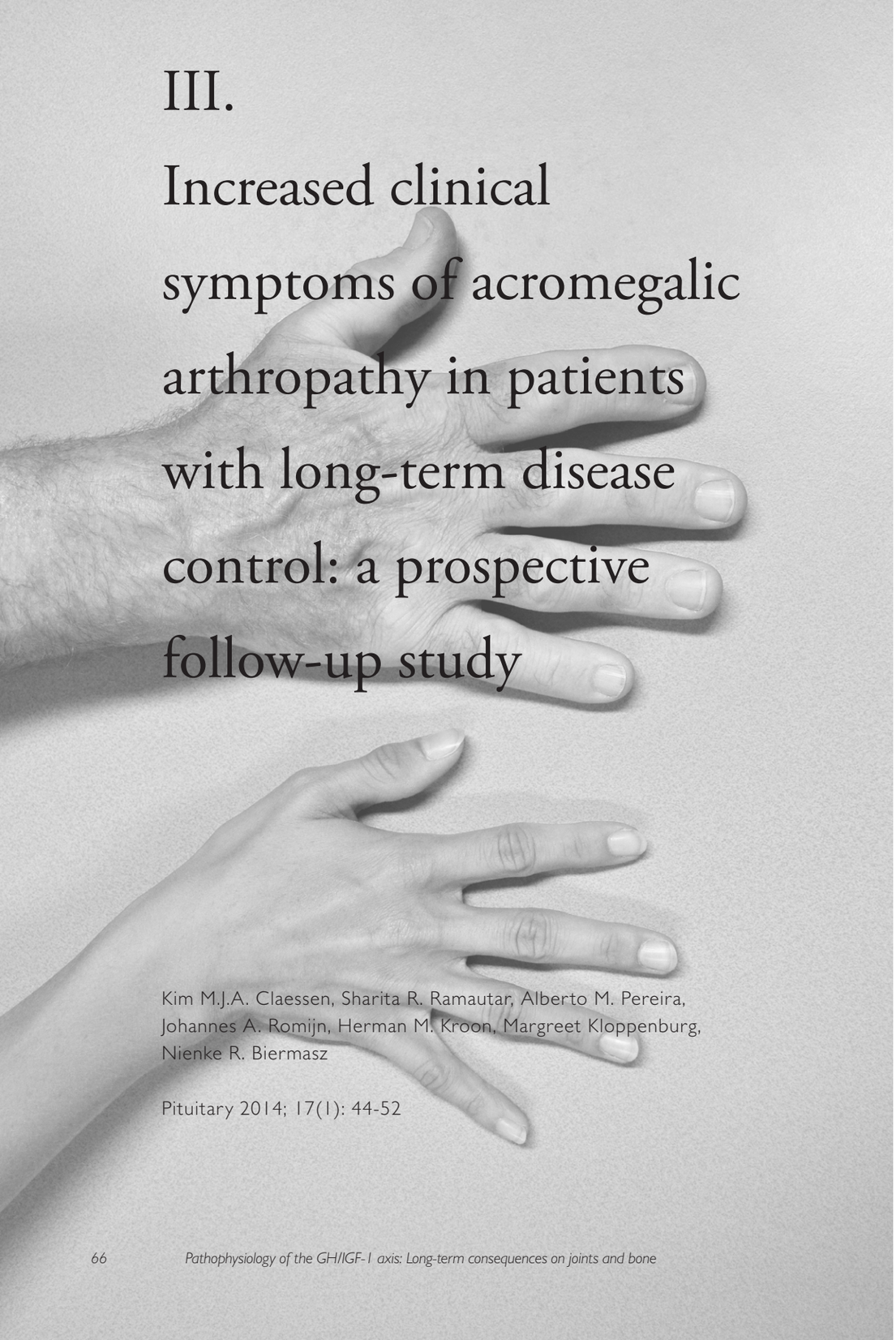
REFERENCE LIST

1. Barkan AL. Acromegalic arthropathy. Pituitary 2001; 4(4):263-264.
2. Wassenaar MJ, Biermasz NR, van DN et al. High prevalence of arthropathy, according to the definitions of radiological and clinical osteoarthritis, in patients with long-term cure of acromegaly: a case-control study. Eur J Endocrinol 2009; 160(3):357-365.
3. Wassenaar MJ, Biermasz NR, Kloppenburg M et al. Clinical osteoarthritis predicts physical and psychological QoL in acromegaly patients. Growth Horm IGF Res 2010; 20(3):226-233.
4. Wassenaar MJ, Biermasz NR, Bijsterbosch J et al. Arthropathy in long-term cured acromegaly is characterised by osteophytes without joint space narrowing: a comparison with generalised osteoarthritis. Ann Rheum Dis 2011; 70(2):320-325.
5. Biermasz NR, Wassenaar MJ, van der Klaauw AA et al. Pretreatment insulin-like growth factor-I concentrations predict radiographic osteoarthritis in acromegalic patients with long-term cured disease. J Clin Endocrinol Metab 2009; 94(7):2374-2379.
6. Wassenaar MJ, Biermasz NR, Pereira AM et al. The exon-3 deleted growth hormone receptor polymorphism predisposes to long-term complications of acromegaly. J Clin Endocrinol Metab 2009; 94(12):4671-4678.
7. Dos Santos C, Essioux L, Teinturier C, Tauber M, Goffin V, Bougneres P. A common polymorphism of the growth hormone receptor is associated with increased responsiveness to growth hormone. Nat Genet 2004; 36(7):720-724.
8. Biermasz NR, Pereira AM, Smit JW, Romijn JA, Roelfsema F. Morbidity after long-term remission for acromegaly: persisting joint-related complaints cause reduced quality of life. J Clin Endocrinol Metab 2005; 90(5):2731-2739.
9. Biermasz NR, van DH, Roelfsema F. Ten-year follow-up results of transsphenoidal microsurgery in acromegaly. J Clin Endocrinol Metab 2000; 85(12):4596-4602.
10. Roelfsema F, van DH, Frolich M. Long-term results of transsphenoidal pituitary microsurgery in 60 acromegalic patients. Clin Endocrinol (Oxf) 1985; 23(5):555-565.
11. van der Klaauw AA, Kars M, Biermasz NR et al. Disease-specific impairments in quality of life during long-term follow-up of patients with different pituitary adenomas. Clin Endocrinol (Oxf) 2008; 69(5):775-784.
12. Janssen YJ, Frolich M, Roelfsema F. A low starting dose of genotropin in growth hormone-deficient adults. J Clin Endocrinol Metab 1997; 82(1):129-135.
13. Cole TJ. The LMS method for constructing normalized growth standards. Eur J Clin Nutr 1990; 44(1):45-60.
14. Rikken B, van DJ, Ringeling A, Van den Brande JL, Massa G, Wit JM. Plasma levels of insulin-like growth factor (IGF)-I, IGF-II and IGF-binding protein-3 in the evaluation of childhood growth hormone deficiency. Horm Res 1998; 50(3):166-176.
15. Pantel J, Machinis K, Sobrier ML, Duquesnoy P, Goossens M, Amselem S. Species-specific alternative splice mimicry at the growth hormone receptor locus revealed by the lineage of retroelements during primate evolution. J Biol Chem 2000; 275(25):18664-18669.
16. Peterfy C, Li J, Zaim S et al. Comparison of fixed-flexion positioning with fluoroscopic semi-flexed positioning for quantifying radiographic joint-space width in the knee: test-retest reproducibility. Skeletal Radiol 2003; 32(3):128-132.
17. Altman RD, Hochberg M, Murphy WA, Jr., Wolfe F, Lequesne M. Atlas of individual radiographic features in osteoarthritis. Osteoarthritis Cartilage 1995; 3 Suppl A:3-70.
18. Botha-Scheepers S, Watt I, Breedveld FC, Kloppenburg M. Reading radiographs in pairs or in chronological order influences radiological progression in osteoarthritis. Rheumatology (Oxford) 2005; 44(11):1452-1455.
19. Bruynesteyn K, Boers M, Kostense P, van der LS, van der HD. Deciding on progression of joint damage in paired films of individual patients: smallest detectable difference or change. Ann Rheum Dis 2005; 64(2):179-182.
20. Fortin PR, Stucki G, Katz JN. Measuring relevant change: an emerging challenge in rheumatologic clinical trials. Arthritis Rheum 1995; 38(8):1027-1030.
21. Sharma L, Kapoor D, Issa S. Epidemiology of osteoarthritis: an update. Curr Opin Rheumatol 2006; 18(2):147-156.

22. Colao A, Marzullo P, Vallone G et al. Reversibility of joint thickening in acromegalic patients: an ultrasonography study. J Clin Endocrinol Metab 1998; 83(6):2121-2125.
23. Colao A, Cannavo S, Marzullo P et al. Twelve months of treatment with octreotide-LAR reduces joint thickness in acromegaly. Eur J Endocrinol 2003; 148(1):31-38.
24. Barkan A. Acromegalic arthropathy and sleep apnea. J Endocrinol 1997; 155 Suppl 1:S41-S44.
25. Neogi T, Zhang Y. Osteoarthritis prevention. Curr Opin Rheumatol 2011; 23(2):185-191.
26. Nishimura A, Hasegawa M, Kato K, Yamada T, Uchida A, Sudo A. Risk factors for the incidence and progression of radiographic osteoarthritis of the knee among Japanese. Int Orthop 2011; 35(6):839-843.
27. Kallman DA, Wigley FM, Scott WW, Jr., Hochberg MC, Tobin JD. The longitudinal course of hand osteoarthritis in a male population. Arthritis Rheum 1990; 33(9):1323-1332.
28. Bijsterbosch J, Watt I, Meulenbelt I, Rosendaal FR, Huizinga TW, Kloppenburg M. Clinical and radiographic disease course of hand osteoarthritis and determinants of outcome after 6 years. Ann Rheum Dis 2011; 70(1):68-73.
29. van Thiel SW, Bax JJ, Biermasz NR et al. Persistent diastolic dysfunction despite successful long-term octreotide treatment in acromegaly. Eur J Endocrinol 2005; 153(2):231-238.
30. Biermasz NR, Pereira AM, Frolich M, Romijn JA, Veldhuis JD, Roelfsema F. Octreotide represses secretory-burst mass and nonpulsatile secretion but does not restore event frequency or orderly GH secretion in acromegaly. Am J Physiol Endocrinol Metab 2004; 286(1):E25-E30.
31. Neggers SJ, van Aken MO, de Herder WW et al. Quality of life in acromegalic patients during long-term somatostatin analog treatment with and without pegvisomant. J Clin Endocrinol Metab 2008; 93(10):3853-3859.
32. Rubeck KZ, Madsen M, Andreasen CM, Fisker S, Frystyk J, Jorgensen JO. Conventional and novel biomarkers of treatment outcome in patients with acromegaly: discordant results after somatostatin analog treatment compared with surgery. Eur J Endocrinol 2010; 163(5):717-726.
33. Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. Ann Rheum Dis 1957; 16(4):494-502.
34. Biermasz NR, van Thiel SW, Pereira AM et al. Decreased quality of life in patients with acromegaly despite long-term cure of growth hormone excess. J Clin Endocrinol Metab 2004; 89(11):5369-5376.
35. Neggers SJ, Kopchick JJ, Jorgensen JO, van der Lely AJ. Hypothesis: Extra-hepatic acromegaly: a new paradigm? Eur J Endocrinol 2011; 164(1):11-16.
36. Weiss RE, Reddi AH, Nimni ME. Somatostatin can locally inhibit proliferation and differentiation of cartilage and bone precursor cells. Calcif Tissue Int 1981; 33(4):425-430.
37. Ferrandez MA, Carrascosa A, Audi L, Ballabriga A. Somatostatin effects on cultured human fetal epiphyseal chondrocytes. Pediatr Res 1992; 32(5):571-573.
38. Zapf J, Gosteli-Peter M, Weckbecker G, Hunziker EB, Reinecke M. The somatostatin analog octreotide inhibits GH-stimulated, but not IGF-I-stimulated, bone growth in hypophysectomized rats. Endocrinology 2002; 143(8):2944-2952.
39. Johansson O, Madsen K. Stimulative effect of somatostatin on cell proliferation in cultured chondrocytes. Med Biol 1987; 65(1):57-60.
40. Mackie EJ, Trechsel U, Bruns C. Somatostatin receptors are restricted to a subpopulation of osteoblast-like cells during endochondral bone formation. Development 1990; 110(4):1233-1239.
41. Miller A, Doll H, David J, Wass J. Impact of musculoskeletal disease on quality of life in long-standing acromegaly. Eur J Endocrinol 2008; 158(5):587-593.
42. Scarpa R, De BD, Pivonello R et al. Acromegalic axial arthropathy: a clinical case-control study. J Clin Endocrinol Metab 2004; 89(2):598-603.
43. Riyazi N, Meulenbelt I, Kroon HM et al. Evidence for familial aggregation of hand, hip, and spine but not knee osteoarthritis in siblings with multiple joint involvement: the GARP study. Ann Rheum Dis 2005; 64(3):438-443.
44. Botha-Scheepers S, Riyazi N, Watt I et al. Progression of hand osteoarthritis over 2 years: a clinical and radiological follow-up study. Ann Rheum Dis 2009; 68(8):1260-1264.
45. Botha-Scheepers SA, Watt I, Slagboom E et al. Influence of familial factors on radiologic disease progression over two years in siblings with osteoarthritis at multiple sites: a prospective longitudinal cohort study. Arthritis Rheum 2007; 57(4):626-632.
46. Bagge E, Bjelle A, Svanborg A. Radiographic osteoarthritis in the elderly. A cohort comparison and a longitudinal study of the "70-year old people in Goteborg". Clin Rheumatol 1992; 11(4):486-491.
47. Busby J, Tobin J, Ettinger W, Roadarmel K, Plato CC. A longitudinal study of osteoarthritis of the hand: the effect of age. Ann Hum Biol 1991; 18(5):417-424.
48. Kalichman L, Kobyliansky E, Seibel MJ, Livshits G. Repeated measurement study of hand osteoarthritis in an apparently healthy Caucasian population. Am J Hum Biol 2005; 17(5):611-621.
49. Reijman M, Pols HA, Bergink AP et al. Body mass index associated with onset and progression of osteoarthritis of the knee but not of the hip: the Rotterdam Study. Ann Rheum Dis 2007; 66(2):158-162.

III.

Increased clinical symptoms of acromegalic arthropathy in patients with long-term disease control: a prospective follow-up study



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ABSTRACT

OBJECTIVE: Arthropathy is an invalidating complication of acromegaly. This arthropathy deteriorates radiographically despite long-term disease control. However, the clinical course and its relationship to the radiographic course are currently unknown. We aimed to investigate the clinical course of arthropathy during follow-up and its relationship to radiographic progression in long-term controlled acromegaly patients.

DESIGN: Prospective follow-up study.

METHODS: We studied 58 patients (mean age 62 years, women 41%) with controlled acromegaly for a mean of 17.6 years. Clinical progression of joint disease was defined at baseline and after 2.6 years, by the Western Ontario McMaster Universities Osteoarthritis Index (WOMAC) and Australian/Canadian Osteoarthritis Index (AUSCAN) questionnaires for lower limb and hand OA, respectively, and performance tests. Potential risk factors for progression were assessed. The clinical course of arthropathy was related to the radiographic course.

RESULTS: On average, hand and lower limb function deteriorated during follow-up, despite large interindividual variations. Joint pain was stable over time. High levels of pain and functional impairment at baseline were related to clinical progression of hand pain and functional limitations. High baseline BMI was a risk factor for functional deterioration in the lower limb. The changes in symptoms and radiographic progression during follow-up were not related.

CONCLUSIONS: In treated acromegaly patients, joint function deteriorates during prolonged follow-up, despite biochemical disease control, although there was interindividual variation. Clinical and radiographic course of arthropathy were not related. Therefore, in clinical practice, a combination of clinical and radiographic assessment is necessary to evaluate the course of acromegalic arthropathy.

INTRODUCTION

In active acromegaly, patients have pathologically high growth hormone (GH) and insulin-like growth hormone-1 (IGF-1) levels, caused by a GH-producing pituitary adenoma in most cases. The increased GH/IGF-1 activity is associated with a variety of complaints, increased morbidity and mortality, resulting in reduced quality of life (QoL). Reversal of GH excess ameliorates clinical symptoms and life expectancy. However, other acromegalic features persist due to irreversible changes, for instance in bone and cartilage (1).

One of the most invalidating complications of acromegaly, which is at least partially irreversible, is arthropathy (2). Joint-related complaints, such as pain, stiffness or functional limitations, present at very young ages, frequently in combination with typical radiological abnormalities (3). Both weight and non-weight bearing joints are affected. Remarkably, even after long-term remission of acromegaly, the prevalence of clinical and radiographic osteoarthritis (OA) is persistently high, up to 12-fold higher compared to that in the general population (4). The presence of joint problems is associated with a considerable impairment of physical functioning and psychological well-being, reducing QoL (5).

Recently, arthropathy was reported to progress radiographically in acromegaly patients, even despite biochemical disease control (6). However, at present the clinical disease course of arthropathy during prolonged follow-up has not been studied. The determinants of outcome are not fully elucidated. Knowledge on these topics provides more insight in the pathophysiological processes that play a role in chronic acromegalic arthropathy. In addition, the knowledge of modifiable determinants can lead to new-targeted therapies.

Therefore, we designed a prospective follow-up study to assess the clinical course of acromegalic arthropathy and to identify risk factors for clinical progression. Furthermore, we aimed to evaluate the clinical OA course in relation to radiographic disease progression in order to propose a clinically useful tool to score arthropathy in acromegaly.

PATIENTS AND METHODS

Study design and patient selection

PATIENTS: All consecutive patients with acromegaly, who were referred to the Leiden University Medical Center for treatment from 1977 onwards, are collected in a database. In 2007, 89 acromegaly patients with long-term biochemical remission for a mean of 15.0 years (range 2.0-31.0 years) were assessed in a cross-sectional study (baseline visit) (4). All patients were re-invited in 2010 for a follow-up visit, of which 58 consented to participate. Thirty-one (35%) declined to participate, with health problems not related to OA (N=16) and travel distance (N=6) as most frequent reasons. There were no differences in demographic and acromegalic disease characteristics, nor in (radiographic) severity of arthropathy between patients participating in the follow-up study and those who did not (*data not shown*), except for more females among non-participators (p=0.025) (4;5).

Detailed yearly biochemical and clinical follow-up had been performed from the onset of acromegaly treatment. From 1977 onwards, the first treatment option in the majority of patients was transsphenoidal surgery (TPS) performed by a single specialized neurosurgeon. If necessary, adjuvant treatment consisted of radiotherapy (prior to 1985) or SMS analogs (from 1985 onwards). From 1998, in some patients, primary treatment consisted of depot formulations of long-acting SMS analogs. This treatment approach resulted in early postoperative control in 66% and late control in more than 90% of the patients (7). From 2003, Pegvisomant was available for treatment-resistant acromegaly.

Disease activity was assessed yearly by oral glucose tolerance tests (except in medically-treated patients), fasting serum GH and IGF-1 levels. Remission was defined as a normal glucose-suppressed serum GH<1.25 (RIA assay until 1992) or 0.38µg/l (immunofluorometric assay (IFMA) from 1992 onwards), serum GH levels of <1.9µg/L (all years), and normal IGF-1 levels for age (from 1986 onwards) (1;8;9). Patients not meeting these criteria were offered additional treatment.

Hypopituitarism was supplemented with estrogens/testosterone, thyroxine, hydrocortisone according to the following definitions (1;8;10). Estrogen deficiency in women was present in case of LH/FSH deficiency in premenopausal women with prolonged amenorrea >1 year without adequate replacement therapy or by low serum oestradiol concentration of <70nmol/l and all postmenopausal women. In men, LH/FSH deficiency was defined as testosterone level <8.0nmol/l. Thyroid stimulating hormone

(TSH) deficiency was defined as a free thyroxine level below the reference range (<10pmol/l). Adrenocorticotrophic hormone (ACTH) deficiency was defined as an insufficient increase of cortisol (peak <0.55μmol/l) after corticotrophin releasing hormone test or insulin tolerance test. GH deficiency was not routinely assessed.

The Medical Ethics Committee approved the study protocol, and all subjects gave written consent for their participation.

STUDY DESIGN: Patients were included for baseline cross-sectional assessment between September and December 2007. Between March and September 2010 participants who consented for a follow-up evaluation were assessed. At both study visits, patients completed standardized questionnaires (*vide infra*), conventional radiographs of hands, hips and knees were obtained in all patients (*vide infra*), and blood samples were taken in the post-absorptive state to assess actual GH and IGF-1 concentrations.

Study parameters

QUESTIONNAIRES: A standardized questionnaire was completed concerning demographic data, medical history and symptoms and signs of OA. Self-reported pain, stiffness and functional limitations were assessed with the corresponding subscales of the Australian/Canadian Osteoarthritis Hand Index (AUSCAN) and Western Ontario McMaster Universities Osteoarthritis Index (WOMAC), assessing the hand and lower limb (knee and hip together), respectively (11;12). Samples of questions include: pain during rest, pain during walking stairs, pain which disturbs sleep, pain during sitting, stiffness after a time of inactivity, problems with bending, problems with pulling on socks, problems with opening of a cooking-pot, and problems when picking up heavy things. Using the AUSCAN, items are rated on a 5-point Likert scale ranging from 0 (none) to 4 (extreme), using a 48-h time frame, total scores ranging from 0 to 20, 0 to 4 and 0 to 36, for pain, stiffness and function, respectively. WOMAC scores on the subscales pain, stiffness and function range from 0 to 100, using a 100mm visual analogue scale (VAS) format. Higher scores indicate worse outcome.

PERFORMANCE TESTS: Cylinder and pinch grip strength of both hands was assessed using a cylinder and pinch grip meter, respectively, in kilograms (13;14).

RADIOGRAPHIC PROTOCOL AND RADIOGRAPHIC SCORING: Conventional radiographs of the hands (dorsovolar), knees (posterior-anterior (PA), in weight-bearing/semi-flexed and lateral) and hips (PA, supine) were obtained by a single experienced radiographer, according to a standardized protocol (15). These radiographic data have been recently published (6).

Radiographs were scored paired in chronological order blinded for patient characteristics by a single trained reader (K.C.), using the Osteoarthritis Research Society (OARSI) atlas (16). Osteophytes and JSN were graded 0-3 in the hands (distal interphalangeal (DIP), proximal interphalangeal (PIP), metacarpophalangeal (MCP), first interphalangeal (IP), and first carpometacarpal (CMC1) joints), knees (medial and lateral tibiofemoral) and hips. Total scores were calculated by adding left and right sites, with maximum scores ranging from 0 to 108 for JSN and from 0 to 120 for osteophytes. Intra-class correlation coefficients (ICCs) for intra-reader reproducibility based on 15 randomly selected radiographs, were 0.98 and 0.98 in the hands, 1.00 and 0.99 in the knee, 0.98 and 1.00 in the hip, for JSN and osteophytes respectively.

DEFINITION OF CLINICAL PROGRESSION: Clinical relevant change in hand OA was evaluated by the minimum clinically important improvement (MCII) of 1.49 and 1.25 for pain and function, respectively (17). Those with a change on AUSCAN pain and function below -1.49 and -1.25, respectively, were classified as improved. Patients with change on AUSCAN pain and function above 1.49 and 1.25, respectively, were classified as deteriorated.

Clinical progression of lower limb OA was defined as an increase in self-reported (WOMAC) pain, stiffness or functional limitations above the predefined minimum perceptible clinical improvement (MPCI). MPCI was originally developed as threshold value to define treatment response in OA. Threshold values were 9.7 for WOMAC pain, 10.0 for WOMAC stiffness and 9.3 for WOMAC function (18). These threshold values with negative sign were used to define clinical improvement.

DEFINITION OF RADIOGRAPHIC PROGRESSION: For osteophytes and JSN the smallest detectable change (SDC) was used to assess change over 2.6 years above the measurement error (0.85 and 0.57 resp.), and therefore, radiographic progression was defined as ≥1-score increase in JSN or osteophyte total scores (19). At joint site level, radiographic progression was defined as ≥1-score increase in JSN or osteophyte score at the specific site (SDCs for osteophytes and JSN: knee, both 0.3; hip, both 0.4; hands, 0.8 and 0.4, resp.), only in those joints with baseline features of radiographic OA.

PARAMETERS OF ACROMEGALIC DISEASE: Active disease duration was estimated using the start of symptoms and signs to the date of serum IGF-1 normalization after treatment. Duration of remission was calculated from the date of biochemical remission until the start of the present study. Cure of acromegaly was defined by normal glucose-suppressed GH levels and IGF-1 levels for age after surgery and/or irradiation. Biochemical acromegaly control was defined by normal serum IGF-1 levels for age during SMS analog treatment. Both cured and biochemically controlled patients were referred to as 'in remission'.

BIOCHEMICAL PARAMETERS: Serum GH was measured with a sensitive IFMA (Wallac, Turku, Finland), specific for the 22kDA GH protein, calibrated against World Health Organisation International Reference Preparation (WHO IRP) 80/505 (detection limit: 0.01µg/l, interassay coefficient of variation (CV): 1.6-8.4% of 0.01-15.38µg/l) from 1992 onwards. For the conversion of µg/l to mU/l, multiply by 2.6. Before 1992, GH was measured by RIA (Biolab, Serona, Coissins, Switzerland), calibrated against WHO IRP 66/21 (detection limit: 0.5mU/l, with an interassay CV<5%; for the conversion of µg/l to mU/l, multiply by 2).

Serum IGF-1 concentrations (nmol/l) were measured using an immunometric technique on an Immulite 2500 system (Diagnostic Products Corporation, Los Angeles, CA, USA). The intra-assay variations at mean plasma levels of 8 and 75nmol/l were 5.0 and 7.5%, respectively. IGF-1 levels were expressed as SD score, using lambda-mu-sigma smoothed reference curves based on measurements in 906 healthy individuals (20;21).

Statistical analysis

SPSS for Windows, version 17.0 (SPSS Inc., Chicago, IL, USA), was used for data analysis. Mean changes with 95% confidence intervals (CI) for the pain, stiffness and function subscales of the AUSCAN and WOMAC questionnaires, hand grip strength, and osteophytes and JSN were calculated. Cumulative probability plots were used to visualise changes in AUSCAN and WOMAC pain and function, respectively.

Binary logistic regression analyses were used to evaluate determinants for clinical OA progression with respect to pain and function, for the hand and lower limb, with clinical progression as dependent variable. Age, sex, BMI, acromegalic-specific parameters (*i.e.* type of treatment, baseline IGF-1 SDS, active disease duration), baseline self-reported pain and function scores, and structural abnormalities at baseline (osteophytes/JSN) were studied. Adjustments were made for the use of pain medication.

The association between clinical change and radiographic progression of OA was assessed by estimating mean differences between patients with and without radiographic progression, using Independent *T*-tests. ANCOVA analyses were performed to adjust for age, sex, BMI, baseline total AUSCAN and WOMAC scores for hand and lower limb OA, respectively, and baseline osteophytes and JSN, with radiographic progression as dependent variable.

RESULTS

Patient description

Fifty-eight patients with long-term remission of acromegaly were included and followed for a mean of 2.6 years (range 2.3-2.9 years). Patient characteristics are shown in *Table 1*. Mean age was 61.8±10.9 years and 41% were women. Patients were in remission for a mean of 17.6±7.2 years (minimum 2 years) and mean actual IGF-1 SDS was 0.51±1.51. No recurrences occurred during longitudinal follow-up. In 40 patients (69%) remission was achieved by surgery, and, if necessary, by additional radiotherapy. The other 18 patients (31%) were treated during the observation period by either primary and/or postoperative long-acting SMS analogs. One patient was co-treated with Pegvisomant. Any pituitary deficiency was present in 20 patients (35%).

At baseline, structural abnormalities on radiographs were highly prevalent. In the knee, osteophytes and JSN were present in 78% and 31% of patients, respectively; in the hip, prevalence of osteophytes and JSN was 72% and 14%, respectively, and in the hands 84% and 70%, respectively.

Clinical course of acromegalic arthropathy over 2.6 years

Self-reported pain and disability

HANDS: Mean self-reported pain did not significantly change over time (*Table 2*). However, there was a great variation on the individual level, as depicted in a cumulative probability plot (*Figure 1A*). Clinically relevant increase in pain was found in 15 patients (27%), whereas 6 patients (10%) reported less pain. With respect to change in functional limitations, 26 patients (45%) reported more functional limitations and 4 patients (7%) improved (*Figure 1A*). Mean AUSCAN function scores deteriorated significantly over time (p<0.001) with a mean change (2.6±4.8) above the clinically relevant deterioration threshold (*i.e.* 1.25), although there was large individual variety. Especially on the questions regarding muscle power and fine motor control, patients reported worse scores over time.

LOWER LIMB: WOMAC stiffness and function scores, not pain scores, deteriorated significantly over time (*Table 2*). Patients deteriorated especially on the following items: descending stairs, putting on and taking off socks, rising from bed and getting in/out bath. As shown in *Figure 1B*, clinically relevant progression was shown in 27 patients (47%), based on changes in WOMAC pain (N=12), stiffness (N=21) or function scores (N=10) above the MCPI. Twenty-one patients improved, based on changes in WOMAC pain (N=9), stiffness (N=9) or function score (N=14) below the MCPI.

AUSCAN and WOMAC change scores of pain, stiffness and function subscales were not significantly different between younger (<45 years) and older (≥65 years) patients, nor between males and females (*data not shown*). Also when the clinical course of arthropathy was only assessed in patients with radiographic presence of OA at baseline, we found similar heterogeneous clinical changes during follow-up (both for AUSCAN and WOMAC).

At baseline and follow-up 6 (10.3%) and 7 (12.1%) patients used medication for joint symptoms, respectively. Non-steroidal anti-inflammatory drugs (NSAIDs) and paracetamol were most frequently used: at baseline by 8.6% and 1.7% of the patients, respectively, and at follow-up by 3.4% and 8.6%, resp. Joint surgery was performed in 4 patients (6.9%) (knee surgery N=3, hip surgery N=1).During follow-up, 3 patients (5.2%) received a joint replacement (2 knees, 1 hip). In the small subgroup of patients with joint prostheses, pain did not change over time, and function scores improved in 1 patient.

Performance tests (Table 2)

Cylinder grip strength decreased over 2.6 years (p=0.052), especially in the right hand (p=0.021), which was the dominant hand in 83% of patients. In addition, pinch grip strength deteriorated considerably, with a mean decrease of 2.5±2.2 kilograms (p<0.001).

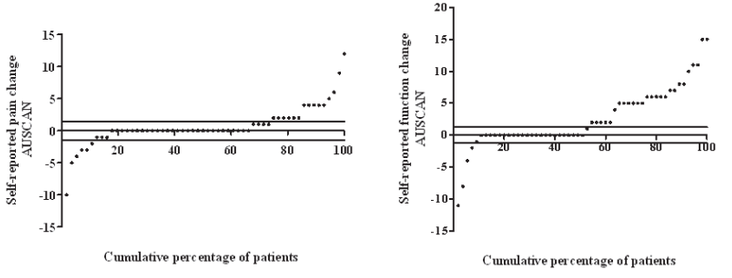

Figure 1A

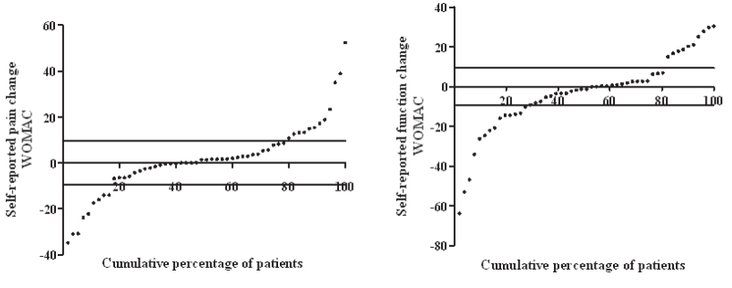

Figure 1B

Cumulative probability plots of change in self-reported pain and functional limitations of the hand (A) and lower limb (B), respectively, over 2.6 years in 58 patients with long-term controlled acromegaly.

A. Change in self-reported pain and functional limitations in the hand. The horizontal lines represent the cut-off for deterioration and improvement based on the minimum clinically important improvement. Patients above the upper horizontal line have deterioration of pain or functional limitations. Patients below the lower dotted line have improvement of pain or functional limitations.

B. Change in self-reported pain and functional limitations in the lower limb. The horizontal line above is the line set at minimal perceptible clinical improvement (MCPI) score which is used as the cut-off to define clinical progression, and the horizontal line below is the line set to define improvement.

Table 1. Clinical characteristics of 58 patients with acromegaly

Clinical characteristics	Patients (N=58)
Age (years)	61.8 (10.9)
Sex, female (n (%))	24 (41.4)
BMI (kg/m^2)	28.9 (4.5)
Treatment (n (%))	
Surgery only	31 (53.4)
Surgery + RT	9 (15.5)
SMS analogues	
Primary	2 (3.4)
Following surgery *	13 (22.4)
Following RT	1 (1.7)
Following surgery + RT	2 (3.4)
Disease duration (years)	9.2 (8.1)
Duration of remission (years)	17.6 (7.2)
Pre-treatment GH (µg/L)	33.7 (45.4)
IGF-1 SD scores	
Pre-treatment	6.9 (3.6)
Actual	0.5 (1.5)
Hypopituitarism (n (%))	20 (34.5)
Corticotrope failure	15 (25.8)
Thyreotrope failure	10 (17.2)
Gonadotrope failure	
Males	8 (13.8)
Pre-menopausal	0 (0.0)
Post-menopausal	22 (37.9)

Values are means (SD) unless stated otherwise.

*GH, growth hormone; IGF-1, insulin-like growth factor 1; BMI, body mass index; RT, radiotherapy; SMS, somatostatin (analogs), long-acting. *, one patient is co-treated with Pegvisomant.*

Table 2. Baseline, follow-up and change scores on self-reported pain and functional limitations, grip strength of the hands, and radiographic OA features in 58 patients with long-term controlled acromegaly followed for 2.6 years

	Baseline	Follow-up	Change*	95% CI**
AUSCAN				
Pain (0-20)	2.1 (3.7)	2.8 (4.2)	0.7 (3.1)	-0.2 to 1.5
Stiffness (0-4)	0.7 (0.9)	0.8 (0.9)	0.1 (0.8)	-0.1 to 0.3
Function (0-36)	3.4 (5.4)	6.0 (7.3)	2.6 (4.8)	1.3 to 3.9
WOMAC				
Pain (0-100)	16.8 (20.4)	18.3 (19.2)	1.5 (15.7)	-2.7 to 5.8
Stiffness (0-100)	24.0 (24.2)	31.0 (27.6)	7.0 (21.9)	0.8 to 13.0
Function (0-100)	16.4 (19.2)	20.7 (20.4)	4.3 (13.4)	0.7 to 8.1
Grip strength (kg)				
Cylinder grip	39.0 (15.7)	37.3 (15.6)	-1.7 (6.4)	-3.4 to 0.02
Pinch grip	8.1 (3.5)	5.6 (2.7)	-2.5 (2.2)	-3.1 to -2.0
Radiographic OA				
OP (0-120)	18.0 (12.9)	20.0 (13.5)	2.0 (1.9)	1.6 to 2.3
JSN (0-108)	5.4 (4.9)	7.1 (5.9)	1.7 (1.7)	1.4 to 2.0

*Data are shown as mean (SD). *, mean change (SD) over 2.6 years. **, 95% CI of the change.*

AUSCAN, Australian/Canadian Osteoarthritis Hand Index; WOMAC, Western Ontario McMaster Universities Osteoarthritis Index; kg, kilograms; OP, osteophytes; JSN, joint space narrowing.

Table 3. Risk factors for clinical progression of acromegalic arthropathy, as defined by an increase in pain or functional impairment over time, in 58 patients with controlled disease, for hand and lower limb OA, respectively

		Hand	Lower limb
Risk factors	Clinical OA progression	OR (95%CI)	OR (95%CI)
Age	Pain	(0.93-1.08)	0.98 (0.93-1.04)
	Function	1.06 (0.96-1.17)	1.02 (0.95-1.09)
Female sex	Pain	0.53 (0.09-2.98)	1.01 (0.28-3.68)
	Function	1.48 (0.27-8.03)	0.93 (0.23-3.74)
BMI	Pain	1.14 (0.96-1.36)	1.10 (0.95-1.27)
	Function	1.10 (0.91-1.32)	1.20 (1.02-1.41)*
Estimated disease duration	Pain	0.96 (0.84-1.09)	0.95 (0.84-1.06)
	Function	0.96 (0.83-1.10)	0.92 (0.79-1.07)
Baseline IGF-1 SDS	Pain	1.26 (0.73-2.20)	1.17 (0.77-1.78)
	Function	1.13 (0.67-1.92)	1.39 (0.85-2.27)
Medically controlled *vs.* cured disease	Pain	1.07 (0.19-6.15)	2.50 (0.49-12.90)
	Function	2.29 (0.25-21.20)	0.99 (0.22-4.39)
Baseline pain	Pain#	1.29 (1.08-1.53)*	0.98 (0.94-1.02)
	Function#	1.33 (1.09-1.60)*	0.99 (0.95-1.03)
Baseline functional impairment	Pain#	1.18 (1.04-1.33)*	0.98 (0.95-1.02)
	Function#	1.32 (1.11-1.58)*	0.98 (0.96-1.03)

Risk factors were analyzed with binary logistic regression analysis with progression of clinical OA as dependent variable, independently assessed for the hand and lower limb. Baseline IGF-1 SDS were IGF-1 SD scores at the time of the study visit in 2007. Disease cure is defined as normal glucose-suppressed GH levels and IGF-1 levels for age after surgery and/or irradiation.

*CI, confidence interval; BMI, body mass index; NA, not applicable. *, p<0.05.*
#, adjustments were made for the use of pain medication.

Determinants of clinical progression in hand and lower limb OA after 2.6 years

HANDS: Both clinical progression of hand pain and functional limitations, defined by the predefined cut-off values of MCII for hand OA, were related to severe functional impairment (OR=1.29 (1.08-1.53), p=0.005 and OR=1.18 (1.04-1.33), p=0.009 for baseline pain and function, resp.) and pain at baseline (OR=1.33 (1.09-1.60), p=0.004 and OR=1.32 (1.11-1.58), p=0.002) for baseline pain and function, resp.), as reflected by AUSCAN function and pain scores, respectively, also when adjusted for the use of pain medication. Age, female sex, BMI, determinants reflecting acromegaly disease activity (*i.e.* baseline IGF-1 SDS, active disease duration, type of treatment for acromegaly) and structural abnormalities at baseline (*i.e.* osteophytes/JSN) were not associated with clinical deterioration (*Table 3*).

LOWER LIMB: Demographic characteristics, acromegaly-specific parameters and structural abnormalities were not related to clinical progression of lower limb OA, defined as WOMAC pain or function scores above the predefined thresholds of the MPCI (*Table 3*). A higher BMI, however, was positively associated to functional progression (OR=1.20 (1.02-1.41), p=0.031), meaning that BMI was 1.2-fold higher in patients with functional deterioration over time. Clinical progression was not associated with the severity of self-reported pain or functional limitations at baseline (as reflected by WOMAC scores).

Relationship between clinical change and radiographic progression in acromegalic arthropathy

As previously published, total scores of osteophytes and JSN deteriorated over time (*Table 2*), reflected by mean changes of total scores of 2.0±1.9 and 1.7±1.7, respectively (3;6). Radiographic progression of osteophytes and JSN at any joint site was present in 42 (72%) and 43 (74%) patients, respectively.

HANDS: The mean change in self-reported pain and functional limitations in the respective AUSCAN subscales was not different between patients with and without radiographic progression of hand OA, with mean differences (95%CI) in pain and functional limitations of -0.14 (-2.36, 2.08) and -0.32 (-3.52, 2.87), respectively, between patients with and without osteophyte progression; mean differences between patients with

and without JSN progression were -0.02 (-1.72, 1.68) and -0.26 (-2.89, 2.36) for pain and function, resp. Adjustments for age, sex, BMI, baseline AUSCAN total score, baseline osteophyte and JSN scores in the hand did not change these results. This indicates no relationship between clinical change and radiographic progression.

LOWER LIMB: In addition, there was no association between clinical change and radiographic progression in the lower limb, neither when adjusted for age, sex, BMI, baseline WOMAC total score, baseline osteophytes and JSN in the lower limb. Mean differences in pain and functional limitations (4.12 (-4.62, 12.86) and 0.91 (-9.39, 11.21), respectively) did not differ between patients with and without osteophyte progression, nor between patients with and without JSN progression (-2.68 (-11.43, 6.06) and -6.31 (-17.23, 4.60) for pain and function, resp.).

DISCUSSION

The present study evaluates the clinical course of acromegalic arthropathy in well-controlled patients and the relationship with radiographic changes. Although there was large interindividual variation, on average we found significant deterioration of hand and lower limb function, but not of pain, over time, as reflected by higher AUSCAN and WOMAC function scores and decreased grip strength after 2.6 years of prospective follow-up. There was no clear association between the clinical and radiographic changes of acromegalic arthropathy over time.

The prevalence of acromegalic arthropathy is high in patients with both active and well-controlled acromegaly, and is significantly increased when compared to general population (4). Acromegalic arthropathy affects both weight and non-weight-bearing joints, which is associated with decreased QoL (22). The pathophysiology 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. Furthermore, IGF-1 is involved in the initiation and regulation of osteophyte development (23). This early stage is thought to be at least partially reversible by adequate treatment (24). However, when this GH/IGF-1 excess persists, the pathophysiological process becomes irreversible and self-perpetuating, eventually leading to joint failure. Recently, acromegalic arthropathy was reported to progress radiographically despite long-term biochemical remission (6). However, clinical disease course in treated patients have not been assessed before.

Several issues are of interest with respect to clinical arthropathy in acromegalic patients. First, scores on the WOMAC and AUSCAN questionnaires, especially on pain and function subscales, were relatively low when compared to primary OA patients. This might be explained by the typical radiographic phenotype of acromegalic arthropathy, with predominantly osteophytosis in combination with preserved or even widened articular cartilage (3). This is in accordance with previous observations reporting less joint prostheses when compared to primary OA patients, suggesting that cartilage hypertrophy may protect against pain caused by osteophytes and, therefore, protect acromegalic patients against a decrease in functional capability. In addition, patients in our acromegaly cohort were not selected on the presence of arthropathy, but we included all consecutive patients that gave informed consent. This may probably explain the lower clinical OA scores in our patients. Second, there was no relation between clinical deterioration and radiographic progression of arthropathy. This discordance between clinical and radiographic OA course is also present in primary OA (3;25). We demonstrated that the clinical course of arthropathy was very heterogeneous: some of the acromegalic patients remained stable and in a substantial proportion of patients even improvement of symptoms was seen. Thus, clinical deterioration is not inevitable for each patient. In contrast, radiographic abnormalities worsen over time. It is important to bear in mind and inform patients that the evolution of symptoms and radiographic abnormalities are not related.

This study might suffer from several limitations. First, the number of patients is relatively small, probably resulting in a power problem to identify risk factors for clinical progression over time. Therefore, larger patient cohorts have to be followed prospectively in order to draw firm conclusions. Second, it has to be kept in mind that the WOMAC and AUSCAN questionnaires are used primarily in primary OA research, and are not developed or validated for acromegalic arthropathy. However, since there are currently no acromegaly-specific scoring methods for arthropathy, we are limited to use these primary OA methods. Further study has to point out whether modification of present methods is needed in order to fit the typical phenotype of acromegalic arthropathy. Third, several patients received analgesic and NSAID therapy, and 31% of patients used SMS analogs which might have analgesic properties.

These factors might have lessened their joint complaints. However, the clinical course, as far as we know, is not influenced by any type of medication; and therefore we do not believe that the used medication will interfere with our results. On the other hand, this indicates that the optimal management of acromegalic arthropathy requires further study. In this respect, further investigation has to assess whether specific intervention therapies, for example with physiotherapy, could be beneficial. Fourth, since a considerable proportion of patients have multiple pituitary hormone deficiencies, it is difficult to examine to what extent the changes in joint problems can be attributed to (previous) GH excess. It could also be the consequence of suboptimal or excessive replacement therapy of other hormones, although, at present, no such associations with arthropathy are reported. Finally, ideally, we would prefer to include a control group of uncontrolled acromegaly patients that gives us insight into the course of arthropathy during active disease. Unfortunately, such a control group is not available in our center. A prospective study with a comparable study design among patients with primary generalized OA is the GARP Study (Genetics, ARthrosis and Progression) (26). The results of our study suggest that especially joint function deteriorates faster in acromegaly than in GARP patients, as reflected by higher mean changes in AUSCAN and WOMAC function scores over 2 years (27;28). However, based on these limited data, no firm conclusions can be drawn.

In conclusion, hand and lower limb function, not joint pain, deteriorated over time in a considerable proportion of patients with well-controlled acromegaly, although there was large variation between individuals. Clinical and radiographic course were not related, and, therefore, we believe that in clinical practice a combination of clinical and radiographic assessment is necessary to evaluate the course of acromegalic arthropathy. Additional studies in larger patient cohorts have to identify potential modifiable risk factors associated with clinical deterioration.

REFERENCE LIST

1. Biermasz NR, Pereira AM, Smit JW, Romijn JA, Roelfsema F. Morbidity after long-term remission for acromegaly: persisting joint-related complaints cause reduced quality of life. J Clin Endocrinol Metab 2005; 90(5):2731-2739.
2. Barkan A. Acromegalic arthropathy and sleep apnea. J Endocrinol 1997; 155 Suppl 1:S41-S44.
3. Wassenaar MJ, Biermasz NR, Bijsterbosch J et al. Arthropathy in long-term cured acromegaly is characterised by osteophytes without joint space narrowing: a comparison with generalised osteoarthritis. Ann Rheum Dis 2011; 70(2):320-325.
4. Wassenaar MJ, Biermasz NR, van DN et al. High prevalence of arthropathy, according to the definitions of radiological and clinical osteoarthritis, in patients with long-term cure of acromegaly: a case-control study. Eur J Endocrinol 2009; 160(3):357-365.
5. Wassenaar MJ, Biermasz NR, Kloppenburg M et al. Clinical osteoarthritis predicts physical and psychological QoL in acromegaly patients. Growth Horm IGF Res 2010; 20(3):226-233.
6. Claessen KM, Ramautar SR, Pereira AM et al. Progression of acromegalic arthropathy despite long-term biochemical control: a prospective, radiological study. Eur J Endocrinol 2012; 167(2):235-244.
7. Biermasz NR, Dekker FW, Pereira AM et al. Determinants of survival in treated acromegaly in a single center: predictive value of serial insulin-like growth factor I measurements. J Clin Endocrinol Metab 2004; 89(6):2789-2796.
8. Biermasz NR, van DH, Roelfsema F. Ten-year follow-up results of transsphenoidal microsurgery in acromegaly. J Clin Endocrinol Metab 2000; 85(12):4596-4602.
9. Roelfsema F, van DH, Frolich M. Long-term results of transsphenoidal pituitary microsurgery in 60 acromegalic patients. Clin Endocrinol (Oxf) 1985; 23(5):555-565.
10. van der Klaauw AA, Kars M, Biermasz NR et al. Disease-specific impairments in quality of life during long-term follow-up of patients with different pituitary adenomas. Clin Endocrinol (Oxf) 2008; 69(5):775-784.
11. Bellamy N, Buchanan WW, Goldsmith CH, Campbell J, Stitt LW. Validation study of WOMAC: a health status instrument for measuring clinically important patient relevant outcomes to antirheumatic drug therapy in patients with osteoarthritis of the hip or knee. J Rheumatol 1988; 15(12):1833-1840.
12. Bellamy N, Campbell J, Haraoui B et al. Clinimetric properties of the AUSCAN Osteoarthritis Hand Index: an evaluation of reliability, validity and responsiveness. Osteoarthritis Cartilage 2002; 10(11):863-869.
13. Bourbonnais D, Frak V, Pilon JF, Goyette M. An instrumented cylinder measuring pinch force and orientation. J Neuroeng Rehabil 2008; 5:2.
14. Sancho-Bru JL, Giurintano DJ, Perez-Gonzalez A, Vergara M. Optimum tool handle diameter for a cylinder grip. J Hand Ther 2003; 16(4):337-342.
15. Peterfy C, Li J, Zaim S et al. Comparison of fixed-flexion positioning with fluoroscopic semi-flexed positioning for quantifying radiographic joint-space width in the knee: test-retest reproducibility. Skeletal Radiol 2003; 32(3):128-132.
16. Altman RD, Hochberg M, Murphy WA, Jr., Wolfe F, Lequesne M. Atlas of individual radiographic features in osteoarthritis. Osteoarthritis Cartilage 1995; 3 Suppl A:3-70.
17. Bellamy N, Wilson C. International estimation of minimally clinically important improvement (MCII75): the Reflect Study. Internal Medicine Journal 2007; 37:A36.
18. Ehrich EW, Davies GM, Watson DJ, Bolognese JA, Seidenberg BC, Bellamy N. Minimal perceptible clinical improvement with the Western Ontario and McMaster Universities osteoarthritis index questionnaire and global assessments in patients with osteoarthritis. J Rheumatol 2000; 27(11):2635-2641.
19. Bruynesteyn K, Boers M, Kostense P, van der LS, van der HD. Deciding on progression of joint damage in paired films of individual patients: smallest detectable difference or change. Ann Rheum Dis 2005; 64(2):179-182.
20. Cole TJ. The LMS method for constructing normalized growth standards. Eur J Clin Nutr 1990; 44(1):45-60.
21. Rikken B, van DJ, Ringeling A, Van den Brande JL, Massa G, Wit JM. Plasma levels of insulin-like growth factor (IGF)-I, IGF-II and IGF-binding protein-3 in the evaluation of childhood growth hormone

deficiency. Horm Res 1998; 50(3):166-176.
22. Biermasz NR, van Thiel SW, Pereira AM et al. Decreased quality of life in patients with acromegaly despite long-term cure of growth hormone excess. J Clin Endocrinol Metab 2004; 89(11):5369-5376.
23. Okazaki K, Jingushi S, Ikenoue T et al. Expression of insulin-like growth factor I messenger ribonucleic acid in developing osteophytes in murine experimental osteoarthritis and in rats inoculated with growth hormone-secreting tumor. Endocrinology 1999; 140(10):4821-4830.
24. Barkan AL. Acromegalic arthropathy. Pituitary 2001; 4(4):263-264.
25. Bijsterbosch J, Watt I, Meulenbelt I, Rosendaal FR, Huizinga TW, Kloppenburg M. Clinical and radiographic disease course of hand osteoarthritis and determinants of outcome after 6 years. Ann Rheum Dis 2011; 70(1):68-73.
26. Riyazi N, Meulenbelt I, Kroon HM et al. Evidence for familial aggregation of hand, hip, and spine but not knee osteoarthritis in siblings with multiple joint involvement: the GARP study. Ann Rheum Dis 2005; 64(3):438-443.
27. Botha-Scheepers S, Watt I, Rosendaal FR, Breedveld FC, Hellio le Graverand MP, Kloppenburg M. Changes in outcome measures for impairment, activity limitation, and participation restriction over two years in osteoarthritis of the lower extremities. Arthritis Rheum 2008; 59(12):1750-1755.
28. Botha-Scheepers S, Riyazi N, Watt I et al. Progression of hand osteoarthritis over 2 years: a clinical and radiological follow-up study. Ann Rheum Dis 2009; 68(8):1260-1264.

IV.

Acromegalic arthropathy in various stages of the disease: a Magnetic Resonance Imaging (MRI) study

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In progress

ABSTRACT

BACKGROUND: Arthropathy is a prevalent and invalidating complication of acromegaly with a characteristic radiographic phenotype. We aimed to further characterize cartilage and bone abnormalities associated with acromegalic arthropathy using Magnetic Resonance (MR) imaging.

METHODS: Twenty-six patients (23% women, mean age 56.8±13.4 years), with active (N=10) and controlled acromegaly (N=16) underwent a 3.0T MRI of the right knee. Osteophytes, cartilage defects, bone marrow laesions, and subchondral cysts were assessed by KOSS (Knee Osteoarthritis Scoring System). Cartilage thickness and cartilage T2 relaxation times, in which higher values reflect increased water content, were measured. Fifty-nine controls (80% women, mean age 61.9±6.8 years) with primary knee OA were included for comparison.

RESULTS: In active acromegaly, structural OA defects were already highly prevalent. Active acromegaly patients had thicker cartilage and higher cartilage T2 relaxation times than controlled patients. When compared to primary OA subjects, acromegaly patients seem to have less cysts, but comparable prevalence of osteophytosis, cartilage defects and bone marrow laesions. Acromegalic patients had thicker joint cartilage with higher cartilage T2 relaxation times than primary OA subjects.

CONCLUSIONS: In active acromegaly, prevalence of structural OA abnormalities is high, in combination with thick joint cartilage. In addition, T2 relaxation times of cartilage are high in active patients, indicating unhealthy cartilage with increased water content, which is (partially) reversible by adequate treatment. Distribution of structural abnormalities on MR imaging differs from that observed in subjects with primary OA, with in particular differences in joint cartilage.

INTRODUCTION

Acromegaly is a chronic rare endocrine disease, caused by a Growth Hormone (GH)-producing pituitary adenoma, resulting in elevated GH and Insulin-like Growth Factor-1 (IGF-1) concentrations. Acromegaly patients have an increased risk to develop secondary osteoarthritis (OA), having a considerable impact on physical functioning and psychological well-being (1;2). The pathophysiology of acromegalic joint disease is not fully elucidated, although there is growing evidence for a role of the GH/IGF-1 system both in the initiation and progression of acromegalic arthropathy (3-5).

On conventional radiographs of patients with active acromegaly, joint disease is characterized by widening of joint spaces and severe osteophytosis (6). In a well-characterized cohort of patients with long-term disease control following currently available treatment, *i.e.* transsphenoidal pituitary surgery or GH-lowering medication, we recently observed a 4 to 12-fold increased prevalence of arthropathy, already being present at young ages (1). Remarkably, the distribution of structural OA features differs from that in patients with primary OA. Acromegalic arthropathy is predominantly characterized by osteophytosis, frequently in combination with preserved or even widened joint spaces, suggesting that cartilage hypertrophy is maintained despite long-term biochemical disease control (7). However, 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 (8;9). We have recently reported that several parameters indicating an increased GH/IGF-1 signal were associated with radiographic OA (ROA) and ROA progression in acromegalic patients, *i.e.* high IGF-1 levels at the time of diagnosis and the presence of the common exon 3 deletion (d3-GHR) GH receptor polymorphism (3-5).

To further study the unique phenotype of acromegalic arthropathy, Magnetic Resonance (MR) imaging may give additional information to radiographs. MRI directly visualizes cartilage, enabling assessment of cartilage defects, thickness and quality, osteophytes but also other structural abnormalities of subchondral bone such as cysts and bone marrow edema. Cartilage quality can be measured by cartilage T2 relaxation times, being related to water content and collagen anisotropy, providing information on cartilage biochemical composition (15). A higher T2 value has previously been reported in cartilage of OA patients compared to healthy controls and it was reported that high T2 values correlate with the severity of the disease (16;17).

In the present study, we investigated knees of 26 acromegaly patients by 3.0 Tesla (3.0T) MRI to study structural OA abnormalities. Both acromegaly patients with active and controlled disease were studied, to study the potential relationship between structural OA features and disease activity. We included subjects with primary OA as controls to differentiate which structural abnormalities on MRI were acromegaly-specific.

MATERIALS AND METHODS

Study design and patient selection

STUDY DESIGN: In a cross-sectional study design, we performed 3.0T MRI scans of the knee in acromegaly patients, who were divided into two subgroups: (1) active patients and (2) patients in remission, by either transsphenoidal surgery and/or radiotherapy or SMS analog treatment. We included control subjects with primary knee OA from the geMstoan study (*vide infra*, (18)) to evaluate differences in structural joint abnormalities and cartilage thickness of the knee. In addition, we included literature controls with primary OA (19) to compare cartilage biochemical composition by measuring cartilage T2 relaxation times at different locations in the knee.

PATIENTS: All consecutive patients with acromegaly, who were referred to the Leiden University Medical Center, were collected in a database. Complementary to a cross-sectional and follow-up study evaluating clinical and radiographic arthropathy in long-term biochemically controlled patients, a subset of controlled acromegaly patients was invited to undergo an additional MRI assessment (1;4). In addition to controlled patients, active acromegaly patients were included in the present MRI study, resulting in a total of 26 eligible patients. Patients were divided into two subgroups: (1) active acromegaly, and (2) acromegaly in remission, comprising both patients cured after transsphenoidal surgery and, if required, additional radiotherapy, and patients controlled by SMS analogs. Two patients underwent two knee MRIs at different time points (*i.e.* one MRI before surgery and one MRI >6 months after successful surgery), resulting in 28 available MRIs for analysis.

Detailed yearly follow-up was performed from the onset of 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.

Disease activity was assessed yearly by oral glucose tolerance tests (oGTT) (except in medically treated patients), fasting serum GH and IGF-1 levels. Remission of acromegaly was defined as a normal glucose-suppressed serum GH <1.25 (RIA assay until 1992) or 0.38µg/l (immunofluorometric assay (IFMA) from 1992 onwards), serum GH levels of <1.9µg/l (all years), and normal IGF-1 levels for age (from 1986 onwards) (20-22). Patients not meeting these criteria were offered additional treatment.

Hypopituitarism was supplemented with levothyroxine, hydrocortisone, testosterone/estrogens according to the following definitions (22). Estrogen deficiency in women was present in case of luteinizing hormone (LH)/follicle-stimulating hormone (FSH) deficiency in premenopausal women with prolonged amenorrea >1 year without adequate replacement therapy or by a low serum oestradiol concentration of <70nmol/liter and all postmenopausal women. In men, LH/FSH deficiency was defined as testosterone level below the reference range (8.0nmol/l). Thyroid-stimulating hormone (TSH) deficiency was defined as a free thyroxine level below the reference range (<10pmol/l). Adrenocorticotrophic hormone (ACTH) deficiency was defined as an insufficient increase of cortisol (peak <0.55µmol/l) after corticotrophin releasing hormone (CRH) test or insulin tolerance test (ITT). GH deficiency was not routinely assessed.

The Medical Ethics Committee approved the study protocol, and all subjects gave written consent.

CONTROLS: Three control groups were included for comparison with acromegaly patients.

A: For comparison of the structural OA abnormalities assessed by the Knee Osteoarthritis Scoring System (KOSS), acromegaly patients were compared to controls from the geMstoanstudy (GEneration of Models, Mechanisms & Markers for Stratification of OsteoArthritis patients). The geMstoan study (N=62) is a longitudinal study among primary OA patients with established symptomatic and radiographic knee OA, aiming on identification of new biomarkers for OA progression (18). Of 62 geMstoan subjects, two MRIs were missing (*vide infra*), and one subject was diagnosed with rheumatoid arthritis, resulting in 59 eligible controls. The geMstoan study is approved by the Medical Ethics Committee, and all patients provided written informed consent.

B: Cartilage thickness measurements were compared with a random selection of 10 controls from the geMstoan study.

C: For comparison of cartilage T2 relaxation times, we used a literature reference from Stahl *et al.* describing 17 controls (9 females/8 males) with mild primary OA, since reference values of cartilage T2 relaxation times were not available in our center (19). In this literature reference, OA was defined as radiographic OA (Kellgren-Lawrence (KL) 1 or 2) and clinical OA according to the clinical ACR criteria (mean age of 54.0±10.0 years; mean BMI 23.6±7.1 kg/m^2).Cartilage T2 relaxation times were measured at the same knee locations as in the acromegalic patients (*vide infra*).

STUDY PROTOCOL: Acromegaly patients were seen on the outpatient clinic for a single study visit. All patients completed standardized questionnaires on demographic data, medical history and OA signs and symptoms, and the validated WOMAC questionnaire on pain, stiffness and functional disability of the lower limb (23). Conventional knee radiographs were obtained according to a standard protocol (*vide infra*), and all patients underwent an MRI scan of the right knee. Physical examination of the knee was performed by a single physician (K.M.J.A.C.), trained in structured joint assessment.

GeMstoan controls underwent an MRI of the knee with symptomatic OA, and conventional knee radiographs were obtained. Self-reported pain was assessed by the visual analogue scale (VAS, 0-100) within two weeks of MRI acquisition and the WOMAC questionnaire was completed.

Study parameters

PARAMETERS OF ACROMEGALIC DISEASE: Duration of active disease was estimated using the start of symptoms and signs to the date of normalization of serum IGF-1 concentration after treatment. Duration of remission was calculated from the date of biochemical remission until the start of the present study. Cure was defined by normal glucose-suppressed GH levels and IGF-1 levels for age after surgery and/or irradiation. Biochemical control was defined by normal serum IGF-1 levels for age during SMS analog treatment. Both cured and biochemically controlled patients were referred to as 'in remission'.

ASSAYS: Serum GH was measured with a sensitive IFMA (Wallac, Turku, Finland), specific for the 22 kDA GH protein (detection limit: 0.01µg/l, interassay coefficient of variation (CV): 1.6-8.4% of 0.01-15.38µg/l) from

1992 onwards. For the conversion of µg/l to mU/l, multiply by 2.6. Before 1992, GH was measured by RIA (Biolab, Serona, Coissins, Switzerland), detection limit: 0.5mU/l, with an interassay CV <5%; for the conversion of µg/l to mU/l, multiply by 2.

Serum IGF-1 concentrations (nmol/l) were measured using an immunometric technique on an Immulite 2500 system (Siemens Healthcare Diagnostics, Deerfield, IL, USA). The intra-assay variations at mean plasma levels of 8 and 75nmol/l were 5.0 and 7.5%, respectively. IGF-1 levels were expressed as SDS, using lambda-mu-sigma smoothed reference curves based on 906 controls (23;24).

RADIOGRAPHIC PROTOCOL: Both in acromegaly patients and geMstoan controls, conventional knee radiographs (posterior-anterior (PA), in weight-bearing/semi-flexed and lateral) were obtained, employing the same standardized protocol with a fixed film-focus distance and fixed flexion-position (25). Radiographic examinations were performed by a single experienced radiographer. Radiographs were available in 24 of 28 acromegaly patients, and in all geMstoan controls.

Radiographic knee OA was assessed according to the KL scale by an experienced musculoskeletal radiologist (H.M.K.) (26), and was defined as KL≥2. The reproducibility, depicted by the intra-class correlation coefficient (ICC), was 0.99 and was based on a randomly selected sample of 36 radiographs (17 right and 17 left knees).

MRI PROTOCOL / ACQUISITION: *Patients*: MRI scans were obtained using an eight-channel knee coil and a 3.0 Tesla (3.0T) superconducting magnet (Gyroscan Achieva; Philips Medical Systems, Best, the Netherlands), and were performed by a single experienced radiology technician. The scan protocol consisted of a series of standard knee sequences: PDW (proton-density weighted) frequency selective fat suppressed transverse (TR/TE 1900/18ms, TSE factor 6, FOV 150x150x115, matrix 288x228, slice thickness 3mm), PDW DRIVE sagittal and coronal (TR/TE 2225/25ms, TSE factor 12, FOV 150x150x86, matrix 432x336, slice thickness 3mm), and a 3D gradient echo fat-suppressed sagittal scan (details below). In addition, a sagittal T2-mapping scan (*vide infra*) was performed.

Controls: GeMstoan controls underwent an MRI using the same 3.0T MRI scanner and eight-channel knee coil as the acromegalic patients. MRIs were available of 60 controls. Both axial and sagittal CE, T1-weighted, turbo spin echo (TSE), and spectral presaturation with inversion recovery (SPIR) sequences were acquired. The control MRI exam did not include a T2-mapping scan.

Study parameters MRI

Evaluation of structural OA changes on MRI: Knee Osteoarthritis Scoring System (KOSS)

MRIs of both patients and geMstoan controls were scored according to the KOSS, which is a validated scoring system for quantifying OA changes in the knee, developed by Kornaat *et al.* (27). For the present study, cartilaginous defects (diffuse and focal), osteophytes, subchondral cysts and bone marrow edema were graded on a scale from 0 (absent) to 3 (severe). Lesions were localized to any of five regions: medial femoral compartment, medial tibiofemoral compartment, lateral femoral compartment, lateral tibiofemoral compartment, and patellofemoral compartment. An osteoarthritic defect was present when a score ≥1 was given; a severe osteoarthritic defect was defined as KOSS ≥2. MRI scans of patients were scored by two experienced readers (A.W.V., E.Y.), blinded for any patient characteristics. Reproducibility was good, as reflected by ICCs of 0.67, 0.92, 1.00 and 1.00 for osteophytes, (diffuse and focal) cartilage defects, cysts and bone marrow laesions, respectively. MRI scans of controls were scored by another experienced reader (B.d.L.), according to the same protocol. ICCs for controls were 0.97, 0.94, 0.98 and 0.93 for osteophytes, (diffuse and focal) cartilage defects, cysts and bone marrow laesions, respectively.

Cartilage thickness measurements

In patients and geMstoan controls, cartilage thickness was measured by the same experienced reader (P.d.B.) in a 3D fat-suppressed spoiled gradient echo sequence with ProSet fat suppression (pulse type 1331), acquisition matrix 304x304, FOV 150, pixel size 0.5x0.5mm^2. The sequence was slightly different between the acromegaly patients and geMstoan controls. Acromegaly patients: TR/TE 20/5.2ms, 60 slices, slice thickness 3.5mm, and acquisition time 6min 2s. Controls: TR/TE 16/9.2ms, 125 slices, slice thickness 1.5mm, and acquisition time 5min 9s. In the center of each ROI, the thickness of a non-degenerated cartilage section was measured perpendicular to the subchondral bone. All measurements were performed using Osirix (Version 5.6) (28). Reproducibility was good, as reflected by an ICC of 0.802, and was based on a random selection of 5 knee MRIs.

T2-MAPPING: T2-mapping in acromegaly patients was performed using a sagittal 2D turbo spin-echo sequence with TR 3307ms, 7 echoes with TE1/ΔTE/TE7 13/13/91ms, acquisition matrix 480x300, in-plane resolution of 0.31x0.5mm^2, slice thickness 3mm, FOV 150mm, and acquisition time 7 min 7 s. T2-maps were fitted using the on-scanner vendor-provided software based on a maximum likelihood approach.

ROIs were drawn on sagittal slices approximately through the center of the medial and lateral condyles in three locations: the weight-bearing and non-weight-bearing femoral cartilage, and the cartilage of the tibia plateau (*Figure 1*). Obvious defects in the cartilage were avoided. In 23 patients, T2 maps of sufficient quality (motion- and artifact-free) were available, and were hence included in the present analysis. Reproducibility of cartilage T2 relaxation times was moderate, as reflected by an ICC of 0.530, based on random selection of 5 knee MRIs throughout the scoring process, blinded for any patient characteristics.

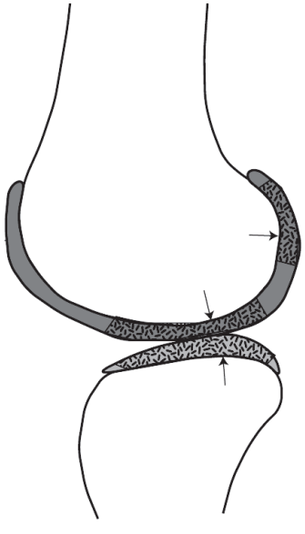


Figure 1. Schematic representation of the ROIs drawn in the medial and lateral condyles of the knee

The arrows indicate the approximate locations of the knee where the cartilage thickness was measured.

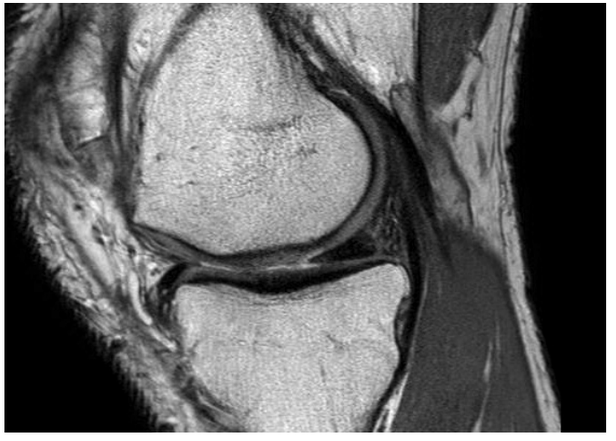


Figure 2. MRI scan of the medial compartment of the right knee of an active acromegalic patient showing preserved joint cartilage and small osteophytes.

Statistical analysis

SPSS for Windows, version 20.0 (SPSS Inc., Chicago, IL, USA), was used for data analysis. Data are presented as mean±SD, unless otherwise stated. Prevalence of structural abnormalities was compared between active and controlled acromegaly patients, using a logistic regression model, adjusting for age, sex and BMI. The relationship between different acromegaly-specific parameters and structural abnormalities was also assessed in a logistic regression model. Cartilage thickness and cartilage T2 relaxation times were compared between active and controlled acromegaly patients by a linear regression model, adjusted for age, sex and BMI.

For the comparison of structural OA changes between acromegaly patients and controls with primary OA, we used a logistic regression model with adjustments for age and sex. Cartilage thickness was compared between patients and geMstoan controls in a linear regression model, adjusting for age, sex and BMI. For comparison of cartilage T2 relaxation times between acromegaly patients and 17 controls with mild OA from the literature reference of Stahl *et al.* (19), a pooled variance *T* test was performed.

RESULTS

Characteristics of patients and controls

Twenty-six acromegaly patients were included, comprising 10 patients with active acromegaly, of whom 9 patients were treatment-naïve, (mean age 50.6±13.8 years, 40% female) and 16 patients in biochemical remission (mean age 60.6±11.9 years, 12.5% female), achieved by transsphenoidal surgery, radiotherapy and/or SMS analogs. Mean remission duration in the latter group was 13.2±10.6 years. Two patients, both in remission, had a history of knee arthroscopy surgery in the scanned knee.

Patients were compared with 59 controls diagnosed with primary OA (mean age 61.9±6.8 years, 80% female). Clinical characteristics of patients and controls were shown in *Table 1*. Mean age was significantly higher in controls (p<0.001), and the control group comprises more females (p<0.001). Mean BMI was comparable between patients and controls. Definite radiographic knee OA of the scanned knee, defined as KL ≥2, was present in 7 patients (29%) and 45 (76%) controls.

Table 1. Clinical characteristics of acromegaly patients with active disease, acromegaly patients in remission, and controls with primary OA

	ACRO Active disease (N = 10)	**ACRO Remission (N = 16)**	**Primary OA (N = 59)**
Age (yr)	50.6 (13.8)	60.6 (11.9)	61.9 (6.8)
Female sex (N (%))	4 (40%)	2 (13%)	47 (80%)
BMI (kg/m2)	28.1 (2.3)	30.0 (4.8)	29.8 (5.4)
Active disease duration (yr)	7.8 (5.6)	8.3(6.5)	NA
Remission duration (yr)	NA	13.2 (10.6)	NA
GH levels (mU/l)			
At diagnosis	163.6 (310.7)	48.0 (47.0)	NA
Current	27.3 (24.7)	3.6 (5.3)	
IGF-1 SDS			
At diagnosis	7.4 (1.7)	7.0 (4.7)	NA
Current	5.6 (3.6)	1.0 (1.5)	
Therapy (N (%))			
Surgery	1 (10%)	13 (81%)	NA
Radiotherapy	0 (0%)	2 (13%)	
SMS analogs	0 (0%)	8 (50%)	
Pegvisomant	0 (0%)	4 (25%)	
D2-agonists	0 (0%)	2 (13%)	
Hypopituitarism (N (%))			
ACTH deficiency	0 (0%)	2 (13%)	NA
TSH deficiency	0 (0%)	1 (6%)	
LH/FSH deficiency	2 (20%)	3 (19%)	
GH deficiency	0 (0%)	0 (0%)	
ADH deficiency	1 (10%)	0 (0%)	
Previous knee surgery (N (%)) *	0 (0%)	2 (13%)	10 (17%)
KL grade (N (%)) **	1.13 (0.84)	0.94 (1.34)	2.20 (1.10)
Grade 0	2 (20%)	9 (56%)	4 (7%)
Grade 1	3 (30%)	3 (19%)	10 (17%)
Grade 2	3 (30%)	1 (6%)	23 (39%)
Grade 3	0 (0%)	2 (13%)	14 (24%)
Grade 4	0 (0%)	1 (6%)	8 (14%)
Missing X-ray	2 (20%)	2 (13%)	0 (0%)

Data are reported as mean ± SD, unless stated otherwise. Control subjects were diagnosed with primary knee OA, and were derived from the geMstoan Study (18).

N, number of patients; SMS, somatostatin analogs; OA, osteoarthritis; BMI, body mass index; D2 agonists, dopamine 2 agonists; ACTH, adrenocorticotrophic hormone; TSH, thyroid-stimulating hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; GH, growth hormone; ADH, anti-diuretic hormone; KL, Kellgren-Lawrence score for presence of radiographic OA; NA, not applicable.

**, Previous knee surgery of the scanned knee.*
***, KL score of the scanned knee (ACRO, right knee; Controls, knee with symptomatic OA).*

Acromegaly patients: active *versus* controlled acromegaly

STRUCTURAL ABNORMALITIES IN THE KNEE ACCORDING TO KOSS: Structural abnormalities were already present in a high proportion of patients with active acromegaly, with comparable high prevalence of cartilage defects, subchondral cysts and bone marrow laesions in controlled patients, but osteophyte prevalence seemed to be lower after achievement of disease remission (*Table 2A*).

Next, we incorporated age of diagnosis, active disease duration, and pre-treatment IGF-1 SDS in a logistic regression model in addition to age, sex and BMI to study the relationship of acromegaly-specific parameters with OA changes in the total acromegaly group. None of the parameters reflecting disease activity was significantly related to structural abnormalities on MRI.

CARTILAGE MORPHOMETRY: Mean cartilage thickness was significantly higher in patients with active acromegaly than in patients with controlled disease at several locations in the knee, adjusted for age, sex and BMI (*Table 2B*). Adjusted total cartilage thickness (*i.e.* sum of all measured sites) was 7% higher in active than in controlled patients (18.8±3.2mm and 17.5±2.7mm in active and controlled patients, respectively, p=0.008).

In the total acromegaly group, total cartilage thickness correlated to the current IGF-1 SDS value (r=0.481, p=0.017), but not to pre-treatment IGF-1 SDS values. Cartilage thickness was not associated to the presence of structural abnormalities according to KOSS (*data not shown*).

BIOCHEMICAL CARTILAGE COMPOSITION: cartilage T2 relaxation times: Patients with active acromegaly had higher cartilage T2 relaxation times than controlled acromegalics at the non-weight-bearing medial femoral cartilage and at the lateral tibial plateau (*Table 2C*), adjusted for age, sex and BMI, but not at other knee locations. Cartilage T2 relaxation times were not related to pre-treatment IGF-1 SDS, current IGF-1 SDS or active disease duration (*data not shown*).

Table 2A. Prevalence of joint defects on a 3.0T MRI of the knee using KOSS, in acromegaly patients with active disease (N=10) vs acromegaly patients in biochemical remission (N=16)

Joint defects N (%)	**Active disease (N=10)**	**Remission (N=16)**
Cartilage defects		
PF	8 (80%)	10 (63%)
TF	7 (70%)	12 (75%)
PF and/or TF	9 (90%)	13 (81%)
Osteophytes		
PF	8 (80%)	9 (56%)
TF	10 (100%)	10 (63%)
PF and/or TF	10 (100%)	14 (88%)
Subchondral cysts		
PF	0 (0%)	2 (13%)
TF	0 (0%)	1 (6%)
PF and/or TF	0 (0%)	3 (19%)
BM laesions		
PF	1 (10%)	2 (13%)
TF	1 (10%)	1 (6%)
PF and/or TF	1 (10%)	3 (19%)

OA defects were scored according to the KOSS score, and were defined as KOSS ≥ 1.

KOSS, Knee Osteoarthritis Scoring System; PF, patellofemoral; TF, tibiofemoral; BM, bone marrow.

Table 2B. Comparison of cartilage thickness in the knee between patients with active acromegaly vs patients in remission of acromegaly

Cartilage thickness (mm)	Active disease (N = 9)	Remission (N = 14)	Adjusted B (95%CI)	P value*
Femur				
Medial femoral condyle (wb)	2.48 ± 0.34	2.09 ± 0.70	-0.426 (-0.956; 0.105)	0.109
Medial femoral condyle (nwb)	2.93 ± 0.45	2.63 ± 0.45	-0.657 (-1.134; -0.179)	0.010
Lateral femoral condyle (wb)	2.80 ± 0.91	2.87 ± 0.57	-0.247 (-1.093; 0.598)	0.545
Lateral femoral condyle (nwb)	3.19 ± 0.72	3.03 ± 0.74	-0.793 (-1.541; -0.044)	0.039
Tibia				
Medial plateau	3.13 ± 0.86	2.72 ± 0.87	-0.515 (-1.523; 0.492)	0.296
Lateral plateau	4.29 ± 0.94	3.82 ± 0.80	-0.932 (-1.761; -0.150)	0.030
Total cartilage thickness	18.82 ± 3.17	17.16 ± 2.30	-3.569 (-6.085; -1.054)	0.008

Data are presented as mean ± SD. Cartilage thickness measurements were analyzed using a linear regression model with adjustments for age, sex and BMI. Total cartilage thickness was defined as the sum of all measured sites. Cartilage thickness measurements were available from 23 patients.

Mm, millimeters; wb, weight-bearing; nwb, non-weight-bearing; B (95%CI), beta with corresponding 95% confidence interval.
**, Adjusted for age, sex and BMI.*

Table 2C. Comparison of cartilage T2 relaxation times between acromegaly patients with active disease vs patients in biochemical remission

T2 relaxation times (ms)	Active disease (N = 9)	Remission (N = 14)	P value *
Femur			
Medial femoral condyle (wb)	39.6 ± 4.3	44.4 ± 8.3	NS
Medial femoral condyle (nwb)	48.4 ± 5.6	45.5 ± 5.7	**0.044**
Lateral femoral condyle (wb)	33.3 ± 4.1	32.9 ± 4.7	NS
Lateral femoral condyle (nwb)	42.9 ± 2.9	41.9 ± 4.2	NS
Tibia			
Medial plateau	42.7 ± 7.4	39.6 ± 7.1	NS
Lateral plateau	31.3 ± 5.5	30.7 ± 6.8	**0.029**

Data are presented as mean ± SD. Cartilage T2 relaxation times were compared between patients with active and controlled acromegaly using a linear regression model with adjustments for age, sex and BMI.

Ms, milliseconds; wb, weight-bearing; nwb, non-weight-bearing.
**, Adjusted for age, sex and BMI.*

Table 3A. Prevalence of joint defects on a 3.0T MRI of the knee using KOSS, in acromegaly patients vs controls with primary OA

Joint defects N (%)	Acromegaly (N=26)	Primary OA (N=59)
Cartilage defects		
PF	18 (69%)	54 (92%)
TF	19 (73%)	58 (98%)
PF and/or TF	22 (85%)	58 (98%)
Osteophytes		
PF	17 (65%)	59 (100%)
TF	20 (77%)	58 (98%)
PF and/or TF	24 (92%)	59 (100%)
Subchondral cysts		
PF	2 (8%)	21 (36%)
TF	1 (4%)	28 (48%)
PF and/or TF	3 (12%)	36 (61%)
BM laesions		
PF	3 (12%)	34 (58%)
TF	2 (8%)	29 (49%)
PF and/or TF	4 (15%)	48 (81%)

OA defects were scored according to the KOSS score, and were defined as KOSS ≥ 1. Control subjects were diagnosed with primary knee OA, and were derived from the geMstoan Study (18).

KOSS, Knee Osteoarthritis Scoring System; PF, patellofemoral; TF, tibiofemoral; BM, bone marrow.

Table 3B. Comparison of cartilage thickness in the knee between acromegaly patients and subjects with primary OA

Cartilage thickness (mm)	Acromegaly patients (N = 23)	Primary OA (N = 10)	P value *
Femur			
Medial femoral condyle (wb)	2.24 ± 0.61	2.21 ± 0.25	NS
Medial femoral condyle (nwb)	2.75 ± 0.46	2.34 ± 0.14	0.109
Lateral femoral condyle (wb)	2.84 ± 0.70	2.49 ± 0.59	NS
Lateral femoral condyle (nwb)	3.09 ± 0.72	2.45 ± 0.46	0.182
Tibia			
Medial plateau	2.88 ± 0.87	2.18 ± 0.41	NS
Lateral plateau	4.00 ± 0.875	2.52 ± 1.09	0.049
Total cartilage thickness	17.81 ± 2.73	14.19 ± 1.79	0.098

Data are presented as mean ± SD. Control subjects were diagnosed with primary knee OA, and were derived from the geMstoan Study (18). Mean age of controls was 64.3±7.1yr and 90% was female. Cartilage thickness measurements were between patients and controls compared using a linear regression model with adjustments for age, sex and BMI.

Mm, millimeters; wb, weight-bearing; nwb, non-weight-bearing.
**, Adjusted for age, sex and BMI*

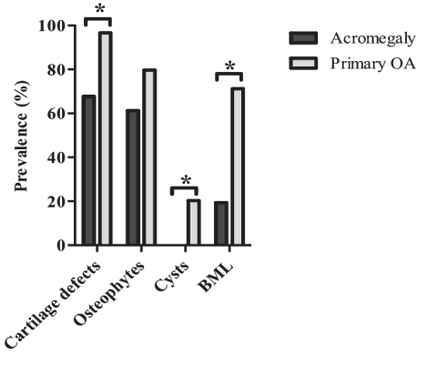


Figure 3. Severe structural OA changes detected on MRI, defined as KOSS ≥2, in acromegaly patients versus controls with primary OA

Data were presented as prevalence (%) of severe structural joint defects, according to the KOSS score. Severe OA changes were defined as KOSS ≥2.
KOSS, Knee Osteoarthritis Scoring System; BML, bone marrow laesions.

Comparison with subjects with primary OA

STRUCTURAL OA CHANGES ACCORDING TO KOSS: As depicted in *Table 3A*, acromegaly patients seem to have less cysts than subjects with primary OA, but comparable prevalence of osteophytosis, cartilage defects and bone marrow laesions. Severe cartilage defects, cysts and bone marrow laesions, defined as KOSS ≥2, were less frequently seen in acromegaly patients than controls with primary OA, whereas the prevalence of severe osteophytosis was comparable (*Figure 3*). KOSS 3 was present less frequently in patients than in controls (4.2 *vs* 8.1 times per knee, p<0.001). Cartilage thickness: Cartilage thickness was higher in acromegaly patients than in primary OA subjects, although not statistically significant after adjusting for age, sex and BMI, except for the lateral tibia plateau (59% increase) (*Table 3B, Figure 4*). All acromegaly patients, except for one, had total cartilage thickness values above the mean value of controls.

BIOCHEMICAL COMPOSITION: cartilage T2 relaxation times: Cartilage T2 relaxation times were compared between acromegaly patients and controls with mild OA from literature to assess the biochemical composition of cartilage. Patients had higher cartilage T2 relaxation times than controls at both the femoral and tibial level, at all measured sites (all p<0.01; *Table 3C*), indicating changes in cartilage quality.

Case series: pre- and postoperative knee MRIs in two acromegaly patients

The first acromegaly patient was a 56-year old male with an estimated active disease duration of 8 years (pre-treatment GH levels and pre-treatment IGF-1 SDS were, respectively, 45.0ug/l and 7.70 SDS). The second patient was a 68-year old male with an estimated active disease duration of 15year (pre-treatment GH levels and pre-treatment IGF-1 SDS were, respectively, 17.8ug/l and 7.91 SDS). Both patients underwent two MRIs of the knee: the first scan during the active, treatment-naïve, phase of acromegaly and the second scan 6 months after achievement of biochemical remission.

One patient, having no cartilage abnormalities on MRI in the active acromegaly phase, developed cartilage defects (grade 2 and 3) after achieving remission. Other structural OA abnormalities did not change. After establishment of biochemical remission, cartilage thickness regressed in both patients when compared to the pre-operative phase (decreases from, respectively, 26.6 to 17.4 and 23.5 to 16.3). Cartilage T2 relaxation times did not change.

Table 3C. Comparison of cartilage T2 relaxation times between acromegaly patients and literature controls with primary OA

T2 relaxation times (ms)	Acromegaly patients (N = 25)	Literature controls (N = 17)	P value
Femur			
Medial femoral condyle (wb)	42.1 ± 7.0	33.7 ± 3.8	**<0.01**
Medial femoral condyle (nwb)	46.9 ± 6.0	33.8 ± 3.0	**<0.01**
Lateral femoral condyle (wb)	42.4 ± 3.8	31.2 ± 3.0	**<0.01**
Lateral femoral condyle (nwb)	40.9 ± 6.9	33.3 ± 2.7	**<0.01**
Tibia			
Medial plateau	33.3 ± 4.3	27.5 ± 2.8	**<0.01**
Lateral plateau	31.0 ± 6.0	27.9 ± 3.1	**<0.01**

Data are presented as mean ± SD. Control subjects have mild radiographic OA (KL 1 or 2) and clinical OA according to the clinical ACR criteria, and were derived a literature reference from Stahl et al. (19).

Ms, milliseconds; wb, weight-bearing; nwb, non-weight-bearing.

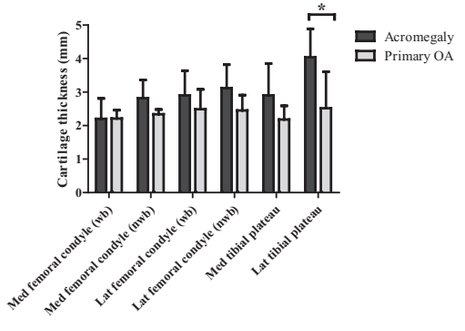


Figure 4. Cartilage thickness in the knee visualized on MRI in acromegaly patients vs controls with primary OA

Med, medial; lat, lateral.

**, p<0.01*

DISCUSSION

The present study is the first to evaluate acromegalic arthropathy by the use of MRI both in acromegaly patients with active and controlled disease. We found that structural OA abnormalities on MRI were already highly prevalent during the active acromegaly, especially of osteophytosis, when compared to controlled patients. In addition, in active patients articular knee cartilage was thicker and cartilage T2 relaxation times were higher than in controlled patients, reflecting differences in cartilage quality between these patients. When compared to primary OA subjects, acromegalic arthropathy seem to be predominantly characterized by alterations in joint cartilage, being thicker cartilage and changes in cartilage biochemical composition. In addition, acromegaly patients showed less cysts, whereas prevalence of cartilage defects, osteophytosis and bone marrow laesions was comparable. Severe OA defects were less prevalent in acromegaly patients.

Arthropathy is one of the most invalidating complications in acromegaly, despite biochemical disease control (1;21), significantly impairing QoL. The exact pathogenesis of acromegalic arthropathy is currently unknown, but there are some similarities with primary OA. There is evidence that GH/IGF-1 activity is associated with both the onset and progression of acromegalic arthropathy (3-5). Interestingly, acromegaly patients have a characteristic radiographic phenotype with severe osteophytosis with preservation of joint cartilage (7;29). Until now, these characteristics were only observed in radiography studies and in a few studies using ultrasonography.

The present study shows that structural OA defects are already highly prevalent during the active acromegaly phase. In addition, we found that 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. Cartilage T2 relaxation times are influenced by several factors, such as the orientation of collagen fibers to the static magnetic field, water content, alterations in water proton mobility and the integrity of collagenous structures in the extracellular cartilage matrix (30-32). In previous studies, primary OA patients were shown to have higher T2 relaxation times than healthy controls (16;17), with a clear correlation between these values and OA severity, indicating increased water content in these patients. The findings of the present study could introduce the hypothesis that in thickened joint cartilage in active acromegaly patients consists of two different components: a structural component of cartilage hypertrophy, being (partially) irreversible despite long-term biochemical remission (7;29), and a component of edema (reflected by cartilage T2 relaxation times), that decreases after successful treatment. This may explain why joint cartilage of controlled acromegalics is still thickened compared to healthy controls due to persisting cartilage hypertrophy, but is thinner than in the active phase due to a decrease in water content by successful treatment. This hypothesis is underlined by a corresponding decrease in cartilage T2 relaxation times after achievement of biochemical control.

When compared to subjects with primary OA, acromegaly patients seemed to have thicker knee cartilage. These results are in keeping with radiographic studies reporting widened joint spaces (7;29), indicating persistent (protective) effects of previous GH excess on joint cartilage. A new finding is the presence of higher cartilage T2 relaxation times in acromegaly patients at all measured sites, suggesting that average biochemical composition of joint cartilage is altered in these patients. The observation of even higher cartilage T2 relaxation times in acromegaly patients might reflect increased cartilage damage in acromegalics, with more cartilage hydration and collagen breakdown. Observations of these altered cartilage composition should be confirmed in future studies.

There were also differences in the distribution of structural abnormalities on MRI between acromegaly patients and subjects with primary OA. Acromegaly patients seemed to have a lower prevalence of cysts, whereas prevalence of cartilage defects, osteophytosis and bone marrow edema was comparable. In acromegalics, prevalence of severe OA defects was lower than in subjects with primary OA, which may explain why, despite significant joint complaints, joint replacement surgery is less frequently performed in acromegaly patients.

This study may suffer from several limitations. First, due to the relatively small number of patients, we only did explorative analyses in this pilot study. Larger studies are needed to draw firm conclusions. Second, the acromegaly group included in the study is very heterogeneous, including both active and controlled patients. However, we decided to include treated patients next to treatment-naïve patients, in order to assess the effects of adequate acromegaly treatment on joint level. Third, in the absence of T2 relaxation time control data in our center, we were limited to the inclusion of a literature reference with primary OA subjects for comparison. In this respect, differences between MRI scanners, scan protocols and scoring methods may confound these results. Finally, the cartilage thickness and T2 relaxation measurements are a first exploration of the MRI data. In future work, we aim to analyze the

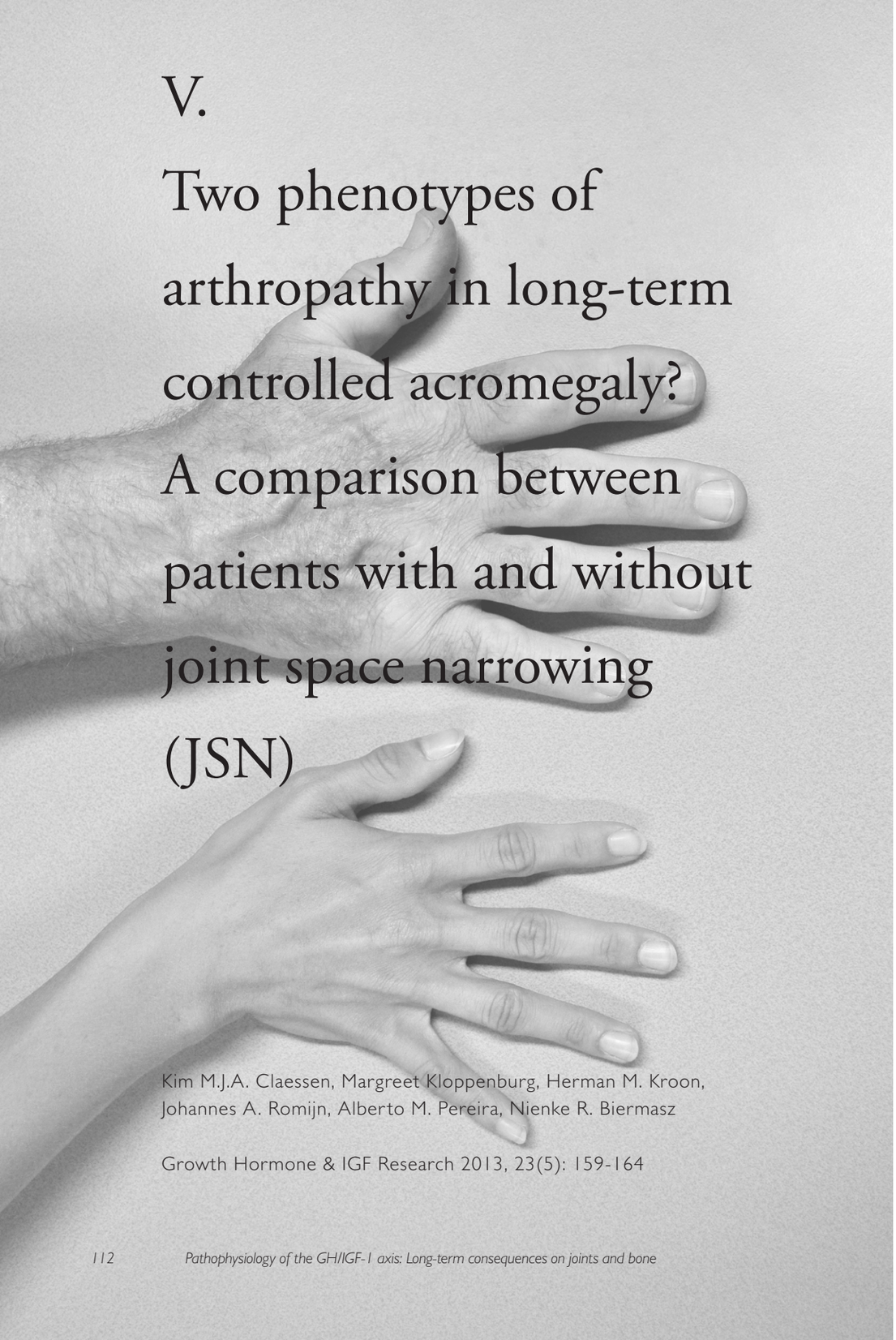
data in more depth, including thickness measurements over the entire cartilage surface and T2-value assessment in the different cartilage layers. For future scans, these analyses may benefit from the higher resolution, both in the spatial and in the contrast domain, which can be achieved using the 7.0T MRI scanner of the C.J. Gorter Center in our hospital. In addition, the moderate ICC of the T2-mapping might be improved by the implementation of a (semi-)automatic analysis algorithm.

In conclusion, this first MRI study on acromegalic arthropathy demonstrates that in the active acromegaly phase structural OA defects are already highly prevalent. Active acromegaly have thicker joint cartilage with larger water content than patients with controlled disease, as reflected by increased cartilage T2 relaxation times. When compared to primary OA subjects, acromegalic arthropathy especially differs with respect to joint cartilage, which is thicker and from different biochemical composition. The findings of the present study underline that acromegalic arthropathy is a clinical entity with a unique phenotype. Future studies have to point out whether acromegaly-specific interventions can be beneficial in the management of acromegalic arthropathy.

REFERENCE LIST

1. Wassenaar MJ, Biermasz NR, van DN et al. High prevalence of arthropathy, according to the definitions of radiological and clinical osteoarthritis, in patients with long-term cure of acromegaly: a case-control study. Eur J Endocrinol 2009; 160(3):357-365.
2. Wassenaar MJ, Biermasz NR, Kloppenburg M et al. Clinical osteoarthritis predicts physical and psychological QoL in acromegaly patients. GrowthHorm IGF Res 2010; 20(3):226-233.
3. Biermasz NR, Wassenaar MJ, van der Klaauw AA et al. Pretreatment insulin-like growth factor-I concentrations predict radiographic osteoarthritis in acromegalic patients with long-term cured disease. J ClinEndocrinolMetab 2009; 94(7):2374-2379.
4. Claessen KM, Ramautar SR, Pereira AM et al. Progression of acromegalic arthropathy despite long-term biochemical control: a prospective, radiological study. Eur J Endocrinol 2012; 167(2):235-244.
5. Wassenaar MJ, Biermasz NR, Pereira AM et al. The exon-3 deleted growth hormone receptor polymorphism predisposes to long-term complications of acromegaly. J ClinEndocrinolMetab 2009; 94(12):4671-4678.
6. Barkan AL. Acromegalic arthropathy. Pituitary 2001; 4(4):263-264.
7. Wassenaar MJ, Biermasz NR, Bijsterbosch J et al. Arthropathy in long-term cured acromegaly is characterised by osteophytes without joint space narrowing: a comparison with generalised osteoarthritis. Ann Rheum Dis 2011; 70(2):320-325.
8. Colao A, Marzullo P, Vallone G et al. Reversibility of joint thickening in acromegalic patients: an ultrasonography study. J ClinEndocrinolMetab 1998; 83(6):2121-2125.
9. Colao A, Cannavo S, Marzullo P et al. Twelve months of treatment with octreotide-LAR reduces joint thickness in acromegaly. Eur J Endocrinol 2003; 148(1):31-38.
10. Claessen KM, Kloppenburg M, Kroon HM, Romijn JA, Pereira AM, Biermasz NR. Two phenotypes of arthropathy in long-term controlled acromegaly? A comparison between patients with and without joint space narrowing (JSN). Growth Horm IGF Res 2013; 23(5):159-164.
11. Biermasz NR, Pereira AM, Frolich M, Romijn JA, Veldhuis JD, Roelfsema F. Octreotide represses secretory-burst mass and nonpulsatile secretion but does not restore event frequency or orderly GH secretion in acromegaly. Am J PhysiolEndocrinolMetab 2004; 286(1):E25-E30.
12. Mackie EJ, Trechsel U, Bruns C. Somatostatin receptors are restricted to a subpopulation of osteoblast-like cells during endochondral bone formation. Development 1990; 110(4):1233-1239.
13. Weiss RE, Reddi AH, Nimni ME. Somatostatin can locally inhibit proliferation and differentiation of cartilage and bone precursor cells. Calcif Tissue Int 1981; 33(4):425-430.
14. Zapf J, Gosteli-Peter M, Weckbecker G, Hunziker EB, Reinecke M. The somatostatin analog octreotide inhibits GH-stimulated, but not IGF-I-stimulated, bone growth in hypophysectomized rats. Endocrinology 2002; 143(8):2944-2952.
15. Stahl R, Luke A, Li X et al. T1rho, T2 and focal knee cartilage abnormalities in physically active and sedentary healthy subjects versus early OA patients--a 3.0-Tesla MRI study. EurRadiol 2009; 19(1):132-143.
16. Blumenkrantz G, Lindsey CT, Dunn TC et al. A pilot, two-year longitudinal study of the interrelationship between trabecular bone and articular cartilage in the osteoarthritic knee. Osteoarthritis Cartilage 2004; 12(12):997-1005.
17. Dunn TC, Lu Y, Jin H, Ries MD, Majumdar S. T2 relaxation time of cartilage at MR imaging: comparison with severity of knee osteoarthritis. Radiology 2004; 232(2):592-598.
18. de Lange-Brokaar BJE, Ioan-Facsinay A, Yusuf E et al. Degree of synovitis on MRI is correlated with histological and macroscopic features of synovial tissue inflammation in knee osteoarthritis. Submitted . 2013.
19. Stahl R, Blumenkrantz G, Carballido-Gamio J et al. MRI-derived T2 relaxation times and cartilage morphometry of the tibio-femoral joint in subjects with and without osteoarthritis during a 1-year follow-up. Osteoarthritis Cartilage 2007; 15(11):1225-1234.

20. Biermasz NR, van DH, Roelfsema F. Ten-year follow-up results of transsphenoidal microsurgery in acromegaly. J ClinEndocrinolMetab 2000; 85(12):4596-4602.
21. Biermasz NR, Pereira AM, Smit JW, Romijn JA, Roelfsema F. Morbidity after long-term remission for acromegaly: persisting joint-related complaints cause reduced quality of life. J ClinEndocrinolMetab 2005; 90(5):2731-2739.
22. Roelfsema F, van DH, Frolich M. Long-term results of transsphenoidal pituitary microsurgery in 60 acromegalic patients. ClinEndocrinol (Oxf) 1985; 23(5):555-565.
23. Cole TJ. The LMS method for constructing normalized growth standards. Eur J ClinNutr 1990; 44(1):45-60.
24. Rikken B, van DJ, Ringeling A, Van den Brande JL, Massa G, Wit JM. Plasma levels of insulin-like growth factor (IGF)-I, IGF-II and IGF-binding protein-3 in the evaluation of childhood growth hormone deficiency. Horm Res 1998; 50(3):166-176.
25. Peterfy C, Li J, Zaim S et al. Comparison of fixed-flexion positioning with fluoroscopic semi-flexed positioning for quantifying radiographic joint-space width in the knee: test-retest reproducibility. Skeletal Radiol 2003; 32(3):128-132.
26. Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. Ann Rheum Dis 1957; 16(4):494-502.
27. Kornaat PR, Ceulemans RY, Kroon HM et al. MRI assessment of knee osteoarthritis: Knee Osteoarthritis Scoring System (KOSS)--inter-observer and intra-observer reproducibility of a compartment-based scoring system. Skeletal Radiol 2005; 34(2):95-102.
28. Rosset A, Spadola L, Ratib O. OsiriX: an open-source software for navigating in multidimensional DICOM images. J Digit Imaging 2004; 17(3):205-216.
29. Biermasz NR, van 't KR, Wassenaar MJ et al. Automated image analysis of hand radiographs reveals widened joint spaces in patients with long-term control of acromegaly: relation to disease activity and symptoms. Eur J Endocrinol 2012; 166(3):407-413.
30. Lusse S, Claassen H, Gehrke T et al. Evaluation of water content by spatially resolved transverse relaxation times of human articular cartilage. MagnReson Imaging 2000; 18(4):423-430.
31. Mosher TJ, Dardzinski BJ, Smith MB. Human articular cartilage: influence of aging and early symptomatic degeneration on the spatial variation of T2--preliminary findings at 3 T. Radiology 2000; 214(1):259-266.
32. Nissi MJ, Rieppo J, Toyras J et al. T (2) relaxation time mapping reveals age- and species-related diversity of collagen network architecture in articular cartilage. Osteoarthritis Cartilage 2006; 14(12):1265-1271.



V.

Two phenotypes of arthropathy in long-term controlled acromegaly? A comparison between patients with and without joint space narrowing (JSN)

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ABSTRACT

BACKGROUND: Arthropathy is an invalidating complication of acromegaly, also in long-term controlled patients, and is radiographically characterized by osteophytes and preserved joint spaces. However, joint space narrowing (JSN) is observed in the minority of patients. It is unknown whether JSN is the end-stage of acromegalic arthropathy or whether this feature develops independently of acromegaly.

OBJECTIVE: To gain insight into the pathophysiology of acromegalic arthropathy, and, more specifically, in the process of JSN, risk factors for radiographic JSN were studied in a cross-sectional study.

METHODS: We studied hips and knees of 89 well-controlled acromegaly patients (mean age 58.3 years, 51% female). Joints were divided into two groups based on the presence of JSN, defined as an Osteoarthritis Research Society (OARSI) score ≥1. Potential risk factors for JSN were assessed, and its relationship to joint complaints. Individual knees and hips were analyzed in a Generalized Estimating Equations model, adjusted for age, sex, BMI and intra-patient effect.

RESULTS: In controlled acromegaly, JSN was found in, respectively, 10.3% and 15.4% of the hips and knees. Increasing age and female sex were associated with more JSN; acromegaly-specific risk factors for JSN were joint-site specific. In the hip, JSN was related to more active disease: higher pre-treatment GH/IGF-1, longer and more severe GH exposure and immediate postoperative cure was less frequently achieved. In the knee, especially previous knee surgery, not acromegaly-specific characteristics, was associated with JSN. The presence of JSN was associated with more joint complaints.

CONCLUSIONS: JSN is an infrequent finding in patients with acromegalic arthropathy, but is associated with more symptoms. This study indicates that, at least in the hip, early and ongoing GH/IGF-1 activity play a role in JSN development.

INTRODUCTION

Acromegaly is a chronic endocrine disease with high prevalence of arthropathy in active and cured disease, resulting in considerable functional disability (1). It is suggested that increased exposure to Insulin-like Growth Factor-1 (IGF-1), the main mediator of Growth Hormone (GH) action, is the driving mechanism in secondary osteoarthritis (OA) in acromegaly (2). In a hypothetical model, acromegalic arthropathy has a bi-phasic pattern with initially reversible endocrine changes, followed by mechanical changes. First, elevated GH and/or IGF-1 levels promote the growth of articular cartilage and peri-articular ligaments, leading to cartilage hypertrophy with a limited range of movements. This early stage is thought to be, at least partially, reversible with adequate biochemical disease control. Subsequently, when GH excess persists, changes become irreversible. At this late stage, acromegalic joints acquire the characteristics of degenerative joint disease (3). However, little clinical studies are available to support this hypothesis.

Previously, we have demonstrated that the prevalence of arthropathy is high, also in patients with long-term biochemical disease control (4). These patients have a 4- to 12-fold increased risk to develop OA, even at very young ages, in comparison to the general population (5). GH/IGF-1 activity at diagnosis is related to the prevalence of radiographic OA (ROA) (6). Interestingly, despite long-term cure, the distribution of radiological abnormalities remained different from regular degenerative joint disease, *i.e.* primary OA. The radiographic phenotype in acromegaly is predominantly characterized by severe osteophytosis, frequently in combination with preserved normal or even widened joint spaces. Therefore, it is suggested that GH hypersecretion is especially involved in bone formation, but may protect against cartilage loss (7). This observation conflicts with the previous hypothesis that there is a final common pathway with primary OA, at least in the majority of patients.

Nonetheless, a minority of acromegaly patients shows radiographic joint space narrowing (JSN), mimicking primary OA, instead of the characteristic joint space widening of acromegaly. This suggests that in acromegaly, there are two types of arthropathy: first, osteophytosis in combination with preserved/widened joint spaces, and second, in a small group, JSN with or without osteophytes. At present, the process of JSN is not fully understood, and it is unknown whether radiographic JSN is the end-stage of acromegalic arthropathy when the GH/IGF-1 excess has exceeded a critical threshold or whether it is a radiographic feature which develops independently of acromegaly, caused by, for example, high biomechanical forces or previous joint surgery.

In order to gain insight in the pathophysiology of acromegalic arthropathy and, more specifically, in the process of JSN, we compared patients with and without radiographic JSN in the knee and hip with respect to patient and treatment characteristics and physical stress. Both patients with and without JSN were derived from the same long-term controlled acromegaly cohort.

METHODS

Patients

All consecutive patients with acromegaly, who were referred for treatment from 1977 onwards to the Leiden University Medical Center, were collected in a database. For the present study, 126 consecutive patients with long-term controlled acromegaly (defined as ≥2 years) were invited for participation. Thirty-seven patients preferred not to participate for various reasons such as illness, travel distance, lack of time or psychological reasons. Consequently, 89 patients were included in the present analysis. The 37 non-included patients did not differ from the participating patients with respect to age, sex, BMI, active disease duration, pre-treatment GH/IGF-1 levels, type of treatment, follow-up duration and self-reported joint complaints (6).

Detailed yearly follow-up was performed from the onset of acromegaly treatment. The first treatment option in the majority of patients was transsphenoidal surgery (TPS) performed by a single specialized neurosurgeon. If necessary, adjuvant treatment consisted of radiotherapy (RT) (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.

Disease activity was assessed yearly by oral glucose tolerance tests (except in medically treated patients), fasting serum GH and IGF-1 levels. Remission of acromegaly was defined as a normal glucose-suppressed serum GH <1.25 (RIA assay until 1992) or 0.38μg/l (immunofluorometric assay (IFMA) from 1992 onwards), serum GH levels of <1.9μg/l (all years), and normal IGF-1 levels for age (from 1986 onwards) (4;8;9). Patients not meeting these criteria were offered additional treatment.

Hypopituitarism was supplemented with thyroxine, hydrocortisone,

testosterone/estrogens according to the following definitions (10). Estrogen deficiency in women was present in case of LH/FSH deficiency in premenopausal women with prolonged amenorrea >1 year without adequate replacement therapy or by a low serum oestradiol concentration of <70 nmol/l and all postmenopausal women. In men, LH/FSH deficiency was defined as testosterone level below the reference range (8.0 nmol/l). Thyroid stimulating hormone (TSH) deficiency was defined as a free thyroxine level below the reference range (<10 pmol/l). Adrenocorticotrophic hormone (ACTH) deficiency was defined as an insufficient increase of cortisol (peak <0.55 μmol/l) after corticotrophin releasing hormone test or insulin tolerance test. GH deficiency was not routinely assessed.

Patients were seen at the outpatient clinic for a single visit. The study protocol was approved by the Medical Ethics Committee, and all subjects gave written consent.

Study parameters

QUESTIONNAIRES: A standardized questionnaire was completed concerning demographic data, medical history, OA symptoms and signs and information on type of occupation and type of sport. Other relevant details of treatment and patient characteristics were derived from patient records.

PHYSICAL EXAMINATION: Physical examination was performed by a single physician (M.W.) trained in structured joint assessment. Internal rotation and flexion of the hip and extension of the knees was assessed, in combination with both pain and crepitation.

RADIOGRAPHIC PROTOCOL: Radiographs were obtained from all patients between September and December 2007. Conventional radiographs of the knee (posterior-anterior (PA), weight-bearing, fixed-flexion (11;12) and hips (PA, supine) were obtained from all patients, according to a standardized protocol with a fixed film-focus distance and fixed joint position. All radiographs were performed by a single experienced radiology technician.

ASSESSMENT OF RADIOGRAPHIC OA: For a semi-quantitative assessment of the radiographic cartilage damage, JSN was graded in the knee (both medial and lateral femorotibial compartments) and hip on a scale from 0 to 3, using the Osteoarthritis Research Society (OARSI) atlas (13). Radiographs were scored by consensus opinion of two experienced readers (M.W. & K.M.J.A.C.), blinded for patient characteristics. In cases of disagreement, the lower, more conservative score was adopted. The reproducibility for JSN in the hip and knee, reflected by the intra-class correlation coefficient (ICC), was good (0.89 and 0.82, respectively, for the hip and knee). The reproducibility was based on the repeat reading of 15 randomly selected radiographs. JSN was defined as an OARSI score of ≥1 at a particular joint site. Based on the presence of JSN, patients were divided into two groups (i.e. OARSI ≥1 vs OARSI 0), independently for the knee and hip joint. Joint prostheses could not be scored with OARSI, and therefore, these joints (i.e. 3 knees, 2 hips) were excluded from the analyses.

Radiographic knee and hip OA were also scored according to the Kellgren-Lawrence (KL) scale, including other OA features, by a single experienced musculoskeletal radiologist (H.M.K.) (14). ICCs were 0.89 and 1.00 for the knee and hip, respectively. Radiographic OA was defined as KL ≥2 or presence of a knee or hip prosthesis.

DEFINITION OF CLINICAL OA: We used the clinical American College of Rheumatology (ACR) criteria for the assessment of clinical hip and knee OA. Criteria for clinical hip OA were pain in combination with internal rotation of ≥15° and morning stiffness for ≤60 min (15). Clinical criteria for knee OA were pain, crepitation on physical examination and morning stiffness ≤30 min in combination with bony enlargements (16).

PARAMETERS OF ACROMEGALIC DISEASE: Active disease duration was calculated from the estimated date of onset, using start of signs and symptoms, and facial changes on photographs to the date of normalization of serum IGF-1 levels after surgery or additional therapy. Remission duration was calculated from the date of normalization of serum IGF-1 concentrations until start of the present study, supported by the findings during the oral glucose tolerance test (oGTT). Both surgically and/or irradiation cured patients and patients with controlled disease by treatment with SMS analogs were collectively referred to as 'in remission'.

ASSAYS: Blood samples were taken in the post-absorptive state to assess the actual GH and IGF1 concentrations. Serum GH was measured with a sensitive IFMA (Wallac, Turku, Finland), specific for the 22 kDA GH protein, calibrated against World Health Organisation International Reference Preparation (WHO IRP) 80/505 (detection limit: 0.01ug/l, interassay coefficient of variation (CV): 1.6-8.4% of 0.01-15.38ug/l) from 1992 onwards. For the conversion of ug/l to mU/l, the values have to be multiplied by 2.6. Prior to 1992, GH was measured by RIA (Biolab, Serona, Coissins, Switzerland), calibrated against WHO IRP 66/21 (detection limit: 0.5mU/l, with an interassay CV less than 5%; for the conversion of ug/l to mU/l, multiply by 2).

Serum IGF-1 concentration (nmol/l) was measured using an immunometric technique on an Immulite 2500 system (Diagnostic Products Corporation, Los Angeles, CA, USA). The intra-assay variations at mean plasma levels of 8 and 75nmol/l were 5.0 and 7.5%. IGF-1 levels were expressed as SDS, using lambda-mu-sigma smoothed reference curves based on the measurement in 906 healthy individuals (17;18).

Statistical analysis

SPSS for Windows, version 17.0 (SPSS Inc., Chicago, IL, USA), was used for data analysis. Data are presented as a mean±SD, unless specified otherwise. P<0.05 was considered to reflect statistical significance. Because hip and knee OA may be associated with different risk factors, these joints were analyzed independently (19), and, therefore, results are presented separately. For both hip and knee, joints were divided into two groups based on the radiographic presence of JSN in order to assess risk factors for JSN. Left and right hips, and left and right knees, respectively, were analyzed independently in a Generalized Estimating Equations (GEE) analysis, taking into account intra-patient effect. A binary logistic model was performed with adjustments for age, sex and BMI.

RESULTS

PATIENT CHARACTERISTICS: We studies 89 acromegaly patients (mean age 58.3 ± 11.5 years, 51% female), who were all in remission for a mean of 14.1 ± 6.2 years (*Table 1*). Fifty patients were cured by surgery alone, 11 patients were cured by surgery and postoperative radiotherapy, and 28 patients were medically controlled by long-acting SMS analogs, either as primary or secondary treatment. Three patients were co-treated with Pegvisomant. Mean estimated duration of active disease was 8.9 ± 7.4 years. Mean actual IGF-1 SDS was 0.6 ± 1.5.

Hip joint: We studied hips of 87 acromegaly patients (n=174), of which 56 hips (32.2%) had radiographic OA (*i.e.* KL ≥2). With respect to cartilage damage, 18 hips (10.3%) had JSN and 156 hips (89.7%) had preserved joint spaces. JSN in the hip was associated with age (p<0.001). There were no significant relationships between JSN and sex, BMI or menopausal status.

DURATION AND SEVERITY OF GH OVERPRODUCTION: Patients with JSN in the hip did not differ in age of diagnosis, active disease duration or remission duration from patients without JSN. As shown in *Table 1*, pre-treatment GH levels were higher in patients with JSN (153.6±170.2 ug/l *vs* 83.3±108.1 ug/l, p=0.025). In addition, patients with JSN had higher pre-treatment IGF-1 SDS than patients with normal joint spaces (9.7±5.6 *vs* 7.0±4.5, p=0.038). As a measure for GH exposure, we studied the interaction term of pre-treatment GH levels and active disease duration. Patients with JSN had higher GH exposures compared to patients without JSN, indicating that JSN was related to longer GH excess and higher GH levels (p=0.04).

TREATMENT OF ACROMEGALY (*TABLE 2*): Patients with and without JSN in the hip differed significantly with respect to the type of acromegaly treatment. Patients with JSN received SMS analogs more frequently than patients without JSN (50% *vs* 22.2%, OR=8.16 (1.1-61.8), p=0.042), and immediate postoperative cure was less frequently achieved (OR=0.18 (0.03-0.98), p=0.047). There was a trend of higher SMS exposure, calculated by the interaction term between duration of SMS treatment and SMS dose, in patients with JSN than patients without JSN in the hip, although not-significant after adjustments for age, sex and BMI (p=0.091).

Table 1. Clinical characteristics of 89 patients with acromegaly

Clinical characteristics	**Patients (N = 89)**
Age (years)	58.3 (11.5)
Sex, female (n (%))	45 (51)
BMI (kg/m^2)	28.5 (4.7)
Treatment (n (%))*	
Surgery only	50 (56)
Surgery + RT	11 (13)
SMS analogues	
Primary	5 (6)
Following surgery	19 (21)
Following RT	1 (1)
Following surgery + RT	3 (3)
Disease duration (years)	8.9 (7.4)
Duration of remission (years)	14.1 (6.2)
Pre-treatment GH (µg/L)	36.8 (48.0)
IGF-1 SD scores	
Pre-treatment	7.4 (4.7)
Actual	0.6 (1.5)
Hypopituitarism (n (%))	
Corticotrope failure	22 (25)
Thyreotrope failure	18 (20)
Gonadotrope failure**	49 (55)

Values are means (SD) unless stated otherwise.

GH, growth hormone; IGF-1, insulin-like growth factor 1; BMI, body mass index; RT, radiotherapy; SMS, somatostatin (analogs).

* *Three patients (3%) were co-treated with Pegvisomant.*

** *Including natural menopause (N = 40 (45%)) and hypogonadotropic hypogonadism*

Table 2. Acromegaly-specific risk factors for JSN in the hip, studying 87 well-controlled acromegaly patients

	No JSN (OARSI 0) n = 156	**JSN (OARSI ≥1) n = 18**	**Adjusted OR (95%CI)**	**Adjusted P value**
Age at diagnosis (yr)	40.2 ± 12.6	44.2 ± 9.1	1.0 (0.9-1.0)	0.259 #
Active disease duration (yr)	8.6 ± 7.6	10.1 ± 4.6	1.0 (0.9-1.1)	0.949
Duration of remission (yr)	13.7 ± 5.9	16.6 ± 7.5	1.1 (0.9-1.2)	0.318
Pre-treatment GH (µg/L)	83.3 ± 108.1	153.6 ± 170.2	1.01 (1.01-1.01)	0.025
Pre-treatment IGF-1 SDS	7.0 ± 4.5	9.7 ± 5.6	1.1 (1.01-1.3)	0.038
Treatment				
Surgery and/or Radiotherapy (%)	77.6	50.0	8.2 (1.1-61.8)	0.042 *
SMS analogs (%)	22.4	50.0		
Immediate postoperative cure (%)	57.3	21.1	0.2 (0.03-0.98)	0.047

n represents total number of hip joints included. Data are shown as mean, unless mentioned otherwise. Immediate cure was defined as immediate postoperative cure, without requiring additional radiotherapy and/or postoperative medical treatment for persisting disease activity. Data were analyzed by Generalized Estimating Equations (GEE) analysis taking into account intra-patient effect, with additional adjustments for age, sex and BMI (except for #).

CI, confidence interval; BMI, body mass index; SMS, somatostatin analogs.

* *SMS analogs vs other therapies for acromegaly disease.*

LOCAL BIOMECHANICAL FACTORS: Three patients had previous hip fractures or hip surgery for another reason. There was no association between physical intensive jobs or sports and JSN.

RELATIONSHIP BETWEEN JSN IN THE HIP AND JOINT COMPLAINTS: Seventy-three % of the patients with JSN of the hip met the ACR criteria for clinical hip OA, indicating a much higher prevalence of clinical OA than in patients with preserved joint spaces, of which only 17.6% met the clinical ACR criteria (OR=14.0 (2.5-79.3), p<0.001). Pain/stiffness at physical examination did not differ between patients with and without JSN.

Knee joint: We studied knees of 88 patients (n=175), of which 48 knees (27.4%) had radiographic OA (i.e. KL ≥2). With regard to cartilage damage, twenty-seven knees had JSN (15.4%) and 148 knees (84.6%) had preserved joint spaces. JSN in the knee was associated with age (65.2±8.0 years vs 57.1±11.6 years, p<0.001) and female sex (70.0% vs 56.3%, p=0.018). There were no differences in BMI or menopausal status, but waist circumference was higher in patients with JSN (p=0.032).

DURATION AND SEVERITY OF GH OVERPRODUCTION: JSN in the knee was not related to age at diagnosis, active disease duration, duration of remission, pre-treatment GH levels or pre-treatment IGF-1 SDS (*Table 3*). However, GH exposure (product of pre-treatment GH levels and active disease duration) was higher in patients with JSN (p=0.029).

TREATMENT OF ACROMEGALY: Acromegaly treatment was not different between patients with and without JSN in the knee. However, immediate postoperative cure was less frequently obtained in patients with JSN (p=0.019), but this did not remain significant after adjustments for age, sex and BMI.

LOCAL BIOMECHANICAL RISK FACTORS: JSN in the knee was associated with a history of knee surgery (p=0.019): especially, the prevalence of meniscectomy was high in patients with JSN (14.8% *vs* 6.1%, p=0.006) (*Table 2*). With respect to physical work load, type of occupation or type of sport were not related to JSN in the knee.

After consideration of age, sex, BMI, pre-treatment IGF-1 levels, surgical cure *vs* medical control and meniscectomy in a multivariate GEE-model, only age and meniscectomy were significantly associated with JSN in the knee (ORs were 1.1 (1.0-1.1) and 16.9 (3.1-92.1), p=0.012 and p=0.001, respectively), but no acromegaly-specific characteristics.

RELATIONSHIP BETWEEN JSN IN THE KNEE AND JOINT COMPLAINTS: Patients with JSN reported more knee pain than patients without JSN (56.5% *vs* 33.6%) (p=0.035). Clinical knee OA, as assessed by the ACR criteria, was more prevalent in patients with JSN than in patients with preserved joint spaces (63.3% *vs* 18.1%; OR=8.2 (2.9-23.1), p<0.001). There were no significant differences found in pain and/or stiffness at physical examination.

Table 3. Acromegalic-specific and general risk factors for JSN in the knee in 88 well-controlled acromegaly patients

	No JSN (OARSI 0) n = 148	**JSN (OARSI ≥1) n = 27**	**Adjusted OR (95%CI)**	**Adjusted P value**
Age at diagnosis (yr)	40.2	43.2	1.0 (0.9-1.0)	0.133 #
Active disease duration (yr)	8.0 ± 4.7	13.1 ± 4.6	1.1 (1.0-1.2)	0.111
Duration of remission (yr)	14.0 ± 6.4	14.6 ± 5.1	1.0 (0.9-1.1)	0.845
Pre-treatment GH (µg/L)	96.5 ± 119.4	93.2 ± 151.1	1.0 (1.0-1.01)	0.559
Pre-treatment IGF-1 SDS	7.2 ± 96.5	8.8 ± 93.2	1.1 (1.0-1.2)	0.146
Treatment				
Surgery and/or Radiotherapy (%)	75.7	70.3	1.4 (0.4-5.0)	0.576 *
SMS analogs (%)	24.3	29.6		
Immediate postoperative cure (%)	56.4	32.1	0.6 (0.2-1.6)	0.295
Knee Surgery (%)	15.2	40.7	3.5 (1.2-9.8)	0.019
History of meniscectomy (%)	6.1	14.8	7.8 (1.8-34.3)	0.006

n represents total number of knee joints included. Data are shown as mean, unless mentioned otherwise. Immediate cure was defined as immediate postoperative cure, without requiring additional radiotherapy and/or postoperative medical treatment for persisting disease activity. Data were analyzed by Generalized Estimating Equations (GEE) analysis taking into account intra-patient effect, with additional adjustments for age, sex and BMI (except for #).

CI, confidence interval; BMI, body mass index; SMS, somatostatin analogs.
** SMS analogs vs other therapies for acromegaly disease.*

DISCUSSION

This is the first study focusing on the process of JSN in acromegaly patients. JSN appears to be an infrequent finding in patients after long-term follow-up of acromegaly, with a prevalence ranging between only 10 and 15 % in our cohort. This low prevalence is in contrast to previous notions on the pathophysiology of this secondary form of OA. We found a clear association with well-known risk factors of OA, such as age, whereas other disease-specific risk factors for JSN were joint-site specific. At the hip site, disease characteristics reflecting more active disease were related to JSN: higher GH/IGF-1 levels at diagnosis, longer and more severe GH exposure, and immediate postoperative cure was less frequently achieved. This suggests that the GH/IGF-1 axis not only plays a role in the early stage of acromegalic arthropathy, but also in the late stage, as reflected by JSN. At the knee site, we did not find acromegaly-specific characteristics to be associated with JSN. Previous knee surgery was a risk factor for JSN in the knee, suggesting that JSN at the knee site is unrelated to GH excess.

In acromegaly, the late effects of arthropathy are striking, even after long-term control of GH overproduction. The assumption is that persistent exposure to pathologically elevated GH and/or IGF-1 levels results in progressive changes in joint geometry. The phenotype of acromegalic arthropathy is characterized by osteophytosis, but most patients have preserved or even widened joint spaces (7). However, in a small subgroup of adequately treated patients radiographic JSN is present, suggesting that in acromegaly probably two types of arthropathy exist. In the present study, JSN was related to both well-known risk factors and acromegaly-specific risk factors. The high prevalence of JSN in older and female patients is in line with previous observations in primary OA (20). In the knee, JSN was strongly associated to previous knee surgery, especially meniscectomy. It is well-known from primary OA that joint dysplasias, fractures of articular surfaces, and tears of menisci and ligaments, which all increase joint instability, frequently precede the development of OA (20). This suggests that the JSN phenotype in the knee is probably unrelated to the previous GH excess.

Remarkably, higher pre-treatment GH and IGF-1 levels were associated with JSN in the hip. These findings suggest that JSN in acromegalic joints can be predicted by a more severe biochemical presentation at diagnosis. An interesting observation is the difference in acromegaly treatment between patients with and without JSN of the hip. JSN was highly prevalent in patients controlled by SMS analogs, in contrast to a relatively low prevalence in patients cured postoperatively. This was not observed in the knee, which is most probably explained by the fact that the hip is the joint site that is most frequently systemically involved. The high prevalence of JSN in SMS-treated patients could be explained by the higher disease activity in patients with SMS analogs, since, during SMS treatment, subtle abnormalities in the GH secretion pattern persist (21). In accordance, previous studies report worse outcome in SMS-treated patients with respect to QoL and diastolic heart function (22;23). Alternatively, SMS analogs possibly could have a direct, IGF-1 independent, effect on joint structure. There is evidence for direct inhibitive local effects of SMS on cartilage (24;25), and, in addition, SMS receptors were demonstrated in bone cells (26). The finding that JSN occurs more frequently in patients with higher SMS-exposure (interaction term of SMS duration and dose) supports this hypothesis. Further studies have to establish the physiological significance of long-term SMS therapy on joint architecture. Another explanation is a generally less favorable previous course of acromegaly in SMS-treated patients, in which other acromegalic-specific factors within the group of SMS-treated patients could probably result in more severe cartilage damage (*i.e.* bias by indication). However, most previous studies failed to demonstrate a relationship between duration of active disease and arthropathy (4;27;28).

JSN appeared to be related to a higher presence of joint complaints (both knee and hip), when applying clinical ACR criteria. Furthermore, JSN was related to more self-reported pain in the knee. Previously, acromegalic patients were reported to have better joint functions and, therefore, have less joint-replacement surgery than patients with primary OA, despite a higher prevalence of osteophytes. It was hypothesized that the preserved joint spaces in acromegaly may protect against pain caused by osteophytes, and, therefore, prevent acromegalic patients from a decrease in functional capability (7).

Some limitations of this study have to be addressed. First, the used scoring methods, such as the OARSI score and clinical ACR criteria are subject to debate, since, at present, there are no acromegaly-specific classification systems for arthropathy available. Therefore, we were limited to the use of primary OA scales for the definition of arthropathy, although these methods are developed and validated for the use in primary OA. With respect to radiographic OA, these primary OA scales can not evaluate a main feature of acromegalic arthropathy, *i.e.* joint space widening. Therefore, we were unable to differentiate between preserved or widened joint spaces, reflecting cartilage hypertrophy. Second, the present study is a cross-sectional study, and therefore, the causality of the association between GH/IGF-1 activity and JSN is difficult to assess.

In conclusion, JSN is an infrequent finding in patients with acromegalic arthropathy. Patients with JSN have more joint complaints. Well-known risk factors, such as age and female sex, are associated with JSN. In addition, especially at the hip site, acromegalic-specific risk factors reflecting more active disease are related to JSN. Remarkable is the high prevalence of JSN in patients who were controlled by SMS analogs when compared to patients immediately cured postoperatively. The findings of the present study suggest that, 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 the late phase of the disease, as reflected by JSN.

REFERENCE LIST

1. Barkan A. Acromegalic arthropathy and sleep apnea. J Endocrinol 1997; 155 Suppl 1:S41-S44.
2. Okazaki K, Jingushi S, Ikenoue T et al. Expression of insulin-like growth factor I messenger ribonucleic acid in developing osteophytes in murine experimental osteoarthritis and in rats inoculated with growth hormone-secreting tumor. Endocrinology 1999; 140(10):4821-4830.
3. Barkan AL. Acromegalic arthropathy. Pituitary 2001; 4(4):263-264.
4. Biermasz NR, Pereira AM, Smit JW, Romijn JA, Roelfsema F. Morbidity after long-term remission for acromegaly: persisting joint-related complaints cause reduced quality of life. J Clin Endocrinol Metab 2005; 90(5):2731-2739.
5. Wassenaar MJ, Biermasz NR, van DN et al. High prevalence of arthropathy, according to the definitions of radiological and clinical osteoarthritis, in patients with long-term cure of acromegaly: a case-control study. Eur J Endocrinol 2009; 160(3):357-365.
6. Biermasz NR, Wassenaar MJ, van der Klaauw AA et al. Pretreatment insulin-like growth factor-I concentrations predict radiographic osteoarthritis in acromegalic patients with long-term cured disease. J Clin Endocrinol Metab 2009; 94(7):2374-2379.
7. Wassenaar MJ, Biermasz NR, Bijsterbosch J et al. Arthropathy in long-term cured acromegaly is characterised by osteophytes without joint space narrowing: a comparison with generalised osteoarthritis. Ann Rheum Dis 2011; 70(2):320-325.
8. Biermasz NR, van DH, Roelfsema F. Ten-year follow-up results of transsphenoidal microsurgery in acromegaly. J Clin Endocrinol Metab 2000; 85(12):4596-4602.
9. Roelfsema F, van DH, Frolich M. Long-term results of transsphenoidal pituitary microsurgery in 60 acromegalic patients. Clin Endocrinol (Oxf) 1985; 23(5):555-565.
10. van der Klaauw AA, Kars M, Biermasz NR et al. Disease-specific impairments in quality of life during long-term follow-up of patients with different pituitary adenomas. Clin Endocrinol (Oxf) 2008; 69(5):775-784.
11. Peterfy C, Li J, Zaim S et al. Comparison of fixed-flexion positioning with fluoroscopic semi-flexed positioning for quantifying radiographic joint-space width in the knee: test-retest reproducibility. Skeletal Radiol 2003; 32(3):128-132.
12. Whitehouse SL, Crawford RW, Learmonth ID. Validation for the reduced Western Ontario and McMaster Universities Osteoarthritis Index function scale. J Orthop Surg (Hong Kong) 2008; 16(1):50-53.
13. Altman RD, Hochberg M, Murphy WA, Jr., Wolfe F, Lequesne M. Atlas of individual radiographic features in osteoarthritis. Osteoarthritis Cartilage 1995; 3 Suppl A:3-70.
14. Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. Ann Rheum Dis 1957; 16(4):494-502.
15. Altman R, Alarcon G, Appelrouth D et al. The American College of Rheumatology criteria for the classification and reporting of osteoarthritis of the hip. Arthritis Rheum 1991; 34(5):505-514.
16. Altman R, Asch E, Bloch D et al. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. Arthritis Rheum 1986; 29(8):1039-1049.
17. Cole TJ. The LMS method for constructing normalized growth standards. Eur J Clin Nutr 1990; 44(1):45-60.
18. Rikken B, van DJ, Ringeling A, Van den Brande JL, Massa G, Wit JM. Plasma levels of insulin-like growth factor (IGF)-I, IGF-II and IGF-binding protein-3 in the evaluation of childhood growth hormone deficiency. Horm Res 1998; 50(3):166-176.
19. Sharma L, Kapoor D, Issa S. Epidemiology of osteoarthritis: an update. Curr Opin Rheumatol 2006; 18(2):147-156.
20. Felson DT, Lawrence RC, Dieppe PA et al. Osteoarthritis: new insights. Part 1: the disease and its risk factors. Ann Intern Med 2000; 133(8):635-646.
21. Biermasz NR, Pereira AM, Frolich M, Romijn JA, Veldhuis JD, Roelfsema F. Octreotide represses secretory-burst mass and nonpulsatile secretion but does not restore event frequency or orderly GH secretion in acromegaly. Am J Physiol Endocrinol Metab 2004; 286(1):E25-E30.

22. Rubeck KZ, Madsen M, Andreasen CM, Fisker S, Frystyk J, Jorgensen JO. Conventional and novel biomarkers of treatment outcome in patients with acromegaly: discordant results after somatostatin analog treatment compared with surgery. Eur J Endocrinol 2010; 163(5):717-726.
23. van Thiel SW, Bax JJ, Biermasz NR et al. Persistent diastolic dysfunction despite successful long-term octreotide treatment in acromegaly. Eur J Endocrinol 2005; 153(2):231-238.
24. Ferrandez MA, Carrascosa A, Audi L, Ballabriga A. Somatostatin effects on cultured human fetal epiphyseal chondrocytes. Pediatr Res 1992; 32(5):571-573.
25. Weiss RE, Reddi AH, Nimni ME. Somatostatin can locally inhibit proliferation and differentiation of cartilage and bone precursor cells. Calcif Tissue Int 1981; 33(4):425-430.
26. Zapf J, Gosteli-Peter M, Weckbecker G, Hunziker EB, Reinecke M. The somatostatin analog octreotide inhibits GH-stimulated, but not IGF-I-stimulated, bone growth in hypophysectomized rats. Endocrinology 2002; 143(8):2944-2952.
27. Colao A, Marzullo P, Vallone G et al. Reversibility of joint thickening in acromegalic patients: an ultrasonography study. J Clin Endocrinol Metab 1998; 83(6):2121-2125.
28. Scarpa R, De BD, Pivonello R et al. Acromegalic axial arthropathy: a clinical case-control study. J Clin Endocrinol Metab 2004; 89(2):598-603.

VI.

Progression of vertebral fractures despite long-term biochemical control of acromegaly: a prospective follow-up study

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ABSTRACT

BACKGROUND: In active acromegaly, pathologically elevated GH and IGF-1 levels are associated with increased bone turnover and a high bone mass, the latter being sustained after normalization of GH values. In a cross-sectional study design, we have previously reported a high prevalence of vertebral fractures (VFs) of about 60% in patients with controlled acromegaly, despite normal mean bone mineral density (BMD) values. Whether these fractures occur during the active acromegaly phase or after remission is achieved is not known.

OBJECTIVE: Our objective was to study the natural progression of VFs and contributing risk factors in patients with controlled acromegaly over a 2.5-year follow-up period.

METHODS: Forty-nine patients (mean age 61.3±11.1 years, 37% female) with controlled acromegaly for ≥2 years following surgery, irradiation and/or medical therapy and not using bisphosphonates were included in the study. Conventional spine radiographs including vertebrae Th4-L4 were assessed for VFs according to the Genant method. VF progression was defined as development of new/incident fractures and/or a minimum 1-point increase in the Genant scoring of pre-existing VFs. BMD was assessed by dual-energy X-ray absorptiometry (Hologic 4500).

RESULTS : Prevalence of baseline VFs was 63%, being highest in men, and fractures were unrelated to baseline BMD. VF progression was documented in 20% of patients, especially in men and in case of ≥2 VFs at baseline. VF progression was not related to BMD values or BMD changes over time.

CONCLUSION: Findings from this longitudinal study show that VFs progress in the long term in 20% of patients with biochemically controlled acromegaly in the absence of osteoporosis or osteopenia. These data suggest that an abnormal bone quality persists in these patients after remission, possibly related to pre-treatment long-term exposure to high circulating levels of GH.

INTRODUCTION

Growth Hormone (GH) and Insulin-like Growth Factor-1 (IGF-1) are important regulators of bone growth modeling and remodeling during an individual's life span (1). IGF-1 mediates most of the effects of GH on skeletal metabolism via the IGF-1 receptor (2). GH and IGF-1 act as anabolic hormones on bone by stimulating proliferation, and to some extent, differentiation of osteoblasts. Osteoclastic bone resorption is also stimulated, resulting in an overall increase in bone remodeling (3;4).

In active acromegaly, pathologically high GH and IGF-1 levels increase bone turnover in favor of bone formation (2;5;6), resulting in an overall increase in bone mineral density (BMD). It has been suggested that high bone mass is sustained after long-term disease control (7-11). However, some studies have also reported a low BMD in patients with acromegaly, especially in the presence of concomitant hypogonadism (9;12). We have recently reported a remarkably high prevalence of vertebral fractures (VFs) (59%) in long-term cured acromegalic patients. The highest VF prevalence was observed in men, particularly in those with hypogonadism, and prevalence was significantly increased compared with that of the general population (13). Three other studies reported a comparably high VF prevalence in controlled acromegaly patients, independently of gonadal status (5;6;14), suggesting that VFs should be included in the panoply of complications of acromegaly. Since BMD has found to be normal in the majority of patients, the high VF risk is likely to be due to alterations in bone quality rather than a decrease in bone quantity. Another important factor in the acquisition of bone mass is the level of sex steroids, being illustrated by the development of osteopenia and an increased fracture risk in hypogonadal men and women and by the preservation of bone mass by restoration of normal endogenous sex steroid levels or by exogenous supplementation (15;16).

Because all studies reported to date have been cross-sectional studies, an important question remaining to be addressed is whether patients solely fracture during the active phase of acromegaly or whether they continue to sustain fractures after achievement of cure (17). Another question to be addressed is how to best identify acromegaly patients at risk for (vertebral) fractures, because BMD is a bad predictor of fracture risk in this form of secondary osteoporosis. In view of the increased morbidity and mortality associated with VFs, these questions are of clinical relevance in the long-term management of acromegaly. In a prospective study design, we evaluated the course of VFs and potential determinants for VF progression in the long-term follow-up of biochemically controlled acromegalic patients.

PATIENTS AND METHODS

PATIENTS: All patients with acromegaly in long-term remission (≥2 years) after surgery (69%) or medical treatment (31%) and regularly followed up at the Department of Endocrinology and Metabolic Diseases of the Leiden University Medical Center were invited to take part in a cross-sectional study in 2007. Eighty-nine patients positively responded and were included in the study (18). All 89 patients were invited for a further evaluation of skeletal status after a mean interval of 31±1.7 months (range 28-35 months), of whom 58 (65%) positively responded. Reasons for nonparticipation in the follow-up evaluation were non-musculoskeletal-related health problems (N=16), travel distance (N=6), lack of time (N=4), psychological reasons (N=3), or moving abroad (N=2). Demographic and disease characteristics did not differ between participants and nonparticipants in the follow-up study (*data not shown*), except for more women among non-participants (p=0.025). We excluded the 9 patients receiving bisphosphonate treatment at any time point during follow-up, so that 49 patients were included in the final analysis.

Clinical follow-up data were available yearly in all patients since initiation of treatment for acromegaly. From 1977 onward, first-line treatment was in the form of transsphenoidal surgery performed by a single experienced neurosurgeon. When required, adjuvant treatment was given in the form of radiotherapy (before 1985) or somatostatin (SMS) analogs (from 1985 onward). Since 1998, a small number of patients received treatment solely in the form of a depot formulation of long-acting SMS analogs. This treatment approach resulted in early postoperative control in 66% and late control in >90% of patients (19). From 2003 onward, Pegvisomant was used for treatment-resistant acromegaly.

Disease activity was assessed yearly by an oral glucose tolerance test (except in medically treated patients) and fasting serum GH and IGF-1 levels. Other pituitary functions were also evaluated yearly. Remission was defined as a normal glucose-suppressed serum GH <1.25μg/L (RIA assay until 1992) or 0.38μg/L (immunofluorometric assay (IFMA) from 1992 onwards), serum GH levels <1.9μg/L, and normal IGF-1 levels for age (from 1986 onwards). Treatment decisions were based on these remission criteria.

STUDY DESIGN: Fasting blood samples were obtained to assess GH and IGF-1 concentrations at baseline and at 2.5 years of follow-up. Conventional spine radiographs were obtained using a standardized protocol (*vide infra*) and BMD was assessed using dual energy X-ray absorptiometry (DXA) (Hologic QDR 4500) at the same time points. All patients had to complete a standardized questionnaire providing demographic data, data on medical history, and data on clinical risk factors for VFs, such as early menopause, previous fractures, glucocorticoid use and diagnosis of rheumatoid arthritis.

The Medical Ethics Committee approved the study protocol, and informed consent was obtained in all patients.

ACROMEGALY DISEASE PARAMETERS: Duration of active acromegaly was calculated from the estimated date of onset of the disease, defined as the date of onset of signs and symptoms or the date of changes in facial features using serial photographs, to the date of normalization of serum IGF-1 after surgery, irradiation and/or medical treatment. Duration of remission of acromegaly was calculated from the date of biochemical remission to the date of baseline evaluation at inclusion in the study. Cure was defined as normal glucose-suppressed age-related GH levels and IGF-1 levels after surgery and/or irradiation. Biochemical control was defined as normal serum IGF-1 levels for age during medical treatment. Both cured and biochemically controlled patients were referred to as being 'in remission'.

ASSESSMENT OF PITUITARY AND GONADAL FUNCTION: Hypopituitarism was diagnosed on the basis of clinically significant hormone deficiencies in at least one axis, which required supplementation with L-T_4, hydrocortisone, testosterone or estrogens (in pre-menopausal women) according to the following definitions (20;21): TSH deficiency was defined as a free T_4 level below the normal laboratory reference range (absolute value <10pmol/l). ACTH deficiency was defined as an insufficient increase in cortisol levels (absolute value <0.55µmol/l) after stimulation by CRH or insulin tolerance test. GH deficiency (GHD) was not routinely assessed.

With respect to gonadal function, patients with adequately treated hypogonadism (*i.e.* gonadal hormone replacement therapy started <1 year after the onset of hypogonadism) were considered eugonadal. Men with a total testosterone concentration of <8.0nmol/l for longer than 1 year during follow-up or documented prior to diagnosis, were considered hypogonadal. Female patients with normal spontaneous menstrual cycle, those using estrogen replacement therapy or the pill, or patients with a history of only short-term amenorrhea who were treated with estrogen within 1 year of diagnosis were considered eugonadal. Females with prolonged untreated amenorrhea in the presence of a serum estradiol concentration of <70nmol/l or natural menopause were considered hypogonadal.

BIOCHEMICAL ASSAYS: Serum GH was measured with a sensitive IFMA (Wallac, Turku, Finland), specific for the 22 kDA GH protein, calibrated against World Health Organisation International Reference Preparation (WHO IRP) 80/505 (detection limit: 0.01µg/l, interassay coefficient of variation (CV): 1.6-8.4% of 0.01-15.38µg/l) from 1992 onwards. For the conversion of µg/l to mU/l, values were multiplied by 2.6. Before 1992, GH was measured by RIA (Biolab, Serona, Coissins, Switzerland), calibrated against WHO IRP 66/21 (detection limit: 0.5mU/l, with an interassay CV less than 5%; for the conversion of µg/l to mU/l, multiply by 2.6).

Until 2005, serum IGF-1 concentrations were determined by a RIA (Incstar; Stillwater, MN, USA) with a detection limit of 1.5nmol/l and an inter-assay CV below 11%. IGF-1 is expressed as standard deviation score (SDS) for age- and sex-related normal levels determined in the same laboratory (32). From 2005 onwards, serum IGF-1 concentrations (nmol/l) were measured using an immunometric technique on an Immulite 2500 system (Diagnostic Products Corporation, Los Angeles, CA, USA). The intra-assay variations at mean plasma levels of 8 and 75nmol/l were 5.0 and 7.5%, respectively. IGF-1 levels were expressed as SDS, using lambda-mu-sigma smoothed reference curves based on measurements in 906 healthy individuals (22;23).

Markers of bone-turnover, beta-crosslaps (bone resorption) and procollagen type 1 amino-terminal propeptide (P1NP) (bone formation), were measured by an electrochemolumiscent immunoassay with a Modular Analytics E-170 system (Roche Diagnostics, Almere, The Netherlands). Serum calcium (adjusted for albumin binding) was measured by a semiautomated technique. 25-hydroxyvitamin D was measured by RIA (Incstar/DiaSorin, Stillwater, MN, USA). Serum concentrations of intact PTH (reference range 1.5-8 pmol/l) were measured using Immulite 2500 (Siemens Diagnostics, Breda, The Netherlands).

BMD MEASUREMENTS: BMD was measured at the lumbar spine (L1-L4) and total hip using DXA (Hologic QDR 4500, Hologic Inc., Waltham, MA, USA) equipped with reference values based on the National Health and Nutrition Examination Survey (NHANES III). World Health Organization (WHO) criteria were used to define osteopenia (T-score between -1.0 and -2.5) and osteoporosis (T-score ≤-2.5). Baseline BMD data have already been published for the original cohort (13). Follow-up BMD data were available in a subset of 15 patients (31%) in the context of standard patient care.

RADIOGRAPHIC PROTOCOL AND VF ASSESSMENT: Conventional lateral radiographs of the thoracic and lumbar spine were performed by an experienced radiology technician following a standardized protocol, with the film centralized on Th7 and L3, respectively, at baseline and after 2.5 years of follow-up. Radiographs obtained in individual patients at both time points were assessed unpaired for presence of VFs in Th4 to L4 according to the validated Genant's semiquantitative method (24). Grade 1 (mild fracture) was defined as approximately 20% to 25% reduction in anterior, middle and/or posterior height; Grade 2 (moderate fracture) was defined as approximately 25% to 40% reduction in anterior, middle and/or posterior height; Grade 3 (severe fracture) was defined as approximately >40% reduction in anterior, middle and/or posterior height. Radiographs were blinded for any patient characteristics and were individually assessed by two independent observers (H.M.K. and K.M.J.A.C.), one of whom is an experienced musculoskeletal radiologist (H.M.K.). In case of discrepancy in assessment, a consensus opinion was obtained. The intra-observer and interobserver variability were good, as depicted by intra-correlation coefficients (ICCs) of, respectively, 0.950 and 0.901. Individual vertebrae with confounding pathology were excluded (5 vertebrae).

Progression of VFs was defined as the development of new/incident VFs (in patients with no VFs or in patients with previous VFs in other vertebrae) and/or the documented minimum 1-point increase in the Genant scoring in pre-existing VFs during the period of follow-up.

Statistical analysis

SPSS for Windows, version 17.0 (SPSS Inc., Chicago, IL, USA), was used for data analysis. Data are presented as mean (SD), unless otherwise stated. $P<0.05$ was considered to be significant. Patient characteristics were grouped according to sex. BMD changes over time were analyzed by a Paired Samples *T* test. Mean baseline BMD, mean current BMD and mean BMD changes were studied compared patients with and without VF progression, using a binary logistic regression analysis, adjusted for age, sex and body mass index (BMI). An X^2 test was used to assess VF progression rate according to the number of prevalent VFs at baseline.

Potential risk factors for VF progression were identified by binary logistic regression analysis, adjusted for age, sex, BMI, parameters of disease activity, and the presence of hypopituitarism.

RESULTS

Patient characteristics

Forty-nine patients with well-controlled acromegaly (mean age 61.3±11.1 years, 37% female) were included in the study (*Table 1*). Patients had been in remission for a mean of 17.0±7.1 years and mean actual IGF-1 SDS was 0.63±1.39. In 36 patients (73%), remission was achieved by surgery with additional radiotherapy, when required. The remaining 13 patients (27%) received long-acting SMS analogs, either as sole treatment or as postoperative treatment after transsphenoidal surgery (mean duration of treatment 105 (range 21-91) months). One patient received additional treatment with Pegvisomant. Thirty male patients were considered eugonadal (24 with preserved gonadal function, 6 with adequate androgen replacement), and only one was hypogonadal. Mean testosterone level at follow-up was 13.4±4.8 nmol/L. Two females were considered eugonadal (pre-menopausal with normal gonadal function), and 16 were post-menopausal. One patient had diabetes mellitus type 1 and two patients had diabetes mellitus type 2.

All patients were Vitamin D replete at baseline and were still so at the end of study evaluation, except for 2 patients (25-hydroxyvitamin D concentrations <25nmol/L). Ten and 8 patients received respectively calcium and Vitamin D supplements during follow-up.

Bone markers

Mean baseline P1NP and β-crosslaps concentrations were, respectively, 39.4±20.9ng/mL and 0.39±0.20ng/mL. All values were within the normal laboratory reference range, except for slightly elevated P1NP levels in 4 postmenopausal women at baseline and 2 postmenopausal women at follow-up.

Table 1. Clinical characteristics of 49 patients with acromegaly, grouped according to sex

Clinical characteristics	Males (N = 31)	Females (N = 18)	P value
Age (yr)	60.1 (11.1)	63.5 (10.9)	0.30
BMI (kg/m²)	29.6 (4.5)	28.0 (4.9)	0.28
Treatment (n (%))			
Surgery only	21 (68%)	8 (44%)	0.11
Surgery + RT	2 (7%)	5 (28%)	**0.04**
SMS analogues			
Primary	0 (0%)	2 (11%)	0.06
Following surgery *	7 (23%)	2 (11%)	0.32
Following RT	1 (3%)	0 (0%)	0.45
Following surgery + RT	0 (0%)	1 (6%)	0.19
Hypopituitarism (n (%))	10 (32%)	6 (35%)	0.83
Corticotrope failure	8 (26%)	4 (22%)	0.78
GHD	4 (13%)	5 (29%)	0.17
Thyreotrope failure	3 (10%)	4 (22%)	0.23
Gonadotrope failure	6 (19%)	0 (0%)	0.05
Hypogonadal/natural menopause (n (%))	1 (3%)	16 (89%)	<0.01
Disease duration (yr)	8.0 (5.3)	9.4 (11.2)	0.60
Duration of remission (yr)	17.3 (6.5)	16.5 (8.2)	0.71
GH (μg/L)			
Pre-treatment	41.2 (55.4)	24.3 (23.5)	0.28
Actual	0.5 (0.5)	1.3 (2.2)	0.21
IGF-1 SDS			
Pre-treatment	7.43 (3.92)	5.22 (1.99)	**0.03**
Baseline	0.63 (2.09)	0.95 (1.55)	0.59
Actual	0.72 (1.32)	0.45 (1.54)	0.58
Calcium (mmol/l)	2.34 (0.10)	2.36 (0.09)	0.61
PTH (pmol/l)	6.0 (3.0)	5.3 (2.1)	0.43
Vitamin D 25(OH) (nmol/L)			
Baseline	73.3 (32.0)	71.9 (24.3)	0.88
Actual	55.7 (21.9)	54.5 (22.4)	0.89
β-crosslaps (ng/mL)	0.24 (0.11)	0.36 (0.16)	0.01
P1NP (ng/mL)	27.2 (8.8)	37.1 (19.2)	0.08
Current smoking (n (%))	7 (23%)	3 (17%)	0.69
Glucocorticoid use (n (%))	8 (26%)	6 (35%)	0.49
RA (n (%))	0 (0%)	0 (0%)	1.00
Diabetes mellitus (n (%))	2 (6%)	1 (6%)	0.35

Values are means (SD) unless stated otherwise. Patient characteristics were grouped according to sex and were analyzed by Independent Samples T test or X2 test, when appropriate. Disease cure is defined as normal glucose-suppressed GH levels and IGF-1 levels for age after surgery and/or irradiation.

Yr, years; GH, Growth Hormone; IGF-1, Insulin-like Growth Factor-1; BMI, body mass index; SMS, somatostatin (analogs); GHD, Growth Hormone Deficiency; PTH, parathyroid hormone; RA, rheumatoid arthritis.
**, one patient is co-treated with Pegvisomant.*

BMD measurements

BASELINE BMD MEASUREMENTS: At baseline, mean BMD at the lumbar spine was 1.05±0.15 g/cm2, mean T-score was -0.12±1.32, and mean Z-score was 0.99±1.53. Mean BMD at the total hip was 0.96±0.16 g/cm2, mean T-score was

-0.53±2.29, and mean Z-score was 1.06±2.51. Three patients (6%) had osteoporosis at baseline, and 13 patients had osteopenia (27%) at one or more measured sites.

LONGITUDINAL BMD MEASUREMENTS AFTER 31 MONTHS OF FOLLOW-UP IN PATIENTS WITH SUSTAINED REMISSION OF ACROMEGALY (N=15): Follow-up DXA (2010) was available in 15 patients (5 M, 10 F), who were in sustained remission for 18.1 (range 11-28) years after treatment. Ten patients (67%) had hypopituitarism with adequate hormone substitution; all females were postmenopausal. Nine and 7 patients received calcium and Vitamin D supplements, respectively.

In this subset, mean BMD did not change at the lumbar spine (1.00±0.20 g/cm^2 at baseline and 1.00±0.19 g/cm^2 at follow-up) or at the total hip (0.89±0.19 g/cm^2 at baseline and 0.88±0.17 g/cm^2 at follow-up) (*Figure 1*). Changes in BMD did not differ between men and women.

Evaluation of vertebral fractures at baseline and at follow-up

BASELINE EVALUATION OF VERTEBRAL FRACTURES (PREVALENT FRACTURES): As previously reported, VF prevalence was high at baseline (63%) (13). Mean number of VFs per patient was 2.3±1.4 (range 1-6). VF prevalence was higher in men than in women (74% *vs* 44%, p=0.039). Most fractures were documented in the thoracic vertebrae Th12 (N=13), Th9 (N=10) and Th8 (N=9). The VF grade varied from mild (Grade 1: 93%) to moderate (Grade 2: 7%); no severe fractures (Grade 3) were observed. *Figure 2a* depicts the VF number at different vertebral levels, showing a typical bimodal pattern with peaks at Th9 and Th11-L1 in males. In females, fractures of vertebrae Th7 and Th8 were the most frequently observed. *Figure 2b* shows the VF severity distribution at different vertebral levels.

Eight patients (14%) sustained a non-vertebral fracture after establishment of biochemical control (prevalence in women *vs* men: 15% *vs* 13%), giving an incidence rate of 7.8 / 1000 person-years for non-vertebral fractures sustained after achieving remission. Most common fracture site was the hip (N=2).

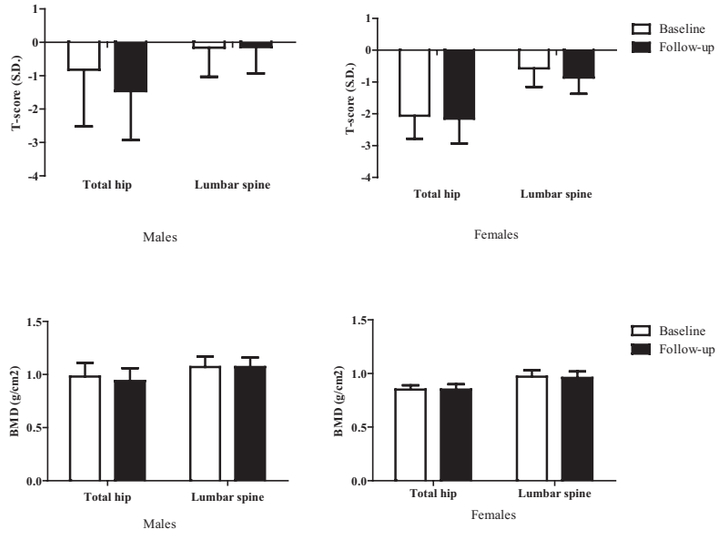


Figure 1. BMD and T-scores in 15 patients with long-term controlled acromegaly, stratified for sex, at baseline and after 31 months of follow-up (N=15)

Data are presented as mean ± SD. Mean BMD and T-scores were presented for 15 acromegaly patients with available follow-up DXA. T-scores reflect the number of standard deviations (SD) above or below the mean for a healthy 30-year old adult of the same sex and ethnicity as the patient. No changes in mean BMD were observed at either the lumbar spine or total hip after a 2.5-year follow-up period.

BMD, bone mineral density; SD, standard deviation.

VERTEBRAL FRACTURE PROGRESSION AND/OR INCIDENT FRACTURES OVER 31 MONTHS OF FOLLOW-UP: VF progression was observed in 10 patients (20%) (29% in men and 5.6% in women), especially at vertebra Th7 (wedge fractures). Eight patients (16%), of whom 3 (6%) had no previous VFs, developed new fractures; one patient showed progression of already existing VFs, and one patient had new fractures in addition to demonstrating progression of preexisting VFs. After excluding the only hypogonadal man, VF progression was seen in 30% of eugonadal men. Excluding the two premenopausal women resulted in VF progression in 6.3% of postmenopausal women.

ANALYSIS OF POTENTIAL RISK FACTORS FOR PROGRESSION OF VERTEBRAL FRACTURES: Patients with ≥2 VFs at baseline had a 9.0-fold increase in risk of VF progression than patients with only one or no VFs at baseline (p=0.005) (*Figure 3*). Risk of progression tended to be higher in males than in females (OR=7.0 (0.8-60.4), p=0.067). Age, BMI, menopausal state, duration of active disease, or pre-treatment/current IGF-1 SDS were not related to VF progression. VF progression rate did not differ between different types of treatment for acromegaly and no effect of Vitamin D status was found.

As assessed in the subset of 15 patients with available follow-up DXA, baseline BMD, current BMD or BMD changes at either the lumbar spine or total hip did not differ between patients with and without VF progression, adjusted for age, sex and BMI.

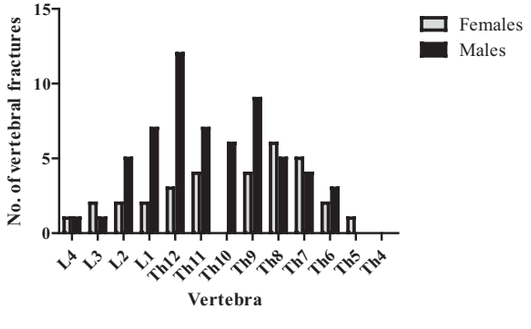


Figure 2A. Distribution of prevalent VFs at different vertebral levels at baseline, for male and female patients with long-term controlled acromegaly

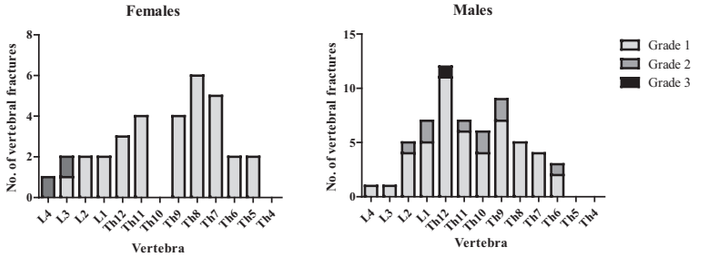


Figure 2B. Severity distribution of VFs at baseline, for male and female patients with long-term controlled acromegaly

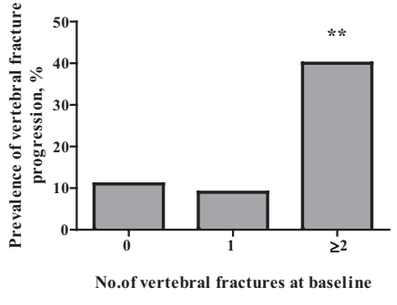


Figure 3. VF progression over 31 months of follow-up according to number of baseline VFs in 49 long-term controlled acromegaly patients

Risk for VF progression in relation to number of baseline VFs is presented. Patients with at least 2 VFs at baseline had a 9.0-fold increased risk of VF progression compared to patients with no or one VF at baseline (p=0.005).

VF, vertebral fracture.

DISCUSSION

Data from this longitudinal study of VFs in patients with acromegaly demonstrate that VFs progress in 20% of patients with long-term biochemically controlled acromegaly during a mean follow-up period of 31 months. These findings suggest that VFs do not solely develop during the active phase of acromegaly, but continue to represent a problem after biochemical control or cure. We have previously shown that normal BMD is maintained during long-term follow-up of up to 17 years after cure. This, however, does not appear to protect against VF progression. Men and patients demonstrating two or more prevalent VFs appear to be more at risk for VF progression.

GH and IGF-1 are important anabolic hormones. Most of the effects of GH is mediated by systemic and/or local IGF-1, enhancing the differentiated function of osteoblasts and bone formation, although GH may also act directly on bone cells (1). On the other hand, IGF-1also induces receptor activator of nuclear factor kB ligand (RANK-L) synthesis and, as a consequence, osteoclastogenesis. In contrast to IGF-1, GH stimulates the production of osteoprotegerin, which competes with the RANK-L receptor, thereby impairing osteoclastogenesis (2). IGF-1 increases the number of remodeling sites, thereby increasing bone activity and diminishing bone strength.

In acromegaly, in which patients are exposed to pathologically high GH and IGF-1 levels for long periods, appropriate treatment by surgery, radiotherapy, medical therapy, or a combination of these treatment modalities considerably improves many of the comorbidities associated with this disorder (25). It is clear, however, that despite long-term biochemical control, a number of skeletal manifestations of acromegaly persist, possibly as a consequence of the severe albeit transient GH excess the skeleton has been originally exposed to. These skeletal complications are associated with increased morbidity and decreased quality of life (26;27). In this respect, a VF is one of the most invalidating (irreversible) complications of acromegaly, occurring in approximately 60% of treated patients (5;6;13;14). Highest VF prevalence has been reported in men, especially in the presence of hypogonadism (13).

Because most previous studies on VFs in acromegaly were of a cross-sectional design, it is difficult to retrospectively discriminate which VFs occurred during the active acromegaly state and which VFs were sustained after disease remission (17). To date, it is still not known whether GH/IGF-1 control reduces this fracture risk. The 20% VF progression rate in adequately controlled acromegaly patients demonstrated in the present study suggests that risk factors for VFs still prevail after achievement of biochemical control. In keeping with earlier studies, progression was greatest in men and in patients with two or more prevalent VFs at baseline (28;29). Our data were consistent with a recent study of Mazziotti *et al.* longitudinally investigating VF progression in a cohort of both active and controlled acromegalics (30). In that particular study, in which control was achieved by medical therapy in the majority , a VF progression rate of 25% over 3 years was observed in their subset of patients with controlled acromegaly.

The high VF prevalence observed in acromegalic patients was interestingly associated with a normal BMD (13), suggesting that BMD is a poor predictor of fracture risk in acromegaly, as is also the case in other forms of secondary osteoporosis (3;4;31). These data also suggest that bone quality may be altered in these patients, potentially irreversibly (32). A note of caution, however, is that BMD at the lumbar spine may be overestimated due to the high prevalence of degenerative changes in acromegaly, induced by prolonged exposure to GH excess (18). Imaging modalities to assess bone quality such as microindentation may allow a better evaluation of fracture risk than DXA in these patients (32;33).

Several comorbidities of acromegaly have been independently associated with fracture risk itself. For example, diabetes mellitus, both type 1 and type 2, is associated with an increased fracture risk, which was reported to be due to increased bone resorption resulting in bone loss and low bone turnover (34). (35). However, in the present study of controlled acromegaly, the number of patients with diabetes was very small. Also hypogonadism is an important factor. However, in the present study, we were not able to analyze the VF progression rate in hypogonadal men nor to explore whether hypogonadism is a risk factor for progression, being limited by the fact that only one man was hypogonadal. However, as described in our previous study, hypogonadal men had the highest VF prevalence of 86% when compared to eugonadal women (19%) and hypogonadal women (49%) (13). These former data strongly suggest that in acromegalic patients in long-term biochemical remission, hypogonadism is also associated with an increased VF risk.

Studies investigating VF progression in general population over 2 to 3 years are scarce; most studies had longer follow-up. Several studies, especially randomized controlled trials of bisphosphonates studying fracture risk in postmenopausal women, reported a VF progression rate of 5-7% over 4 to 5 years of follow-up in the control arm without bisphosphonate use (36-38). Although study designs are not fully comparable, the VF progression rate in acromegaly patients in remission is suggested to be much higher than in the general population. This study has a number of strengths and limitations. Of the study's strengths are the relatively large number of acromegaly patients studies with well-documented follow-up, data including follow-up radiographs, VF scoring by two independent experienced observers with low intra- and interobserver variability, and the adequate supplementation of pituitary hormone deficiencies, which may have otherwise potentially increased fracture risk. We also excluded patients using bisphosphonate treatment to avoid confounding effects of this antiresorptive medication on fracture risk, although by doing so, we also excluded the patients with the worst risk for further fractures. The main limitation of this study may be the inclusion of Grade 1 fractures in our analysis. Although the predictive value of Genant grade 1 fractures for future fractures remains debatable, the prognostic impact of these fractures for future fractures in acromegaly patients is not known. Patients with these mild-grade fractures were therefore included in the final analysis of data from this study.

In conclusion, we demonstrated that VFs progress in 20% of patients with long-term biochemically controlled acromegaly in the absence of osteoporosis or osteopenia. These data suggest persisting poor bone quality possibly related to long-term exposure to high circulating GH levels, although this also remains to be established. Based on our findings and the morbidity and mortality attached to VFs, we recommend the inclusion of VF assessment in the follow-up evaluation of acromegaly patients, also after establishment of biochemical remission, to allow timely therapeutic intervention to prevent further fractures. Future studies are needed to address the pathophysiological basis of the changes in bone quality leading to the high VF risk in long-term controlled acromegaly.

REFERENCE LIST

1. Giustina A, Mazziotti G, Canalis E. Growth hormone, insulin-like growth factors, and the skeleton. Endocr Rev 2008; 29(5):535-559.
2. Canalis E, Giustina A, Bilezikian JP. Mechanisms of anabolic therapies for osteoporosis. N Engl J Med 2007; 357(9):905-916.
3. Manelli F, Carpinteri R, Bossoni S et al. Growth hormone in glucocorticoid-induced osteoporosis. Front Horm Res 2002; 30:174-183.
4. Ohlsson C, Bengtsson BA, Isaksson OG, Andreassen TT, Slootweg MC. Growth hormone and bone. Endocr Rev 1998; 19(1):55-79.
5. Bonadonna S, Mazziotti G, Nuzzo M et al. Increased prevalence of radiological spinal deformities in active acromegaly: a cross-sectional study in postmenopausal women. J Bone Miner Res 2005; 20(10):1837-1844.
6. Mazziotti G, Bianchi A, Bonadonna S et al. Prevalence of vertebral fractures in men with acromegaly. J Clin Endocrinol Metab 2008; 93(12):4649-4655.
7. Battista C, Chiodini I, Muscarella S et al. Spinal volumetric trabecular bone mass in acromegalic patients: a longitudinal study. Clin Endocrinol (Oxf) 2009; 70(3):378-382.
8. Biermasz NR, Hamdy NA, Pereira AM, Romijn JA, Roelfsema F. Long-term maintenance of the anabolic effects of GH on the skeleton in successfully treated patients with acromegaly. Eur J Endocrinol 2005; 152(1):53-60.
9. Bolanowski M, Daroszewski J, Medras M, Zadrozna-Sliwka B. Bone mineral density and turnover in patients with acromegaly in relation to sex, disease activity, and gonadal function. J Bone Miner Metab 2006; 24(1):72-78.
10. Lesse GP, Fraser WD, Farquharson R, Hipkin L, Vora JP. Gonadal status is an important determinant of bone density in acromegaly. Clin Endocrinol (Oxf) 1998; 48(1):59-65.
11. Scillitani A, Battista C, Chiodini I et al. Bone mineral density in acromegaly: the effect of gender, disease activity and gonadal status. Clin Endocrinol (Oxf) 2003; 58(6):725-731.
12. Seeman E, Wahner HW, Offord KP, Kumar R, Johnson WJ, Riggs BL. Differential effects of endocrine dysfunction on the axial and the appendicular skeleton. J Clin Invest 1982; 69(6):1302-1309.
13. Wassenaar MJ, Biermasz NR, Hamdy NA et al. High prevalence of vertebral fractures despite normal bone mineral density in patients with long-term controlled acromegaly. Eur J Endocrinol 2011; 164(4):475-483.
14. Madeira M, Neto LV, Torres CH, de Mendonca LM, Gadelha MR, de Farias ML. Vertebral fracture assessment in acromegaly. J Clin Densitom 2013; 16(2):238-243.
15. Holmes SJ, Shalet SM. Role of growth hormone and sex steroids in achieving and maintaining normal bone mass. Horm Res 1996; 45(1-2):86-93.
16. Tuck SP, Francis RM. Testosterone, bone and osteoporosis. Front Horm Res 2009; 37:123-132.
17. Netelenbos JC, Lems WF, Geusens PP et al. Spine radiographs to improve the identification of women at high risk for fractures. Osteoporos Int 2009; 20(8):1347-1352.
18. Wassenaar MJ, Biermasz NR, van DN et al. High prevalence of arthropathy, according to the definitions of radiological and clinical osteoarthritis, in patients with long-term cure of acromegaly: a case-control study. Eur J Endocrinol 2009; 160(3):357-365.
19. Biermasz NR, Pereira AM, Smit JW, Romijn JA, Roelfsema F. Morbidity after long-term remission for acromegaly: persisting joint-related complaints cause reduced quality of life. J Clin Endocrinol Metab 2005; 90(5):2731-2739.
20. Biermasz NR, van Dulken H, Roelfsema F. Ten-year follow-up results of transsphenoidal microsurgery in acromegaly. J Clin Endocrinol Metab 2000; 85(12):4596-4602.
21. Biermasz NR, Dekker FW, Pereira AM et al. Determinants of survival in treated acromegaly in a single center: predictive value of serial insulin-like growth factor I measurements. J Clin Endocrinol Metab 2004; 89(6):2789-2796.
22. Cole TJ. The LMS method for constructing normalized growth standards. Eur J Clin Nutr 1990; 44(1):45-60.
23. Rikken B, van DJ, Ringeling A, Van den Brande JL, Massa G, Wit JM. Plasma levels of insulin-like growth factor (IGF)-I, IGF-II and IGF-binding protein-3 in the evaluation of childhood growth hormone

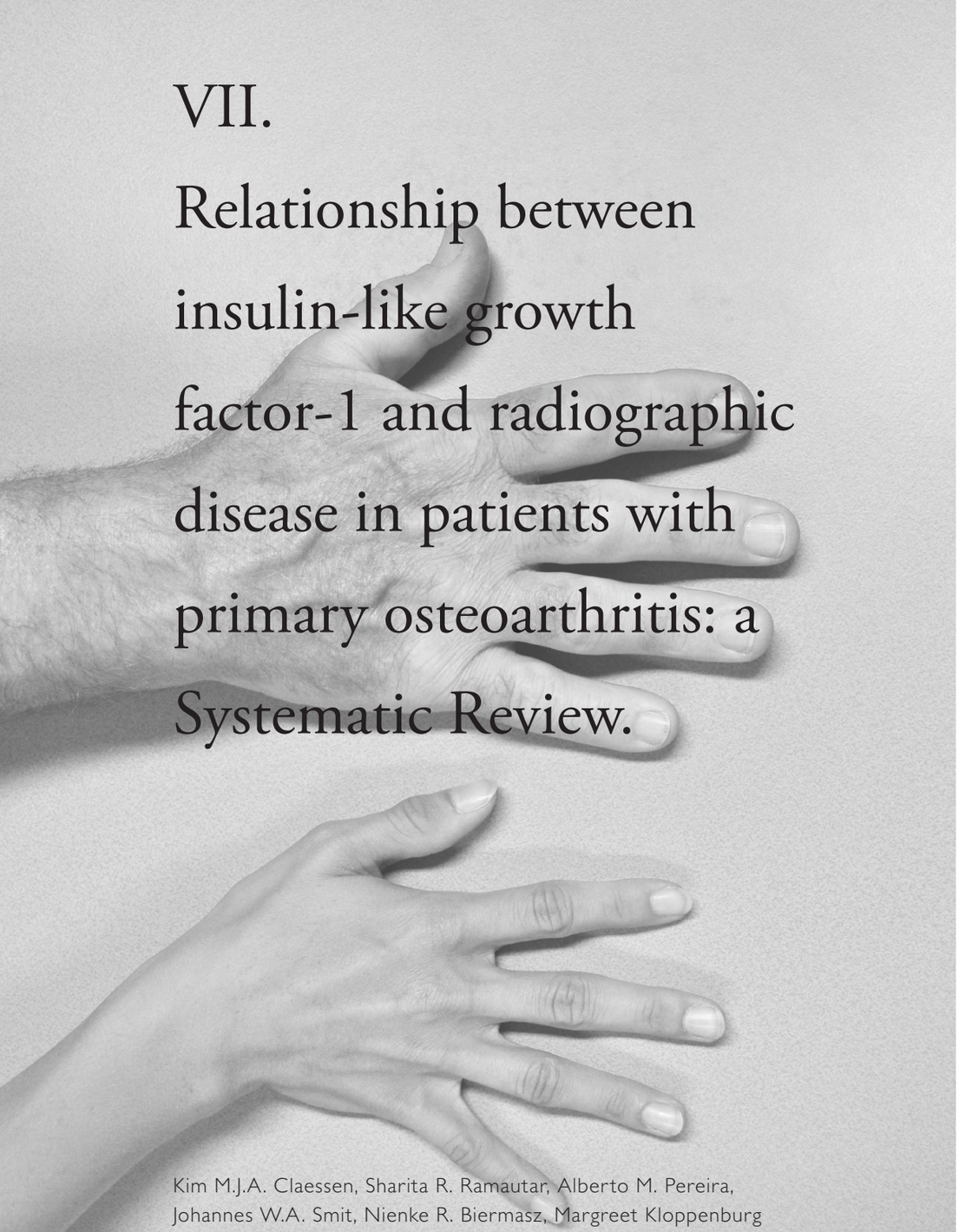
deficiency. Horm Res 1998; 50(3):166-176.
24. Genant HK, Wu CY, van KC, Nevitt MC. Vertebral fracture assessment using a semiquantitative technique. J Bone Miner Res 1993; 8(9):1137-1148.
25. Colao A, Ferone D, Marzullo P, Lombardi G. Systemic complications of acromegaly: epidemiology, pathogenesis, and management. Endocr Rev 2004; 25(1):102-152.
26. Dekkers OM, Biermasz NR, Pereira AM, Romijn JA, Vandenbroucke JP. Mortality in acromegaly: a metaanalysis. J Clin Endocrinol Metab 2008; 93(1):61-67.
27. Wassenaar MJ, Biermasz NR, Kloppenburg M et al. Clinical osteoarthritis predicts physical and psychological QoL in acromegaly patients. Growth Horm IGF Res 2010; 20(3):226-233.
28. Jackson SA, Tenenhouse A, Robertson L. Vertebral fracture definition from population-based data: preliminary results from the Canadian Multicenter Osteoporosis Study (CaMos). Osteoporos Int 2000; 11(8):680-687.
29. Klotzbuecher CM, Ross PD, Landsman PB, Abbott TA, III, Berger M. Patients with prior fractures have an increased risk of future fractures: a summary of the literature and statistical synthesis. J Bone Miner Res 2000; 15(4):721-739.
30. Mazziotti G, Bianchi A, Porcelli T et al. Vertebral fractures in patients with acromegaly: a 3-year prospective study. J Clin Endocrinol Metab 2013; 98(8):3402-3410.
31. Van Staa TP, Laan RF, Barton IP, Cohen S, Reid DM, Cooper C. Bone density threshold and other predictors of vertebral fracture in patients receiving oral glucocorticoid therapy. Arthritis Rheum 2003; 48(11):3224-3229.
32. Ueland T, Ebbesen EN, Thomsen JS et al. Decreased trabecular bone biomechanical competence, apparent density, IGF-II and IGFBP-5 content in acromegaly. Eur J Clin Invest 2002; 32(2):122-128.
33. Dall'Ara E, Schmidt R, Zysset P. Microindentation can discriminate between damaged and intact human bone tissue. Bone 2012; 50(4):925-929.
34. Bonds DE, Larson JC, Schwartz AV et al. Risk of fracture in women with type 2 diabetes: the Women's Health Initiative Observational Study. J Clin Endocrinol Metab 2006; 91(9):3404-3410.
35. Krakauer JC, McKenna MJ, Buderer NF, Rao DS, Whitehouse FW, Parfitt AM. Bone loss and bone turnover in diabetes. Diabetes 1995; 44(7):775-782.
36. Ross PD, Davis JW, Epstein RS, Wasnich RD. Pre-existing fractures and bone mass predict vertebral fracture incidence in women. Ann Intern Med 1991; 114(11):919-923.
37. Nevitt MC, Ross PD, Palermo L, Musliner T, Genant HK, Thompson DE. Association of prevalent vertebral fractures, bone density, and alendronate treatment with incident vertebral fractures: effect of number and spinal location of fractures. The Fracture Intervention Trial Research Group. Bone 1999; 25(5):613-619.
38. Black DM, Arden NK, Palermo L, Pearson J, Cummings SR. Prevalent vertebral deformities predict hip fractures and new vertebral deformities but not wrist fractures. Study of Osteoporotic Fractures Research Group. J Bone Miner Res 1999; 14(5):821-828.

Part B

The role of the GH / IGF-1 axis in primary OA

VII.

Relationship between insulin-like growth factor-1 and radiographic disease in patients with primary osteoarthritis: a Systematic Review.



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ABSTRACT

OBJECTIVE: To evaluate the association between radiographic osteoarthritis (OA) and either serum insulin-like growth factor-1 (IGF-1) levels or IGF-1 gene polymorphisms in patients with primary OA.

METHODS: We conducted a systematic review of reported associations between circulating IGF-1 and/or IGF-1 gene polymorphisms and radiographic OA. Studies were eligible when: 1) investigating serum IGF-1 and/or IGF-1 gene polymorphisms in relation to prevalent or incident radiographic OA; 2) written in English; 3) full-text article or abstract; 4) patients had primary OA in knee, hip, hand or spine; 5) longitudinal, case-control or cross-sectional design. Quality assessment was done using a standardized criteria set. Best-evidence synthesis was performed based on guidelines on systematic review from the Cochrane Collaboration Back Review Group, using five evidence levels: strong, moderate, limited, conflicting and no evidence.

RESULTS: We included 11 studies with more than 3000 primary OA cases. Data on the relationship between serum IGF-1 and radiographic OA were inconsistent. Adjustment for body mass index (BMI) was often omitted. Of four high-quality studies, three studies reported no association, one study found significantly higher IGF-1 levels in OA patients compared to controls. Patients with IGF-1 gene promoter polymorphisms and a genetic variation at the IGF-1R locus had an increased OA prevalence compared to controls.

CONCLUSIONS: Observational data showed no association between serum IGF-1 and occurrence of radiographic OA (moderate level of evidence), and a positive relationship between IGF-1 gene polymorphisms and radiographic OA (moderate level of evidence); however the confounding effect of BMI was insufficiently addressed. Future well-designed prospective studies should further elaborate the role of the complex GH/IGF-1 system in primary OA.

INTRODUCTION

Osteoarthritis (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, body mass index (BMI), hormonal and local biomechanical factors together with genetic predisposition play a role (1-5). In OA, the stable equilibrium between synthesis and breakdown of cartilage components is disrupted and the rate of loss of proteoglycans and other matrix components eventually exceeds their formation rate (6). Factors responsible for preservation or loss of the articular cartilage matrix are of considerable interest to clinicians and scientists. Among chondrotrophic growth factors, insulin-like growth factor-1 (IGF-1) and its response on mature adult cartilage has been studied extensively.

IGF-1 is an important growth promoting peptide with structural and functional homology to pro-insulin. IGF-1 is mainly produced by the liver and mediates the effects of growth hormone (GH) at tissue level. In addition, IGF-1 is a key factor for longitudinal skeletal growth (7). Serum IGF-1 concentrations in adulthood decrease progressively with age and are influenced by many other factors, such as BMI (8,9). Three lines of research suggest IGF-1 involvement in OA pathogenesis.

Firstly, 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* (10-20), also during cytokine exposure, which drives most predominating catabolic processes in cartilage. Furthermore, IGF-1 inhibits cytokine-stimulated degradation of proteoglycans directly in normal cartilage *in vitro* (21,22). These anabolic and protecting properties make IGF-1 an obvious candidate for a major role in cartilage repair.

Secondly, serum IGF-1 is elevated in acromegaly, a rare endocrine disease caused by GH overproduction from a pituitary adenoma. The GH excess results in high serum GH and IGF-1 levels and is associated with an increased risk of secondary OA (23,24). Prevalence and severity of arthropathy worsen with the duration of uncontrolled acromegaly. Furthermore, pre-treatment IGF-1 levels predict radiographic OA in a dose-dependent manner (25).

Finally, earlier studies showed that genes regulating formation, degradation and repair of articular cartilage and subchondral bone remodelling may be involved in OA pathogenesis (26-29). Probably, genetic variations of GH/IGF-1 genes with functional properties may affect the pathogenesis of primary OA.

Several epidemiological studies have explored the role of serum IGF-1 in primary OA, showing various results (30-33). We aimed to systematically review observational studies on the relationship between radiographic OA and either serum IGF-1 and IGF-1 gene polymorphisms in patients with primary OA, taking in account the variable quality of studies.

MATERIALS AND METHODS

IDENTIFICATION AND SELECTION OF THE LITERATURE: To identify studies investigating the relationship between IGF-1 and radiographic primary OA, we searched the following databases, up to May 2011: Medline, EMBASE, Web of Science, the Cochrane Library, ScienceDirect, CINAHL, LWW Journals, Wiley, Academic Search Premier and WHO International Clinical Trials Registration Platform. The following combined key words were used: ("Insulin-Like Growth Factor I" OR "IGF-1" OR "Insulin-Like Somatomedin Peptide I" OR "Somatomedin C" OR "IGF-I-SmC" OR "Receptor, IGF Type 1" OR "IGF-1 Receptor" OR "IGF 1 Receptor" OR "IGF-I Receptor" OR "IGF I Receptor" OR "IGF-1 Receptors" OR "IGF Type 1 Receptor" OR "Insulin-Like-Growth Factor I Receptor") AND ("Osteoarthritis" OR "Osteoarthritides" OR "Osteoarthrosis" OR "Osteoarthroses" OR "Degenerative Arthritides" OR "Degenerative Arthritis"). References of relevant articles were checked for additional articles.

A study was eligible for inclusion with the following criteria: (1) the association between serum IGF-1 levels and/or IGF-1 gene polymorphisms and prevalent or incident radiographic OA was investigated; (2) written in English; (3) full-text article or abstract; (4) patients had primary OA in knee, hip, hand or spine; (5) longitudinal, case-control or cross-sectional design. Incident OA was defined as the development of OA in joints that were previously unaffected during the specified time period in a longitudinal study. Prevalent OA was defined as the presence of OA at the moment of the study visit in a cross-sectional or case-control study.

A study was excluded if the studied population had a specific underlying pathology, such as trauma (fractures), infection, rheumatoid arthritis, or ankylosing spondylitis. Only human studies were evaluated.

DATA EXTRACTION AND ANALYSIS: Data extraction and eligibility were assessed by two independent investigators (K.M.J.A.C. and S.R.R.). Inconsistencies in data extraction were resolved by consensus. We used data comparing serum IGF-1 and/or presence of IGF-1 gene polymorphisms between patients with radiographic OA and controls without radiographic OA.

The following data were extracted: (1) study population (patient characteristics, population size, gender, age, BMI); (2) exposure (serum IGF-1 or IGF-1 gene polymorphisms), (3) outcome (methods of assessment of radiographic OA, reproducibility, blinding); (4) potential confounders (age, sex, BMI, height, race).

METHODOLOGICAL QUALITY ASSESSMENT : The quality of each included paper was assessed by two independent reviewers (K.M.J.A.C. and S.R.R.) using a standardized set of criteria (*Table 1*), which have been used previously in reviews on musculoskeletal disorders (34) and were modified to cover the topic of our review.

When the criterion was met in the article, '1' was given, otherwise '0'. A '0'was also given when no information was given about the specific criterion mentioned in the article. Several items are not applicable to certain types of study design and therefore do not contribute to the total score of that particular study. The maximum score (100%) for each study was based only on the items applicable to that particular study design (cohort and case-control 14, cross-sectional 11). Total scores per study were calculated as the percentage of maximum obtainable scores. For example, a quality score of 57% (8 / 14 x 100%) was assigned to a case-control study with 8 positive items. A study was considered high-quality (HQ) if the methodological quality score was ≥64%, chosen as the median of all quality scores.

Level of evidence synthesis: Because the observational studies were heterogeneous with regard to study population, methodological quality and determinants and measures of radiological OA assessment, we followed standard practice and refrained from statistically pooling the data and performed a 'best evidence' synthesis. A prospective cohort study was judged as the preferred design, followed by a case-control study, and then by a cross-sectional study. Subsequently, the studies were ranked according to their methodological quality score. The following ranking of the levels of evidence was formulated based on the guidelines on systematic review of the Cochrane Collaboration Back Review Group, a method to summarize evidence in observation studies with heterogeneous methodological study characteristics (*Table 2*) (35,36).

Table 1. List of criteria used for methodological quality assessment.

Item	Criterion	CH/CC/CS/GA*
Study population		
1.	Clear description of selection of study subjects.	CH/CC/CS/GA
2.	Sufficient description of characteristics of study groups.	CH/CC/CS/GA
3.	Cases and controls were drawn from the same source	CC
5.	population.	
	Participation rate ≥ 80% for cases/cohort.	CH/CC/CS/GA
	Participation rate ≥ 80% for controls.	CC
Assessment of risk factor: serum IGF-1 levels/IGF-1 gene polymorphism		
6.	Serum IGF-1/IGF-1 gene polymorphism was assessed with standardized or valid instruments.	CH/CC/CS/GA
7.	IGF-1 measurement/IGF-1 gene polymorphism assessment was identical in the studied population.	CH/CC/CS/GA
Radiographic OA assessment		
8.	Radiographic OA measures were valid.	CH/CC/CS/GA
9.	Presence of radiographic OA was assessed reproducibly.	CH/CC/CS/GA
10.	Radiographic OA was assessed identically in all patients.	CH/CC/CS/GA
Analysis and data presentation		
11.	Mean levels of IGF-1 (e.g. serum and/or synovial) were given.	CH/CC/CS
12.	Frequencies of radiographic OA were given.	CH/CCCS/GA
13.	Appropriate analysis techniques were used.	CH/CC/CS/GA
14.	Adjusted for at least age, sex and BMI.	CH/CC/CS/GA

OA=osteoarthritis. IGF-1=insulin-like growth factor 1.
** Applicable to CH=cohort study. CC=case-control study. CS=cross sectional study. GA=genetic association study.*

Table 2. Levels of evidence used in best-evidence synthesis.

Strong	Generally consistent findings were presented in multiple high-quality cohort studies.
Moderate	One high-quality cohort study and two or more high quality case-control studies, or when at least three high-quality case-control studies show generally consistent results.
Limited	Generally consistent findings were found in a single cohort study, or in one or two case- control studies or in multiple cross-sectional studies.
Conflicting	Less than 75% of the studies reported consistent findings.
No evidence	No study could be found.

Sensitivity analyses by defining other cut-offs (mean score of all studies instead of median) of HQ studies were performed. Furthermore, a sensitivity analysis was performed based on all positive studies, regardless of methodological quality. A study was regarded as positive if it showed a significant association between serum IGF-1 and radiographic OA.

RESULTS

IDENTIFICATION AND SELECTION OF THE LITERATURE: From initial 668 potentially relevant studies identified, 634 were excluded on the basis of title and abstract. 34 papers were retrieved for detailed assessment: 7 were excluded because no primary OA patients were included, 10 because OA was not assessed radiographically, and 3 were not original articles. One abstract appeared to be a duplicate of a full text article and was excluded. Another 2 studies were excluded because they studied OA progression and severity, respectively, and not prevalent or incident OA. Consequently, a total of 11 studies were included (*Figure 1*).

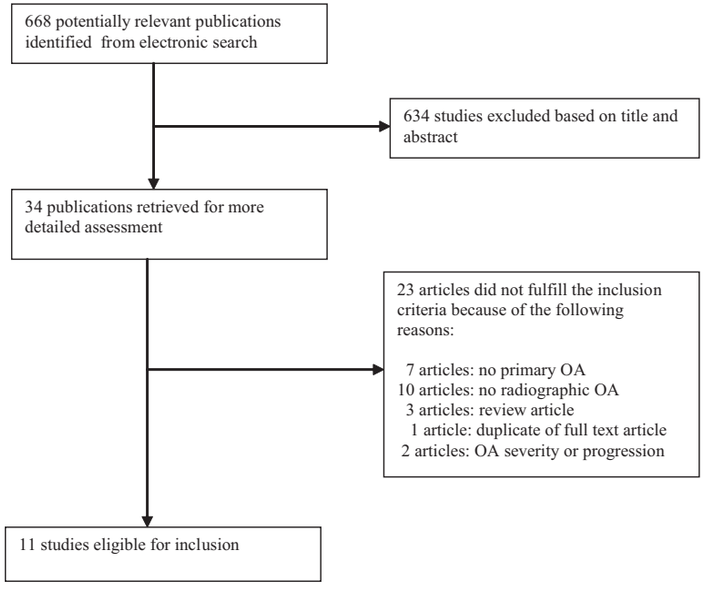

Figure 1. Flow diagram of study selection and exclusion stages.

STUDY DESCRIPTION: A detailed study description is given in *Tables 3a&3b*. These studies provided data on the relationship between serum IGF-1 and radiographic OA of knee (n=5) (30,33,37,38,40), hand (n=1) (32), OA in general (n=3) (31,39), and IGF-1 gene polymorphisms (n=3) (26-28). The studies were published from 1992 onwards. One had a nested case-control design; the other 10 were case-control/cross-sectional studies. All studies used a radio-immuno assay (RIA) for IGF-1 measurement in serum. Assays were generally done blinded to case status.

Most studies investigated a middle-aged population and three exclusively included women (31,32,40). The majority of studies adjusted for age and sex, two studies were matched (30,39). Three studies adjusted for at least three variables (26,28,38).

Six studies assessed radiographic OA according to the Kellgren&Lawrence (KL) score, and two studies used personal scoring systems for individual radiographic OA features (individual score by Scott *et al.* **(33)**, individual score by the principles of KL (30)). Two studies used both KL and a scoring method for individual OA characteristics. Two studies also assessed clinical OA; one by the American College of Rheumatology criteria (ACR) criteria for knee OA (37), the other used a question about knee pain from the Health and Nutrition Examination Survey (30).

METHODOLOGICAL QUALITY ASSESSMENT: The quality of studies was assessed according to the criteria stated in *Table 1* and methodological quality scores are shown in *Table 4*. The scores ranged from 14 to 82% of the maximum obtainable score for each study design, with a median score of 64%. Only four of the eight studies on serum IGF-1 and radiographic OA (one cohort study, three case-control/cross-sectional studies) and all three studies on IGF-1 gene polymorphisms were HQ studies.

Overall, selection of study subjects was not clearly described. In addition, none of studies described participation rates for cases and controls in detail. Reproducibility of radiological OA scoring was described in only three studies with kappa or intracorrelation coefficient. Three studies adjusted for age, sex and BMI; the other studies did not correct for BMI-related IGF-1 changes.

ASSOCIATION BETWEEN SERUM IGF-1 AND RADIOGRAPHIC OA IN PRIMARY OA PATIENTS: The largest study (Lloyd *et al.*, N=761) showed a positive association between high serum IGF-1 and radiographic knee OA (31). In this HQ case-control study, a clear dose-response relationship was shown with the strongest association in patients with bilateral and more

severe knee OA (p<0.0001). High IGF-1 levels were also associated with hand OA, especially in the distal interphalangeal (DIP) joint (p=0.006). Results were adjusted for age, weight and height. Fraenkel *et al.* reported a trend towards significance for higher serum IGF-1 in men with bilateral knee OA, in a HQ longitudinal study with a nested case-control design. Adjustments for age, sex, BMI, height and race were made (30).

In contrast, Denko *et al.* reported lower serum IGF-1 concentration in patients with general radiographic OA (39). In this low-quality (LQ) case-control study (N=111; *Tables 3a&3b*), patients and controls were matched on age, sex, race, height and weight.

Five studies (two HQ (33,38), three LQ (32,37,40)), all case-control studies, reported no association between serum IGF-1 and radiographic OA. Three studies (37,38,40) found no relation between serum IGF-1 and knee OA. In two cross-sectional studies (32,33), Hochberg *et al.* reported significantly lower IGF-1 levels in patients with radiographic hand and knee OA from the Baltimore Longitudinal Study of Aging cohort. However, after adjustments for age-related IGF-1 decline, results were no longer significant. None of these studies corrected for BMI.

By taking the mean of all methodological quality scores as cut-off point, a study was considered high-quality with a score ≥59%. Using this alternative cut-off point didn't change the results. The sensitivity analysis based on all positive studies without quality assessment, showed similar results with moderate evidence for no association between serum IGF-1 and radiographic OA, and a positive association between IGF-1 gene polymorphisms and radiographic OA.

GENETIC ASSOCIATION BETWEEN IGF-1 GENE POLYMORPHISMS AND RADIOGRAPHIC OA: Three studies, all HQ studies, provided data on the outcomes of genetic association studies, in which Single Nucleotide Polymorphisms (SNPs) in the IGF-1 and IGF-1 receptor genes were investigated in relation to radiographic OA. All studies were cross-sectional; one study contained only women from a Japanese population (27).

Meulenbelt *et al.* investigated polymorphisms in the promoter region of the IGF-1 gene in relation to radiographic OA. Nine different alleles were identified, of which allele 3 and 4 (A3, A4) were associated with a higher prevalence of radiographic OA at any joint site, mostly in the hip. Especially A3, which is less prevalent than A4, is from a population genetic point the most likely allele associated with radiographic OA. Subjects heterozygous for A3 had a 1.9 times increased risk of radiographic OA compared to wildtypes (adjustments for age, sex, BMI and BMD). A 3.6 times increased risk of radiographic OA was found in patients homozygous for A3, however non-significant (26).

Zhai *et al.* studied the 192bp allele of the IGF-1 promotor polymorphism in relation to radiographic OA in the knee, hip, hand and spine. Cases had at least one of the four joint sites affected. Previously, absence of 192bp (wildtype) allele was found to be associated with lower serum IGF-1 levels and lower height (41), indicating functional properties. In the overall study population, the polymorphism increased radiographic OA risk, although not statistically significant. In a subgroup aged ≤65years radiographic OA risk was significantly higher in SNP carriers (p=0.03 for trend), with highest prevalence in homozygotes (OR=1.9), suggesting an allele-dose effect. These findings were independent of age, sex, BMI and BMD (28).

Urano *et al.* studied a SNP at intron1 in the IGF-1 receptor gene (IVS1+ 14488C>G, rs11247361) in relation to radiographic features of spinal disc degeneration in healthy postmenopausal Japanese women, assessed semi-quantitatively by the Genant method. In this population-based study, the genetic variation was correlated with spinal disc narrowing (OR=2.0, p=0.0033), especially in homozygotes, suggesting a possible contribution of IGF-1R to human cartilage metabolism. Occurrence of endplate sclerosis and osteophytosis was comparable between SNP genotypes (27).

Table 3a. Study characteristics of studies on serum IGF-1 and radiographic OA.

Author, year (ref.)	N	Study design	Population	Sex	Age (years, mean±SD)	Assessment ROA Hand	Knee	Spine	Total	Adjustments Age	Sex	BMI	Height	Race	Results on IGF-1 & ROA	Quality Score
Hochberg, 1993 (32)	115	Case-control	Baltimore Longitudinal Study of Aging 60 hand OA 55 healthy controls	♀ 100%	53.6 ± 18.2	KL	-	-	-	Yes	NA	No	No	No	NS (P≥0.50)	57%
McAlindon, 1993 (30)	156	Case-control	Bristol Community Survey 78 knee OA 78 healthy controls	♀ 60% ♂ 40%	71.8 ± 7.6	-	TFJ& PFJ OP &JSN &SCL	-	-	Yes	Yes	No	No	No	NS (P 0.48)	64%
Denko, 1994 (39)	111	Case-control	57 generalized OA 54 healthy controls	♀ 68% ♂ 32%	♀ 66 ± 12 ♂ 68 ± 11	-	-	-	JSN& OP	Yes	Yes	No	Yes	Yes	Low IGF-1 P<0.001	43%
Hochberg, 1994 (33)	187	Case-control	Baltimore Longitudinal Study of Aging 59 knee OA 128 healthy controls	♀ 38% ♂ 62%	♀ 54 ± 18 ♂ 56 ± 17	-	KL	-	-	Yes	NA	No	No	No	NS	64%
Fernihough, 1996 (37)	27	Case-control	16 knee OA 11 healthy controls	♀ 44% ♂ 56%	µ69	-	ACR criteria	-	-	No	No	No	No	No	NS	29%
Lloyd, 1996 (31)	761	Case-control	Chingford study, 606 patients with OA at different locations 155 healthy controls	♀ 100%	54.2 ± 6	KL	KL	KL	-	Yes	NA	No	Yes	No	High IGF-1 & DIP P=0.006, knee P<0.001	79%
Fraenkel, 1998 (38)	423	Nested case-control	Framingham OA Study 202 knee OA 221 healthy controls	♀ 66% ♂ 34%	µ69.9	-	KL	-	-	Yes	Yes	Yes	Yes	Yes	NS ♀ OR 0.9 (0.6-1.7) ♂ OR 1.2 (0.6-2.6)	71%
Jubb, 1998 (40)	162	Case-control	74 polyarticular OA 37 knee OA 61 healthy controls	♀ 100%	NA	-	Not specified	-	Not specified	Yes	NA	No	No	No	NS	14%

OA=osteoarthritis. IGF1=insulin-like growth factor1. ROA=radiological osteoarthritis. ACR criteria=American College of Rheumatology criteria. KL=Kellgren&Lawrence. OP=osteophytosis. JSN=joint space narrowing. SCL=sclerosis. DIP=distal interphalangeal. PIP=proximal interphalangeal. CMC=carpometacarpal. NA=not applicable, NS=not significant. µ=mean. TFJ=tibiofemoral joints. PFJ=patellofemoral joint. N=number of studied patients. – =not assessed.

Table 3b. Study characteristics of genetic association studies.

Author, year (ref.)	N	Study design	Population	Sex	Age (years, mean±SD)	BMI (kg/m2, mean ±SD)
Meulenbelt, 1998 (26)	785	Cross-sectional	Rotterdam Study 651 OA cases 135 healthy controls	♀ 60% ♂ 40%	55-65	ROA+ 26.6±3.7 ROA- 25.1±3.1
Zhai, 2004 (28)	1575	Cross-sectional	Rotterdam Study 1355 OA cases 191 healthy controls	♀ 59% ♂ 41%	55-70	ROA+ 26.5±3.7 ROA- 25.0±3.3
Urano, 2008 (27)	434	Cross-sectional	Japanese population-based study 342 cases with disc narrowing	♀ 100%	66.5 ± 8.4	CC 22.3±2.8 GC 21.8±3.0 GG 22.6±3.2

Genetic test	Assessment ROA				Adjustments			Results on IGF-1 & ROA	Quality score
	Hand	Knee	Spine	Hip	Age	Sex	BMI		
IGF-1 gene promoter polymorphisms Allele 1-9 (A1-A9)	KL	KL	KL	KL	Yes	Yes	Yes	A3 at IGF-1 locus associated with ROA at any joint site. OR 1.9 (1.2-3.1)	82%
IGF-1 gene promoter polymorphism: absence of 192bp allele	KL	KL	KL	KL	Yes	Yes	Yes	Positive association, only in a subgroup≤65yr (p=0.03), allele-dose effect. OR 1.9 (1.1-3.3) for homozygotes	82%
C>G SNP (rs11247361) IGF-1R gene at intron1	NA	NA	OP, SCL, DN	NA	Yes	NA	No	Positive association (p=0.0033), mostly with disc narrowing. (OR 2.0, 1.3-3.3)	64%

Table 4. Methodological quality scores of the studies, according to study type.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Individual score	Total obtainable	Total score
Longitudinal (nested case-control)																	
Fraenkel, 1998	0	1	1	0	0	1	1	1	1	1	1	0	1	1	10	14	71%
Case-control																	
Lloyd, 1996	1	1	1	0	0	1	1	1	1	1	1	1	1	0	11	14	79%
McAlindon, 1993	0	1	1	0	0	1	1	0	1	1	1	1	1	0	9	14	64%
Hochberg, 1994	0	1	1	0	0	1	1	1	0	1	1	1	1	0	9	14	64%
Hochberg, 1993	0	1	1	0	0	0	1	1	0	1	1	1	1	0	8	14	57%
Denko, 1994	1	1	0	0	0	1	1	0	0	0	1	0	1	0	6	14	43%
Fernihough, 1996	0	0	0	0	0	1	1	0	0	0	1	0	1	0	4	14	29%
Jubb, 1998	0	0	0	0	0	1	1	0	0	0	0	0	0	0	2	14	14%
Cross-sectional																	
Meulenbelt, 1998	1	1	NA	0	NA	1	1	1	0	1	NA	1	1	1	9	11	82%
Zhai, 2004	1	1	NA	0	NA	1	1	1	0	1	NA	1	1	1	9	11	82%
Urano, 2008	0	1	NA	0	NA	1	1	1	0	1	NA	1	1	0	7	11	64%

Table 4: Each item was scored 1 if it met the methodological criteria listed in Table 1, if not or otherwise not described, a score of 0 was assigned. Positive scores were summed to give an overall internal validity score. NA=not applicable.

OA=osteoarthritis. SNP=single nucleotide polymorphism. IGF-1=insulin-like growth factor ERGO=Erasmus Rotterdam Gezondheid Ouderen. ROA=radiological osteoarthritis. PSM=postmenopausal. μ=mean. KL=Kellgren&Lawrence. PMS=postmenopausal. OP=osteophytosis. SCL, sclerosis. DN, disc narrowing. NS=not significant. NA=not applicable. N=number of studied patients. CC=wildtypes. GC=heterozygotes. GG=homozygotes.

DISCUSSION

A relationship between IGF-1 and radiographic primary OA is suggested from *in vitro* and anecdotal evidence, but is infrequently studied in observational studies. This is the first systematic review that summarizes current data on the relation between radiographic OA and either serum IGF-1 levels or IGF-1 gene polymorphisms, taking into account important factors influencing IGF-1 concentration, such as age, sex and BMI. Using strictly defined criteria, a total of only eight studies were included that studied serum IGF-1 in relation to radiographic OA. Based on these studies, we can conclude that there is moderate evidence for no association between serum IGF-1 and radiographic OA, although there is moderate evidence for a positive relationship between IGF-1 gene polymorphisms and radiographic OA.

Only the HQ study of Lloyd *et al.* showed high serum IGF-1 to be related with radiographic knee and hand OA, especially in bilateral and more severe knee OA (31). Fraenkel *et al.* reported a trend for higher serum IGF-1 levels in men with bilateral knee OA, although non-significant. In contrast, Denko *et al.* found low IGF-1 levels to be associated with general radiographic OA (39)**;** however, this study was ranked as low methodological quality. The remaining six studies (three HQ, three LQ) showed no significant relationship.

We were able to include three HQ studies (26-28) on genetic polymorphisms in the GH/IGF-1 pathway in relation to radiographic OA. All three showed associations between IGF-1 gene polymorphisms and OA, in accordance with the hypothesis that genetic variations in the GH/IGF-1 system may be involved in OA pathogenesis. The SNP in the IGF-1 gene promotor region studied in Zhai *et al.*, was earlier found to be associated with lower IGF-1 levels and lower body height, suggesting that chronic low IGF-1 levels play a role in the pathophysiology of primary OA (41). However, the exact role of the IGF-1 polymorphism in the GH/IGF-1 pathway has not yet been elucidated. The other SNPs (26,27) were not studied in relation to circulating IGF-1 levels. Furthermore, we have to take into account interactions between polymorphisms. Zhai *et al.* studied the interaction with a collagen type II A1 (COL2A1) polymorphism, which is associated with an extreme OA phenotype (29). Highest OA prevalence was found in individuals with both IGF-1 and COL2A1 risk phenotype, suggesting that the IGF-1 effect occurs in interaction with the COL2A1 gene (28).

Our review may suffer from several limitations. Firstly, the review is based mainly on cross-sectional data and therefore the strength of evidence is limited by the quality of the available studies. Unfortunately, only one longitudinal study (nested case-control study) (38), investigated OA incidence in relation to serum IGF-1. There was an additional longitudinal study (44) on IGF-1 and radiographic OA, though this study focuses on OA progression and was therefore excluded. In this 12-year follow-up study, Schouten *et al.* found high serum IGF-1 levels to be a risk factor for knee OA progression, especially of osteophytosis, independently of age, sex and BMI. Because the progression data could underline findings on prevalent and incident OA, more research is needed on the relationship between serum IGF-1 and primary OA progression. Secondly, we cannot rule out publication bias, especially in the genetic field, which can be explained by a selection of positive studies for publication. Thirdly, small study sizes may influence the findings, especially since the IGF-1 concentration is affected by many factors that also influence OA risk. Finally, there is a possibility of misclassification of OA by inclusion of conventional radiographs only, which may contribute to not finding a consistent association between IGF-1 and radiographic OA. Magnetic resonance imaging (MRI) may give additional information to plain films**,** however at this moment no MRI studies have investigated the relationship between IGF-1 and OA.

Several sources of between-study heterogeneity can be identified. First of all, the study design can lead to heterogeneity. Secondly, assessing the role of IGF-1 in OA by serum samples is very complex, because individual IGF-1 levels are liable to temporal variations. Furthermore, IGF-1 levels are inversely correlated to age, estrogen levels and BMI (9,42,43), so adjustments for these factors are essential for interpretation of IGF-1 levels. Most studies corrected for age and sex, however, only three adjusted for BMI. In addition, RIAs for measuring serum IGF-1 are subject to analytical difficulty. This means that large sample sizes are required for demonstrating an IGF-1 effect on radiographic OA at population level; unfortunately, only the study of Lloyd *et al.* (31) reported a large sample size (N=761). Another remaining problem is OA case definition. For epidemiological studies, there is general consensus that radiological features are the preferred method. However, the included studies used different scoring methods (KL, Ahlback, ACR criteria) at different joint sites.

Because of heterogeneity of several study aspects, no meta-analysis could be performed. We provided an alternative by performing a best-evidence synthesis and methodological quality assessment, although no

generally accepted criteria set exists for methodological quality assessment in observational studies and for performing a best-evidence synthesis. Lievense *et al.* presented a reproducible criteria list for methodological quality assessment, which we modified to cover our review topic (36). The use of quality scores could be a technical limitation of our review at the same time, because using different criteria sets could result possibly in different interpretation of our results. According to our criteria set (*Tables 3a&3b*), four of the eight studies on serum IGF-1 and all three genetic association studies were considered as HQ studies.

Knowledge of the association between serum IGF-1 levels and radiographic OA is very important, because this could increase our understanding of the pathogenesis of OA, which subsequently could lead to development of new treatment options.

An important question that needs further research is whether circulating total IGF-1 levels truly reflect tissue concentrations. Preliminary data indicate that measuring free unbound IGF-1 levels and IGF-1 bioactivity better reflect GH/IGF-1 status than serum total IGF-1 (45-47). Free IGF-1 is the major biologically active form of IGF-1; however, because of methodological difficulties in measurement, total serum IGF-1 is often used to assess GH/IGF-1 axis activity (45). This could possibly explain not finding a consistent effect of total IGF-1 on radiographic OA.

In conclusion, *in vitro* studies, genetic association studies and the high secondary risk in pathological IGF-1 states suggest all an effect of the GH/IGF-1 system in OA development. Although the largest cross-sectional study (31) and the longitudinal study on OA progression (44) found a positive association with high serum IGF-1, inconsistent results were shown in studies on serum IGF-1 and radiographic OA. This systematic review concludes that there is moderate evidence that IGF-1 is no risk factor for primary radiographic 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.

REFERENCE LIST

1. Felson DT, Lawrence RC, Dieppe PA, Hirsch R, Helminck CG, et al. Osteoarthritis: new insights. Part 1: the disease and its risk factors. Ann Intern Med 2000;133 (8):635-46.
2. Peyron JG. Osteoarthritis. The epidemiologic viewpoint. Clin Orthop 1986;213:13-19.
3. Spector TD, Cicuttini F, Baker J, Loughlin J, Hart DJ. Genetic influences on osteoarthritis in women: a twins study. BMJ 1996;312:940-944.
4. Kellgren JH, Lawrence S, Bier F. Genetic factors in generalized osteoarthrosis. Ann Rheum Dis 1963;22:237-253.
5. Hart DJ, Spector TD. The relationship of obesity, fat distribution and osteoarthritis in women in the general population: the Chingford Study. J Rheumatol 1993;20(2):331-335.
6. George E, Sharif M, Dieppe P. Editorials, Insulin-like Growth Factor 1 and biochemical markers of Osteoarthritis. British Journal of Rheumatology 1993;32:265-268.
7. LeRoith, moderator. Insulin-like growth factors in health and disease. Ann Intern Med 1992; 116:854-862.
8. Copeland KC, Colletti RB, Devlin JT, McAuliffe TL. The relationship between insulin-like growth factor-I, adiposity, and aging. Metabolism 1990 Jun;39(6):548-7.
9. Juul A. Serum levels of insulin-like growth factor I and its binding proteins in health and disease. Growth Hormone & IGF Research 2003;13:113–170.
10. Guenther HL, Guenther HE, Frosch ER, Flisch H. Effect of insulin-like growth factor on collagen and glycosaminoglycan synthesis by rabbit chondrocytes in culture. Experientia 1982;38:979-981.
11. McQuillan DJ, Handley CJ, Campbell MA, Bolis S, Milway VE, Herington AC. Stimulation of proteoglycan biosynthesis by serum and insulin-like growth factor-I in cultured bovine articular cartilage. Biochem J 1986;240:423-430.
12. Vetter U, Zapf J, Heit W, Helbing G, Heinze E, et al. Human fetal and adult chrondrocytes: effect of insulin like growth factors I and II, insulin, and growth hormone on clonal growth. J Clin Invest 1986;77:1903-1908.
13. Luyten FP, Hascall VC, Nissley SP, Morales TI, Reddi AH. Insulin-like growth factors maintain steady state metabolism of proteoglycans in bovine articular cartilage explants. Arch Biochem Biophys 1988;267:416-425.
14. Makower AM, Wroblewski J, Pawlowski A. Effects of IGF-I, ECF, and FGF on proteoglycans synthesized by fractionated chondrocytes of rat rib growth plate. Exp Cell Res 1988;179:498-506.
15. Sandell LJ, Dudek E. IGF-I stimulates type II collagen gene expression in cultured chondrocytes. Orthop Trans 1988;13:300.
16. Osborne KD, Trippel SB, Mankin HJ. Growth factor stimulation of adult articular cartilage. J Orthop Res 1989;7:35-42.
17. Trippel SB, Corvol MT, Dumontier MF, Rappaport R, Hung HH, Mankin HJ. Effect of somatomedin-C/insulin-like growth factor I and growth hormone on cultured growth plate and articular chondrocytes. Pediatr Res 1989;25:76-82.
18. Barone-Varelas J, Schnitzer TJ, Meng Q, Otten L, Thonar E. Age related differences in the metabolism of proteoglycans in bovine articular cartilage explants maintained in the presence of insulin-like growth factor-1. Connect Tissue Res 1991;26:101-120.
19. Morales TI. Polypeptide regulators of matrix homeostasis in articular cartilage, Articular Cartilage and Osteoarthritis. Edited by Kuettner K, Schleyerbach R, Peyron JG, Hascall VC. New York, Raven Press 1992.
20. Schoenle E, Zapf J, Humbel RE, Froesch ER. Insulin-like growth factor I stimulates growth in hypophysectomized rats. Nature 1982;96:252-3.
21. Tyler J. Insulin-like growth factor 1 can decrease degradation and promote synthesis of proteoglycan in cartilage exposed to cytokines. Biochem J 1989;260:543-548.
22. Fosang AJ, Typler JA, Hardingham TE. Effect of IL-1 and IGF-1 on the release of proteoglycan components and hyaluronan from pig articular cartilage in explant culture.s. Matrix 1991;11:17-24.
23. Barkan AL. Acromegalic arthropathy. Pituitary 2001;4;263-264.
24. Layton MW, Fudman EJ, Barkan A, Braunstein EM, Fox IH. Acromegalic arthropathy. Characteristics and response to therapy. Arthritis Rheum 1998;31:1022-1027.
25. Biermasz NR, Wassenaar MJ, Van der Klaauw AA, Pereira AM, Smit JW, Roelfsema F, et al. Pretreatment insuline-

like growth factor-I concentrations predict radiographic osteoarthritis in acromegalic patients with long-term cured disease. J Clin Endocrinol Metab 2009;94:2374-9.
26. Meulenbelt I, Bijkerk C, Miedema HS, Breedveld FC, Hofman A, Valkenburg HA, et al. A genetic association study of the IGF-1 gene and radiological osteoarthritis in a population-based cohort study (the Rotterdam Study). Ann Rheum Dis 1998;57(6):371-374.
27. Urano T, Narusawa K, Shiraki M, Usui T, Sasaki N, Hosoi T, et al. Association of a single nucleotide polymorphism in the insulin-like growth-factor-1 receptor gene with spinal disc degeneration in postmenopausal Japanese women. Spine 2008;33(11):1256-1261.
28. Zhai G, Rivadeneira F, Houwing-Duistermaat JJ, Meulenbelt I, Bijkerk C, Hofman A, et al. Insulin-like growth factor I gene promoter polymorphism, collagen type II alpha1 (COL2A1) gene, and the prevalence of radiographic osteoarthritis: the Rotterdam Study. Ann Rheum Dis 2004;63(5):544-548.
29. Bijkerk C. Genetic epidemiology of OA. In: Association of the COL2A1 gene with radiological OA in a population-based cohort study: the Rotterdam Study. Erasmus University Rotterdam: Rotterdam, 1999:61-76.
30. McAlindon TE, Taele JD, Dieppe PA. Levels of insulin related growth factor 1 in osteoarthritis of the knee. Ann Rheum Dis 1993;52(3):229-231.
31. Lloyd ME, Hart DJ, Nandra D, McAlindon TE, Wheeler M, et al. Relation between insulin-like growth factor-I concentrations, bone density, and fractures in general population: the Chingford study. Ann Rheum Dis 1996;55(12):870-874.
32. Hochberg MC, Lethbridge-Cejku M, Scott Jr WW, Plato CC, Tobin JD. Decreased serum levels of insulin-like growth factor-1 in women with osteoarthritis of the hand: data from the Baltimore Longitudinal Study of Aging (BLSA). Osteoarthritis and Cartilage 1993;1(1):17.
33. Hochberg MC, Lethbridge-Cejku M, Scott Jr WW, Reichle R, Plato CC, Tobin JD. Serum levels of insulin-like growth factor in subjects with osteoarthritis of the knee. Data from the Baltimore Longitudinal Study of Aging. Arthritis and Rheumatism 1994;37(8):1177-1180.
34. Lievense AM, Bierma-Zeinstra SMA, Verhagen AP, Verhaar JAN, Koes BW. Influence of work on the development of osteoarthtritis of the hip: a systematic review. J Rheumatology 2001;28:2520-8.
35. Van Tulder M, Furlan A, Bombardier C, et al. Updated method guidelines for systematic reviews in the cochrane collaboration back review group. Spine 2003;28:1290-9.
36. Lievense AM, Bierma-Zeinstra SMA, Verhagen AP, van Baar ME, Verhaar JAN, Koes BW. Influence of obesity on the development of osteoarthritis of the hip: a systematic review. Rheumatology 2002;41:1155–1162.
37. Fernihough JK, Billingham ME, Cqyfan-Hughes S, Holly JM. Local disruption of the insulin-like growth factor system in the arthritic joint. Arthritis Rheum 1996;39(9):1556-1565.
38. Fraenkel L, Zhang Y, Trippel SB, McAlindon TE, LaValley MP, Assif A, et al. Longitudinal analysis of the relationship between serum insulin-like growth factor-I and radiographic knee osteoarthritis. Osteoarthritis and Cartilage 1998;6(5):362-367.
39. Denko CW, Boja B, Moskowitz RW. Growth promoting peptides in osteoarthritis and diffuse idiopathic skeletal hyperostosis – Insulin, Insulin-like growth factor-I, growth hormone. J Rheumatol. 1994;21(9):1725-1730.
40. Jubb RW. Cartilage growth factors and post-menopausal osteoarthritis. British Journal of Rheumatology 1998:27(suppl1):21.
41. Vaessen N, Heutink P, Janssen JA, Witteman JC, Testers L, Hofman A, et al. A polymorphism in the gene for IGF-I: functional properties and risk for type 2 diabetes and myocardial infarction. Diabetes 2001 Mar;50(3):637-42.
42. Ursavas A, Karadag M, Ilcol YO, Ercan I, et al. Low level of IGF-1 in obesity may be related to obstructive sleep apnea syndrome. Lung 2007;185(5):309-1
43. Miller KK, Biller BM, Lipman JG, Bradwin G, Rifai N, Klibanski A. Truncal adiposity, relative growth hormone deficiency, and cardiovascular risk. J Clin Endocrinol Metab 2005;90(2):768-74.
44. Schouten JS, Van den Ouweland FA, Valkenburg HA, Lamberts SW. Insulin-like growth factor-1: a prognostic factor of knee osteoarthritis. Br J Rheumatol 1993;32(4):274-280.
45. Janssen JA, van der Lely AJ, Lamberts SW. Circulating free insulin-like growth-factor-I (IGF-I) levels should also be measured to estimate the IGF-I bioactivity. J Endocrinol Invest 2003;26(6):588-94.
46. Brugts MP, Ranke MB, Hofland LJ, van der Wansem K, Weber K, Frystyk J, et al. Normal values of circulating insulin-like growth factor-1 bioactivity in the healthy population: comparison with five widely used IGF-1 immunoassays. J Clin Endocrinol Metab 2008;93(7):2539-45.
47. Varewijck AJ, Lamberts SWJ, Uitterlinden P, Hofland LJ. IGF-1 bioactivity more accurately reflects growth hormone deficiency than total IGF-1. Endocr Rev 2001;32:OR 16-2.

VIII.

High serum Insulin-like Growth Factor-1 (IGF-1) levels are associated with the presence of primary osteoarthritis, but not with radiographic progression: the GARP Study

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Submitted

ABSTRACT

BACKGROUND: Several studies point towards a role of the growth hormone (GH)/insulin-like growth factor-1 (IGF-1) axis in the pathophysiology of primary osteoarthritis (OA). Few epidemiological studies investigated the relationship between serum IGF-1 levels and radiographic OA, reporting inconsistent results.

OBJECTIVE: To investigate the relationship between serum IGF-1 and the presence and progression of radiographic OA in patients with a severe familial form of OA.

METHODS: We studied 340 subjects from the GARP (Genetics, osteoARthritis and Progression) Study with symptomatic OA at multiple joint sites on a familial base. Serum IGF-1 concentrations were measured and expressed as standard deviation scores (SDS), adjusted for age and sex. Radiographs of hands, knees and hips were obtained. Joint space narrowing (JSN) and osteophytes were scored semi-quantitatively using the Osteoarthritis Research Society (OARSI) atlas. Radiographic OA progression was defined as change ≥1 in osteophyte and/or JSN score. Generalized Estimated Equations (GEE) analysis was performed to adjust for intra-patient and intra-family relationships, with additional adjustments for age, sex, and BMI.

RESULTS: Mean (±SD) IGF-1 SDS was +0.79±1.39, which was significantly increased when compared to reference control values ($p<0.01$). Baseline IGF-1 SDS was not associated with the presence of radiographic OA, at none of the investigated joints. In addition, no association was found between IGF-1 SDS and radiographic OA progression or worsening of individual radiographic features after 2 years of follow-up.

CONCLUSIONS: Mean IGF-1 SDS was increased in patients with familial and symptomatic OA at multiple sites when compared to reference values. In this cohort, consisting exclusively of systemically affected patients, no relationship was found between IGF-1 SDS and either the presence or progression of radiographic OA. Further study has to clarify the complex role of the GH/IGF-1 axis in OA pathophysiology.

INTRODUCTION

Primary osteoarthritis (OA) is a debilitating disease, characterized by progressive degradation of articular cartilage and bone remodeling. Etiology is known to be multifactorial, in which age, body mass index (BMI), genetic, hormonal and local biomechanical factors play a role (1-3). With respect to the hormonal system, the interesting role of growth factors in this process is not elucidated. Insulin-like Growth Factor-1 (IGF-1) is the main factor involved in longitudinal skeletal growth (4). Growth Hormone (GH) secreted by the pituitary stimulates IGF-1 secretion mainly at the liver, but also by other tissues. IGF-1 mediates the effects of GH at the tissue level. Serum IGF-1 concentrations are age-dependent, being highest during puberty and adolescence, and declining thereafter with aging. In addition, they are influenced by many other factors, such as estrogens and BMI (5;6).

Several lines of research suggest a role of the GH/IGF-1 axis in the pathogenesis of primary OA. First, IGF-1 enhances chondrocyte proliferation and proteoglycan and collagen synthesis (7;8). Second, genetic functional variations of GH/IGF-1 genes, such as the exon 3 deletion of the GHR (d3-GHR), are associated with primary OA (9-12). The d3-GHR polymorphism is associated with an increased signal transduction, resulting in an enhanced GH responsiveness of the GHR and, thereby, increasing GH/IGF-1 activity. Third, in pathologically high GH/IGF-1 conditions, which is the case in acromegaly, a rare endocrine disease caused by a GH-producing pituitary adenoma, there is a strongly increased risk to develop secondary OA (13). In acromegaly, GH/IGF-1 activity (at diagnosis) has been associated with radiographic severity and progression of arthropathy (14-16), further supporting the hypothesis of GH/IGF-1 axis involvement in OA pathophysiology. Fourth, recent GWAS data show genetic evidence for significant association between height, being largely dependent on IGF-1 concentrations during the process of longitudinal skeletal growth, and OA, being suggestive of a common genetic etiology (17).

Recently, in a systematic review summarizing the evidence for association between serum IGF-1 and primary OA, results among studies were inconsistent (18). However, sample sizes were small and sex and BMI, both important factors for the interpretation of IGF-1 levels, were not taken into account in most studies. Moreover, only two studies had a longitudinal design enabling to study OA progression (19;20).

Based on the evidence in literature, we hypothesize that an increased GH/IGF-1 activity, as reflected by increased serum IGF-1 levels, increases the risk of primary OA. We, therefore, investigated the relationship between serum IGF-1 and the presence and progression of radiographic OA in a well-characterized OA population with a severe OA phenotype.

MATERIALS AND METHODS

PATIENTS: The GARP (Genetics, osteoARthritis and Progression) Study is a prospective cohort study, aimed at identifying determinants of OA susceptibility and progression. The GARP Study consists of 192 sib pairs (N=384) between 40 and 70 years of age of Dutch ancestry, all having symptomatic OA at multiple sites in the hands or in ≥2 of the following joint sites: hand, spine (cervical or lumbar), knee, or hip. Patients with other (secondary) forms of OA were excluded. Details of recruitment and patient selection have been reported elsewhere (21). Sib pairs with at least one subject with symptomatic knee or hip OA (but not in a radiographic end-stage OA) were eligible for the 2-year follow-up visit, resulting in the inclusion of 105 eligible sib pairs (N=210)(22). The GARP Study was approved by the Medical Ethics Committee of the Leiden University Medical Center.

IGF-1 MEASUREMENT: Baseline blood samples were drawn and stored. Serum IGF-1 concentrations (nmol/l) were measured using an immunometric technique on an Immulite 1000 (Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA), since February 2006. The lower limit of detection was 25ng/ml and the inter-assay variation was 6.4%–3.8% in the range of 60–400ng/ml. In order to adjust IGF-1 levels for age and sex, which is common practice in the evaluation of endocrine diseases, IGF-1 levels were expressed as standard deviation scores (SDS), using lambda-mu-sigma smoothed (LMS) reference curves based on the widely accepted method of Rikken and Cole (23;24), with a normal IGF-1 SDS reference range between -2 and +2 SDS. Serum IGF-1 measurement was successfully performed in 340 patients.

RADIOGRAPHIC ASSESSMENT: Conventional radiographs of the hands, knees, hips, and spine were obtained according to a standardized protocol, as previously described in detail (21). Radiographic OA was scored according to the Kellgren-Lawrence (KL) scale by a single experienced musculoskeletal radiologist (H.K.), as described in detail elsewhere. ICCs were good (21;25). Radiographic OA was defined according to the ROA score, which is described in detail elsewhere (26). Radiographic knee and hip OA were defined as KL≥2

in, respectively, at least one knee or hip. Radiographic OA in the hand was defined as KL≥2 in at least 3 of the following joints: distal interphalangeal joint (DIP) 2–5, proximal interphalangeal joint (PIP) 2–5, interphalangeal joints 1, and carpometacarpal (CMC) joint 1. Spinal disc degeneration was defined as KL≥2 at at least 3 levels.

Individual OA features, being joint space narrowing (JSN) and osteophytes were assessed semi-quantitatively (Grade 0-3), using the Osteoarthritis Research Society (OARSI) atlas, in consensus opinion of two experienced readers (26). OARSI≥1 reflects presence of JSN or osteophytes. The reproducibility for JSN and osteophyte grading was based on the repeat reading of 20 radiographs (randomly selected throughout the study period) and was depicted by the intra-class correlation coefficient (ICC). ICCs for, respectively, JSN and osteophytes were 0.92 and 0.98 in the hands, 0.88 and 0.87 in the knees and 1.00 and 1.00 in the hips.

To assess radiographic OA progression, baseline and 2-year radiographs of the knee, hip, hands, and spine were scored in consensus paired without knowledge of chronological order, blinded for patient characteristics, for osteophytes and JSN by two experienced readers, using the OARSI atlas. Progression was defined as the change in JSN or osteophyte scores above the smallest detectable change (SDC), reflecting the change above measurement error (27;28). Since the SDCs for osteophytes and JSN were respectively 0.22 and 0.71 in the knee, 0.00 and 0.00 in the hip, and 0.35 and 0.62 in the hand, a change ≥1 in osteophyte/JSN score was defined as progression at all sites. Also joints (without end-stage (Grade 3) OA at baseline) replaced by a knee or hip prosthesis during follow-up, were considered to have radiographic progression.

STATISTICAL ANALYSIS: SPSS version 20.0 (SPSS inc., Chicago, IL, USA) was used for data analysis. Mean IGF-1 levels were compared between males and females using an Independent Samples T test. To compare mean IGF-1 SDS between GARP patients and 906 healthy literature controls from Rikken *et al.*(24), we performed a pooled variance *T* test, since sample sizes differ substantially between both groups. Mean baseline IGF-1 SDS was studied in relation to presence of radiographic OA at different sites, using a One-way ANOVA test. To assess the risk of radiographic OA at a given IGF-1 SDS, a Generalized Estimated Equations (GEE) analysis was carried out, thereby adjusting for intra-patient and intra-familial effects in GARP. A binary logistic model was used, stratified for sex, with presence of radiographic OA as dependent variable and age, BMI, and IGF-1 SDS divided into tertiles (based on the distribution in the patients) as covariates.

In a subset of patients with available follow-up radiographs, baseline IGF-1 SDS (tertiles) was studied in relation to progression of osteophytes and JSN over 2 years. We performed a GEE-analysis with a binary logistic model to take into account intra-patient and intra-family relationships, with additional adjustments for age, sex, BMI, and radiographic score at baseline.

RESULTS

PATIENT DESCRIPTION: Complete set of radiographs and successful serum IGF-1 measurements were available of 340 patients with symptomatic OA at multiple joint sites (82% female, of which 9.6% premenopausal). Baseline characteristics are shown in *Table 1*. Radiographic knee, hip, hand, and spine OA were, respectively, present in 37%, 26%, 74%, and 89% of the females, and in 38%, 28%, 70%, and 83% of the males. Eight patients from separate families had a unilateral knee replacement; one patient had bilateral knee replacements. In the hip, 22 patients had a unilateral prosthesis and fifteen patients had bilateral prostheses. Mean serum IGF-1 level was 18.2±6.3 nmol/l, being significantly higher in males than in females (p=0.013). As expected, IGF-1 levels were negatively correlated with age (Pearson correlation coefficient r=-0.119, p=0.030) and BMI (r=-0.162, p=0.003).

RELATIONSHIP BETWEEN SERUM IGF-1 SDS AND RADIOGRAPHIC OA: Mean IGF-1 SDS among GARP patients was +0.79±1.39 (95%CI +0.64;+0.93), with 67 patients (20%) above +2 SDS. When compared to 906 literature controls, mean IGF-1 SDS was significantly increased in GARP patients (p<0.01). Mean IGF-1 SDS did not differ between specific joint sites with radiographic OA. In addition, no dose-response relationship between baseline IGF-1 SDS and the presence of radiographic OA was observed, at none of the investigated joint sites, although in females a trend towards associations between higher baseline IGF-1 SDS and both knee and discus OA was observed (*Table 2*). No significant differences were seen between males and females or between patients <65 years and ≥65 years.

RELATIONSHIP BETWEEN SERUM IGF-1 SDS AND PROGRESSION OF RADIOGRAPHIC OA OVER 2 YEARS OF FOLLOW-UP: IGF-1 SDS and complete baseline and 2-yr follow-up radiographs were available in 174 patients (80% female). Radiographic progression of respectively knee, hip, and hand OA was seen in 37 (21.3%), 17 (9.8%) and 60 (34.5%) patients, being comparable between males and females. Baseline IGF-1 SDS was not related to radiographic progression of hip, knee or hand OA (*Table 3*), or worsening of individual radiographic features (*i.e.* osteophyte growth or joint space loss) (*data not shown*).

Table 1. Baseline characteristics of GARP subjects (N = 340)

Clinical characteristics	**Females* (N= 280)**	**Males (N= 60)**
Age, years	59.6 ± 7.5	60.1 ± 6.8
BMI, kg/m^2	27.0 ± 4.9	27.2 ± 3.4
Radiographic OA		
Knee (N (%))	103 (37%)	23 (38%)
Hip (N (%))	72 (26%)	17 (28%)
Hand (N (%))	206 (74%)	42 (70%)
Spine (N (%))	248 (89%)	50 (83%)
IGF-1 (nmol/l)	17.8±6.3	20.1±6.3
IGF-1 SDS	0.69±1.40	1.23±1.26

Data are shown as mean±SD, unless mentioned otherwise, and were stratified for sex. Radiographic OA was defined as ROA≥1, based on KL≥2(26). Spine OA is defined as radiographic disc degeneration of the spine.
**, 27 of the 280 studied females (9.6%) were premenopausal.*

GARP, Genetics, osteoARthritis and Progression Study; N, number of patients; OA, osteoarthritis; BMI, body mass index; IGF-1, Insulin-like Growth Factor-1; SDS, standard deviation score; KL, Kellgren-Lawrence score.

Table 2. Risk of having radiographic OA according to tertiles of IGF-1 SDS for, respectively, 60 male and 280 female patients with familial OA at multiple joint sites

Males

	Radiographic OA risk: adjusted OR (95%CI)*			
IGF-1 SDS (tertiles)	**Knee (N=23)**	**Hip (N=17)**	**Hand (N=42)**	**Discus (N=50)**
1	1.0	1.0	1.0	1.0
2	0.7 (0.2-2.4)	0.8 (0.2-3.8)	0.3 (0.1-1.6)	0.2 (0.0-2.7)
3	0.6 (0.2-2.3)	0.9 (0.2-3.8)	0.3 (0.1-1.5)	0.2 (0.0-2.5)

Females

	Radiographic OA risk: adjusted OR (95%CI)*			
IGF-1 SDS (tertiles)	**Knee (N=103)**	**Hip (N=72)**	**Hand (N=206)**	**Discus (N=248)**
1	1.0	1.0	1.0	1.0
2	1.6 (0.9-3.0)	0.4 (0.2-0.9)	0.8 (0.4-1.6)	1.4 (0.5-3.8)
3	1.7 (0.9-3.2)	0.8 (0.4-1.6)	0.7 (0.3-1.4)	2.8 (1.0-8.3)

** IGF-1 SDS at the time of the baseline study visit was divided into tertiles (based on the distribution among patients). A Generalized Estimating Equations (GEE) analysis was performed to adjust for intra-patient and intra-familial effects, stratified for sex. A binary logistic model was used with radiographic OA as outcome parameter and IGF-1 SDS tertiles, age, and BMI as covariates. The lowest IGF-1 SDS tertile was used as reference level.*

N, number of patients with radiographic OA according to Kellgren-Lawrence ≥2; OR, odds ratio; CI, confidence interval; OA, osteoarthritis; IGF-1, Insulin-like Growth-Factor 1; BMI, body mass index; Disc, disc degeneration.

Table 3. IGF-1 SDS at baseline were not related to radiographic progression of knee, hip or hand OA over 2 years of follow-up in patients with familial OA at multiple joint sites (N = 174)

	Number of patients (N=174)		
Knee OA**			
IGF-1 SDS (tertiles)	**With progression (N=37)**	**Without progression (N=137)**	**Adjusted OR (95%CI)***
1	11	44	1.0
2	14	47	1.3 (0.5-3.3)
3	12	46	1.1 (0.4-2.7)
Hip OA**			
IGF-1 SDS (tertiles)	**With progression (N=17)**	**Without progression (N=157)**	**Adjusted OR (95%CI)***
1	5	50	1.0
2	10	51	0.7 (0.2-2.4)
3	2	56	0.4 (0.1-1.6)
Hand OA**			
IGF-1 SDS (tertiles)	**With progression (N=60)**	**Without progression (N=114)**	**Adjusted OR (95%CI)***
1	20	35	1.0
2	21	40	0.6 (0.2-1.3)
3	19	39	0.7 (0.3-1.7)

** IGF-1 SDS at the time of the baseline visit was divided into tertiles (based on the distribution among patients). A Generalized Estimating Equations (GEE) analysis was performed to adjust for intra-patient and intra-familial effects. A binary logistic model was used with radiographic OA progression as dependent variable and IGF-1 SDS tertiles, age, sex, BMI, and radiographic score at baseline as covariates. The lowest IGF-1SDS tertile was used as reference level.*

*** Radiographic OA progression was defined as an increase of ≥ 1 in OARSI score for JSN and/or osteophytes.*

N, number of patients; OR, odds ratio; CI, confidence interval; IGF-1, insulin-like growth-factor 1; SDS, standard deviation score; BMI, body mass index.

DISCUSSION

In the present longitudinal study, we investigated the association between serum IGF-1 and, respectively, the presence and progression of radiographic OA in patients with familial OA at multiple joint sites from the GARP Study. Mean IGF-1 SDS in OA patients was significantly higher than in reference values from controls, indicating that, overall, serum IGF-1 concentrations are high. When looking at specific joint sites, however, we did not observe a relationship between IGF-1 SDS and the presence of radiographic OA. In addition, baseline IGF-1 SDS was not related to radiographic OA progression over 2 years or worsening of individual OA features.

IGF-1 is an important growth promoting polypeptide belonging to the same family of growth factors as insulin (4). IGF-1 is mainly produced by the liver and mediates the effects of GH in many organs, primarily by the IGF-1 receptor type 1. Serum IGF-1 concentrations reflect the GH concentrations over 24 hours and IGF-1 bioavailability is regulated by IGF-1 binding proteins. Serum IGF-1 concentrations in adulthood decrease progressively with age and are influenced by many other factors, such as estrogen and BMI (5;6). In clinical practice, serum IGF-1 levels are converted into IGF-1 SDS in order to standardize IGF-1 levels for age and sex.

Several functional genetic variants involved in the process of endochondral ossification, being the main process in longitudinal growth, are identified to be associated with primary OA (9;10;12). GH and IGF-1 play important roles in this process (29). Recently, we found evidence for an association between the common d3-GHR polymorphism, associated with an enhanced GH responsiveness, and primary OA in females, indicating that increased activity of the GH/IGF-1 axis might play a role in OA pathophysiology (9). *In vitro* studies also indicate that IGF-1 has stimulatory effects in chondrocyte proliferation and proteoglycan and collagen synthesis (7;8;29), supporting involvement of the GH/IGF-1 axis in OA development. Moreover, in acromegaly disease, GH/IGF-1 activity has been associated with radiographic severity and progression of arthropathy (14-16). With respect to the association between serum IGF-1 levels and primary OA, only few epidemiological studies have been previously performed. A recent systematic review summarizing literature on this association showed moderate evidence for no relationship between serum IGF-1 and radiographic OA (18). However, this review included mainly cross-sectional data and study sizes were relatively small. In addition, most studies did not adjust for age, sex, and BMI, which all independently influence IGF-1 concentrations. In this respect, it

should also be noted that comparisons between studies are difficult, since differences exist in IGF-1 assays, OA scoring methods, reading order of baseline and follow-up radiographs, mean age of participants and statistical analyses used.

In the present study, we found that mean IGF-1 SDS was significantly increased in GARP patients when compared to control/reference values, suggesting that high serum IGF-1 levels are associated with the presence of a severe familial form of OA. Although mean IGF-1 SDS was high in the GARP Study, no clear dose-response relationship was found between IGF-1 SDS and either the presence or progression of OA. This might probably be explained by the fact that per inclusion all GARP patients had severe radiographic and clinical OA at multiple joint sites on a familial base. Furthermore, serum IGF-1 levels of most GARP subjects were within the normal reference range, probably resulting in too less variation in IGF-1 levels to detect a dose-response relationship with radiographic occurrence or progression of OA. In addition, an important question which requires further investigation is whether circulating total IGF-1 levels truly reflect peripheral IGF-1 activity. There is increasing evidence that measuring free unbound IGF-1 levels and IGF-1 bioactivity better reflect the GH/IGF-1 status than total IGF-1 (30;31). However, because of methodological difficulties in measurement, total serum IGF-1 is often used to assess GH/IGF-1 activity (30). Presence of negative endocrine feedback systems further complicates the interpretation of IGF-1 levels.

Strengths of the present study are, first, the relatively large number of patients with a severe, familial form of primary OA. This specific OA phenotype increases the a priori chance to detect an association between IGF-1 levels and OA. Second, we had the availability of a cohort that was longitudinally followed and we were, therefore, able to study IGF-1 in relation to OA progression. Since a population with OA at multiple joint sites, such as the GARP Study, is associated with rapid progression, our study population is suitable to investigate OA progression within a relatively short period. In this respect, we chose the 2-year time point to study progression, since we expect more differentiation in (and faster) OA progression, increasing the sensitivity to detect risk factors. Third, we adjusted for age, sex, and BMI in our analyses, since all these factors are important confounders when studying IGF-1 in relation to primary OA.

This study has a number of limitations. First, since all GARP subjects have a severe OA phenotype of familial OA at multiple joint sites, joint-specific analyses are difficult to perform. In addition, since the GARP Study is a very homogeneous cohort with less variation in OA phenotype (*i.e.* all patients have severe familial symptomatic OA at multiple joint sites), the detection of a clear relationship with OA at different joint sites is very difficult. In this respect, also the generalizability of our results in other population settings has to be investigated (32). Second, it should be noted that the GARP Study consists mainly of females, a phenomenon that is well-known from OA literature (33). To adjust for the female overrepresentation in our cohort, analyses were stratified for sex. Third, IGF-1 measurement by serum samples is very complex, because individual IGF-1 levels are liable to temporal variations and are inversely correlated to age, estrogen levels, and BMI. Fourth, we compared mean IGF-1 SDS of the GARP cohort with literature-based reference values. Ideally, it would be preferred to use a control population of subjects without signs of OA for comparison. Furthermore, radio-immuno assays (RIA) for measuring serum IGF-1 are subject to analytical difficulty. Finally, in general, it should be noted that due to a complex pathophysiology, the extent to which hormones are involved in common diseases is difficult to assess. Therefore, to investigate the individual roles of GH and IGF-1 in joint disease, acromegaly is a unique human model of which findings might possibly be extrapolated to primary OA (14;34;35).

In conclusion, mean IGF-1 SDS was increased in patients with familial OA at multiple joint sites when compared to control values from literature, suggesting that high serum IGF-1 is associated with OA. This is similar to several lines of previous evidence suggesting a role for increased GH/IGF-1 activity in the pathophysiology of OA. However, no clear relationship between IGF-1 SDS and either the presence or progression of radiographic OA at different joint sites was found. Taking into account imperfections in IGF-1 measurement and the homogeneous OA phenotype in the GARP Study thereby prohibiting the detection of a clear dose-response association, further research has to clarify the complex role of the GH/IGF-1 axis in OA pathophysiology.

REFERENCE LIST

1. Felson DT, Lawrence RC, Dieppe PA et al. Osteoarthritis: new insights. Part 1: the disease and its risk factors. Ann Intern Med 2000; 133(8):635-646.
2. Peyron JG. Osteoarthritis. The epidemiologic viewpoint. Clin Orthop Relat Res 1986;(213):13-19.
3. Spector TD, Cicuttini F, Baker J, Loughlin J, Hart D. Genetic influences on osteoarthritis in women: a twin study. BMJ 1996; 312(7036):940-943.
4. Le RD, Bondy C, Yakar S, Liu JL, Butler A. The somatomedin hypothesis: 2001. Endocr Rev 2001; 22(1):53-74.
5. Copeland KC, Colletti RB, Devlin JT, McAuliffe TL. The relationship between insulin-like growth factor-I, adiposity, and aging. Metabolism 1990; 39(6):584-587.
6. Juul A. Serum levels of insulin-like growth factor I and its binding proteins in health and disease. Growth Horm IGF Res 2003; 13(4):113-170.
7. Guenther HL, Guenther HE, Froesch ER, Fleisch H. Effect of insulin-like growth factor on collagen and glycosaminoglycan synthesis by rabbit articular chondrocytes in culture. Experientia 1982; 38(8):979-981.
8. McQuillan DJ, Handley CJ, Campbell MA, Bolis S, Milway VE, Herington AC. Stimulation of proteoglycan biosynthesis by serum and insulin-like growth factor-I in cultured bovine articular cartilage. Biochem J 1986; 240(2):423-430.
9. Claessen KM, Kloppenburg M, Kroon HM et al. Relationship between the functional exon 3 deleted growth hormone receptor polymorphism and symptomatic osteoarthritis in women. Ann Rheum Dis 2013.
10. Meulenbelt I, Bijkerk C, Miedema HS et al. A genetic association study of the IGF-1 gene and radiological osteoarthritis in a population-based cohort study (the Rotterdam Study). Ann Rheum Dis 1998; 57(6):371-374.
11. Urano T, Narusawa K, Shiraki M et al. Association of a single nucleotide polymorphism in the insulin-like growth factor-1 receptor gene with spinal disc degeneration in postmenopausal Japanese women. Spine (Phila Pa 1976) 2008; 33(11):1256-1261.
12. Zhai G, Rivadeneira F, Houwing-Duistermaat JJ et al. Insulin-like growth factor I gene promoter polymorphism, collagen type II alpha1 (COL2A1) gene, and the prevalence of radiographic osteoarthritis: the Rotterdam Study. Ann Rheum Dis 2004; 63(5):544-548.
13. Barkan AL. Acromegalic arthropathy. Pituitary 2001; 4(4):263-264.
14. Biermasz NR, Wassenaar MJ, van der Klaauw AA et al. Pretreatment insulin-like growth factor-I concentrations predict radiographic osteoarthritis in acromegalic patients with long-term cured disease. J Clin Endocrinol Metab 2009; 94(7):2374-2379.
15. Biermasz NR, van 't Klooster R, Wassenaar MJ et al. Automated image analysis of hand radiographs reveals widened joint spaces in patients with long-term control of acromegaly: relation to disease activity and symptoms. Eur J Endocrinol 2012; 166(3):407-413.
16. Claessen KM, Ramautar SR, Pereira AM et al. Progression of acromegalic arthropathy despite long-term biochemical control: a prospective, radiological study. Eur J Endocrinol 2012; 167(2):235-244.
17. Elliott KS, Chapman K, Day-Williams A et al. Evaluation of the genetic overlap between osteoarthritis with body mass index and height using genome-wide association scan data. Ann Rheum Dis 2013; 72(6):935-941.
18. Claessen KM, Ramautar SR, Pereira AM, Smit JW, Biermasz NR, Kloppenburg M. Relationship between insulin-like growth factor-1 and radiographic disease in patients with primary osteoarthritis: a systematic review. Osteoarthritis Cartilage 2012; 20(2):79-86.
19. Fraenkel L, Zhang Y, Trippel SB et al. Longitudinal analysis of the relationship between serum insulin-like growth factor-I and radiographic knee osteoarthritis. Osteoarthritis Cartilage 1998; 6(5):362-367.
20. Schouten JS, Van den Ouweland FA, Valkenburg HA, Lamberts SW. Insulin-like growth factor-1: a prognostic factor of knee osteoarthritis. Br J Rheumatol 1993; 32(4):274-280.
21. Riyazi N, Meulenbelt I, Kroon HM et al. Evidence for familial aggregation of hand, hip, and spine but not knee osteoarthritis in siblings with multiple joint involvement: the GARP study. Ann Rheum Dis 2005; 64(3):438-443.
22. Botha-Scheepers SA, Watt I, Slagboom E et al. Influence of familial factors on radiologic disease progression over two years in siblings with osteoarthritis at multiple sites: a prospective longitudinal cohort study. Arthritis Rheum 2007; 57(4):626-632.
23. Cole TJ. The LMS method for constructing normalized growth standards. Eur J Clin Nutr 1990; 44(1):45-60.
24. Rikken B, van Doorn J, Ringeling A, Van den Brande JL, Massa G, Wit JM. Plasma levels of insulin-like growth factor (IGF)-I, IGF-II and IGF-binding protein-3 in the evaluation of childhood growth hormone deficiency. Horm Res 1998; 50(3):166-176.
25. Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. Ann Rheum Dis 1957; 16(4):494-502.
26. Meulenbelt I, Kloppenburg M, Kroon HM et al. Urinary CTX-II levels are associated with radiographic subtypes of osteoarthritis in hip, knee, hand, and facet joints in subject with familial osteoarthritis at multiple sites: the GARP study. Ann Rheum Dis 2006; 65(3):360-365.
27. Botha-Scheepers S, Watt I, Breedveld FC, Kloppenburg M. Reading radiographs in pairs or in chronological order influences radiological progression in osteoarthritis. Rheumatology (Oxford) 2005; 44(11):1452-1455.
28. Bruynesteyn K, Boers M, Kostense P, van der Linden S, van der Heide D. Deciding on progression of joint damage in paired films of individual patients: smallest detectable difference or change. Ann Rheum Dis 2005; 64(2):179-182.
29. Okazaki K, Jingushi S, Ikenoue T et al. Expression of insulin-like growth factor I messenger ribonucleic acid in developing osteophytes in murine experimental osteoarthritis and in rats inoculated with growth hormone-secreting tumor. Endocrinology 1999; 140(10):4821-4830.
30. Janssen JA, van der Lely AJ, Lamberts SW. Circulating free insulin-like growth-factor-I (IGF-I) levels should also be measured to estimate the IGF-I bioactivity. J Endocrinol Invest 2003; 26(6):588-594.
31. Varewijck AJ, Lamberts SW, Uitterlinden P, Hofland LJ, Janssen JA. IGF-I bioactivity better reflects growth hormone deficiency than total IGF-I. J Clin Endocrinol Metab 2011; 96(7):2248-2254.
32. Ledingham J, Regan M, Jones A, Doherty M. Factors affecting radiographic progression of knee osteoarthritis. Ann Rheum Dis 1995; 54(1):53-58.
33. Dieppe P, Cushnaghan J, Tucker M, Browning S, Shepstone L. The Bristol 'OA500 study': progression and impact of the disease after 8 years. Osteoarthritis Cartilage 2000; 8(2):63-68.
34. Wassenaar MJ, Biermasz NR, van Duinen N et al. High prevalence of arthropathy, according to the definitions of radiological and clinical osteoarthritis, in patients with long-term cure of acromegaly: a case-control study. Eur J Endocrinol 2009; 160(3):357-365.
35. Wassenaar MJ, Biermasz NR, Pereira AM et al. The exon-3 deleted growth hormone receptor polymorphism predisposes to long-term complications of acromegaly. J Clin Endocrinol Metab 2009; 94(12):4671-4678.

IX.

Relationship between the functional exon 3 deleted growth hormone receptor polymorphism and symptomatic osteoarthritis in women

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ABSTRACT

BACKGROUND: Several studies suggest a role of the growth hormone (GH)/insulin-like growth factor-1 (IGF-1) axis in the pathophysiology of primary osteoarthritis (OA). A common polymorphism of the GH receptor (exon 3 deletion, d3-GHR) is associated with increased GH/IGF-1activity.

OBJECTIVE: To study associations between the d3-GHR polymorphism and symptomatic OA.

METHODS: In the GARP (Genetics, osteoARthritis and Progression) Study, we compared the d3-GHR polymorphism between OA patients and controls. GARP patients were genotyped for 7 single nucleotide polymorphisms encompassing the d3-GHR gene, using rs4590183 as proxy for d3-GHR (pairwise r2=1). Binary logistic regression models with robust standard errors were performed, stratified by sex. For replication, rs4590183 was tested in three additional cohorts. Fixed- and random-effects combined analyses were performed.

RESULTS: In female GARP patients with severe familial OA, d3-GHR was associated with OA (adjusted odds ratio (OR) 1.36 (95%CI 1.01-1.83), p=0.043), independently of age and BMI. Combined analysis of all studies showed suggestive evidence for association between d3-GHR and OA (OR=1.17 (95%CI 1.04-1.30), p=0.008). Evidence was strongest in hip OA cases, without any evidence for heterogeneity.

CONCLUSIONS: In women, the d3-GHR polymorphism was associated with symptomatic OA, especially at the hip site.

INTRODUCTION

Osteoarthritis (OA) is a debilitating disease characterized by progressive degradation of articular cartilage and bone remodelling (1). Although the exact pathogenesis remains to be elucidated, genetic studies have identified several variants associated with primary OA, such as the 7q22 containing multiple potential genes, Growth Differentiation Factor 5 (GDF5) gene, frizzled related protein (FRZB) gene, Deiodinase IOdothyronine type II (DIO2) gene and Mothers Against Decapentaplegic homolog 3 (SMAD3) gene (2). Together, these genes provide evidence that endochondral ossification may be involved in OA onset (3).

Endochondral ossification is driven by growth plate chondrocytes, resulting in longitudinal skeletal growth through a combination of proliferation, extracellular matrix (ECM) secretion and hypertrophy. Subsequently, terminally differentiated chondrocytes die and are replaced with bone tissue (4). At all stages, chondrocyte behaviour is tightly regulated by a complex network of interactions between circulating hormones, locally produced growth factors and ECM components. These chondrocytes likely play a role in bone shape and/or the quality of articular cartilage. One of the strongest stimulators of chondrocyte proliferation is Growth Hormone (GH), predominantly via insulin-like growth factor-1 (IGF-1) secretion (5). This qualifies variations within GH/IGF-1 genes as obvious candidates for association studies.

The dimeric GH receptor (GHR) mediates the effect of GH on target tissues and exists of different molecular structures, depending on assortments of coding polymorphisms in the GHR gene. Three GHR variants, differing in the presence or absence of exon 3 (GHRfl-fl, GHRfl-d3, GHRd3-d3), are commonly seen. Exon 3 loss (d3-GHR) results in a truncated receptor with increased GH responsiveness by enhanced signal transduction (6;7).

We hypothesize that the d3-GHR polymorphism increases OA risk by increasing (local) GH/IGF-1 activity. Therefore, we compared the effects of the d3-GHR polymorphism between cases with symptomatic generalized OA and controls. We tested for confirmation in three additional cohorts. A combined analysis was performed in women (2175 OA cases and 2623 controls).

PATIENTS AND METHODS

SUBJECTS: The discovery study was the GARP (Genetics ARthrosis and Progression) Study (8). For replication, the PAPRIKA (PAtients Prospectively Recruited In Knee and hip Arthroplasty)/RAAK (Research Articular osteoArthritis Cartilage), ACRO (acromegaly) and Rotterdam Studies were used (9-11). All patients and controls were from Dutch descent. Details of original study design and phenotype definition were described in *Supplementary File 1*.

GENOTYPING: Genomic DNA was isolated from peripheral blood according to standard procedures. In GARP, the d3-GHR polymorphism was detected as described previously (12), based on specific amplification of the wild type (935 base pairs (bp)) and mutant (532bp) alleles. To allow high-throughput genotyping, we assessed linkage disequilibrium (LD) between d3-GHR and Single Nucleotide Polymorphisms (SNPs) covering the gene as determined in a GWAS, by means of Illumina 660W. GWAS details are published elsewhere (13). Previously, only one SNP (rs6873545) was described to capture the d3-GHR polymorphism (14). We genotyped 373 GARP subjects and 752 controls for 7 other SNPs (rs4590183, rs13354167, rs7721081, rs7701605, rs4242116, rs6878512, rs10941583), all being in high LD with the d3-GHR polymorphism (*Supplementary File 2*). All SNPs were in Hardy-Weinberg equilibrium. In cases and controls, rs4590183 was selected as proxy for d3-GHR genotype (r^2=1). Throughout this report, the d3 allele of rs4590183 was designated as risk allele.

Replication cohorts: Samples of the Rotterdam Study were genotyped with the Illumina HumanHap 550v3 Genotyping BeadChip. GWAS details are published elsewhere (15). Other cohorts were genotyped by mass spectrometry using the homogeneous MassARRAY system of Sequenom (San Diego, California, USA) using standard conditions.

STUDY DESIGN/STATISTICAL ANALYSIS: First, association with d3-GHR with OA was performed in the GARP Study (men and women) since this study consists of genetically enriched patients with symptomatic OA at multiple joint sites. Subsequently, we tested for confirmation in women of three other cohorts, the PAPRIKA/RAAK (joint replacement), acromegaly (signs of clinical and radiographic OA), and Rotterdam (severe radiographic OA) studies.

Logistic regression analyses were performed with STATA Statistical Software version 10.1 (Statacorp, College Station, TX). A dominant genotypic model was applied. To adjust for family relationships within

the GARP Study, robust standard errors were estimated from the variance between sibling pairs (16). Combined analyses were performed in women, using R version 2.15.0(17). If the heterogeneity metric I^2 exceeded 25%, a random-effects model was also used, otherwise only a fixed-effects model was applied. Given that only one polymorphism was studied with well established functional effects, $p<0.05$ was considered as reflecting significance.

RESULTS

Table 1 describes the phenotypic characteristics of the GARP Study (discovery sample). As shown in *Table 2*, we found evidence for association between the d3-GHR polymorphism and OA, only in women of the GARP Study (OR=1.36, 95%CI 1.01-1.83, p=0.043). Adjustment for age and body mass index (BMI) did not significantly affect the genotypic association.

Since women drove the association with d3-GHR, our replication was aimed at women with symptomatic OA of the PAPRIKA/RAAK and ACRO Studies, and severe radiographic OA in the Rotterdam Study (*Table 1)*. For the combined analysis, 2175 cases and 2623 controls were available, and the respective genotype frequencies are shown in *Supplementary File 3*. Although the association with d3-GHR was significant only in the PAPRIKA/RAAK Study, the combined analysis of four studies with OA at any joint location provides evidence for association with d3-GHR, with an OR of 1.17 (95%CI 1.04-1.32,p=0.008), without any evidence for heterogeneity (p=0.470, I^2=0%) *(Table 2)*. In a sensitivity analysis excluding the discovery GARP Study, the association persisted (OR=1.14, 95%CI 1.01-1.30, p=0.042).

When we stratified for joint site in the combined analysis (*Table 2*), we observed consistent effect sizes of approximately 1.2–1.3 among the joint strata, being significant in hip OA cases (p=0.002), without evidence for heterogeneity (*Figure 2B*). Allelic data were presented in *Supplementary File 4*.

Table 1. Clinical characteristics of the discovery and replication studies

	Discovery study				Replication studies					
Clinical characteristics	**GARP Females (N = 305)**	**Males (N = 68)**	**Controls Females (N = 435)**	**Males (N = 317)**	**PAPRIKA/RAAK Females (N = 332)**	**Controls Females (N = 563)**	**ACRO Females (N = 41)**	**Controls Females (N = 361)**	**Rotterdam Females (N = 1497)**	**Controls Females (N = 1264)**
Age, years	59.8 (7.6)	61.3 (7.6)	57.0 (6.9)	61.3 (7.2)	61.9 (6.5)	57.7 (1.4)	60.8 (12.2)	57.7 (1.4)	70.0 (8.1)	66.3 (7.8)
BMI, kg/m²	27.0 (4.9)	27.1 (3.4)	25.4 (3.9)	25.8 (3.2)	NR	NR	28.4 (4.9)	NR	27.1 (4.1)	26.2 (3.9)
ROA, by joint site*										
Knee (n (%))	116 (38%)	26 (38%)	NA	NA	121 (36%)	NA	18 (44%)	NA	284 (19%)	NA
Hip (n (%))	76 (25%)	19 (28%)	NA	NA	211 (64%)	NA	12 (29%)	NA	246 (16%)	NA
Hand (n (%))	261 (86%)	61 (90%)	NA	NA	NA	NA	32 (78%)	NA	1310 (88%)	NA
ROA definition	KL≥2**	KL≥2**	NA	NA	THP/TKP***	NA	KL≥2**	NA	KL≥3**	NA

Data are shown as mean (SD), unless mentioned otherwise. For the replication studies, only female data are shown.

GARP, Genetics osteoARthritis and Progression Study (primary OA); PAPRIKA/RAAK, PAtients Prospectively Recruited In Knee and hip Arthroplasty/Research Articular osteoArthritis Cartilage (primary OA); ACRO, acromegaly patients; Rotterdam, cases of the Rotterdam Study; BMI, body mass index; ROA, radiographic osteoarthritis; KL, Kellgren-Lawrence; THP, total hip prosthesis; TKP, total knee prosthesis; NA, not applicable. NR, not reported.
**, In the GARP Study, 8 patients had a unilateral knee prosthesis, 1 patient had bilateral knee prostheses, 22 patients had a unilateral hip prosthesis and 15 patients had bilateral hip prostheses, due to end-stage OA. All cases with prostheses were from separate families.*
***, Hand OA was defined as KL≥2 in 2 of the following 3 groups: distal interphalangeal (DIP), proximal interphalangeal (PIP), carpometacarpal (CMC) or trapezium scaphoid (TS) joints.*
****, 98% of patients had Kellgren Lawrence ≥2 pre-operatively*

Table 2. Study-specific and combined analyses investigating the association between the d3-GHR polymorphism and symptomatic OA in females for OA at any joint site, and combined analyses after joint stratification

Studies	OA at any joint site*		Heterogeneity***		joint site	Combined analyses*		Heterogeneity***	
	OR (95%CI)**	p-value	I^2	p-value		OR (95%CI)**	p-value	I^2	p-value
GARP*	1.36 (1.01-1.83)	0.043	NA	NA	**Hip OA**	1.34 (1.11-1.62)	0.002	0%	0.709
PAPRIKA / RAAK*	1.32 (1.00-1.73)	0.048	NA	NA	**Knee OA**	1.20 (0.84-1.72)	0.308	66.7%	0.029
ACRO*	1.17 (0.73-1.90)	0.514	NA	NA	**Hand OA**	1.29 (0.99-1.67)	0.055	51.7%	0.126
RDAM*	1.09 (0.94-1.27)	0.244	NA	NA					
Combined analysis	1.17 (1.04-1.32)	0.008	0%	0.470					

**, Data were presented for female OA cases only.*
***, OR (95%CI) and p-values were calculated for the dominant genotypic model, and were presented with the minor allele (exon 3 deletion, d3) as the dominant risk allele. rs4590183 was used as proxy for the d3-GHR polymorphism in all cases and controls. The OR represents the risk of having the d3-GHR polymorphism in female OA cases vs controls, as shown for the total cohort and stratified for joint site. Logistic regression analysis is performed, applying a binary logistic model.*
****, Heterogeneity across the OA studies quantified by the I2 statistic, whereas its statistical significance was determined by the X2 distributed Cochran Q statistic (20).*

OA, osteoarthritis; NA, not applicable; GARP, Genetics ARthrosis and Progression Study; PAPRIKA/RAAK, PAtients Prospectively Recruited In Knee and hip Arthroplasty/Research Articular osteoArthritis Cartilage (primary OA); ACRO, acromegaly patients.

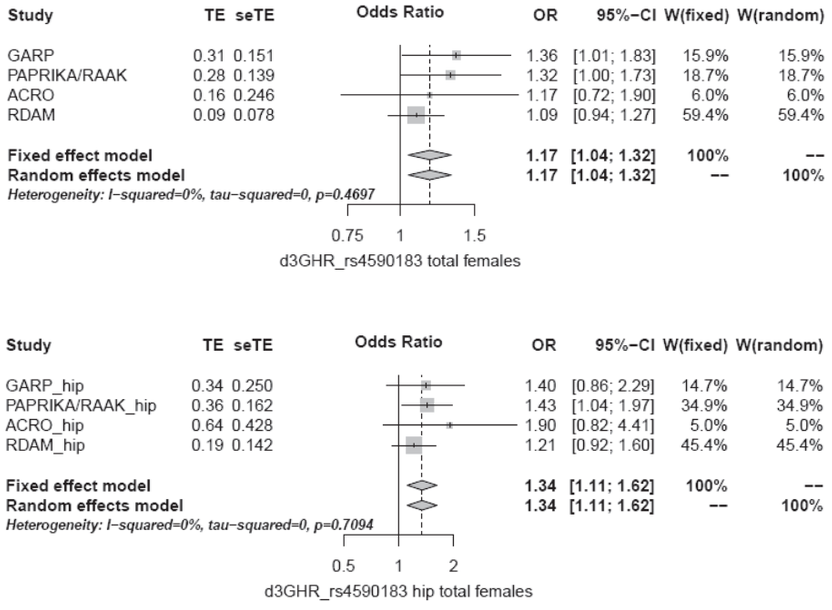


Figure 1. Forest plots for the association between the d3-GHR polymorphism and symptomatic OA in females. Results are presented for the combined analyses of the GARP, PAPRIKA/RAAK, ACRO and Rotterdam Studies, showing the association with OA at any joint site (A), and hip OA (B).

GHR, growth hormone receptor; ROA, radiographic osteoarthritis; GARP, Genetics osteoARthritis and Progression Study; PAPRIKA, PAPRIKA, PAtients Prospectively Recruited In Knee and hip Arthroplasty; RAAK, Research Articular osteoArthritis Cartilage; ACRO, acromegaly patients, ROTTERDAM, cases from the Rotterdam Study.

DISCUSSION

In a combined analysis of 2175 female OA cases and 2623 controls, we found evidence for association between the functional d3-GHR polymorphism and symptomatic OA (pooled OR=1.17,95%CI 1.04-1.32, p=0.008), without evidence of heterogeneity and independently of age and BMI. Stratifying by joint site indicated that the association was most predominant in female cases with hip OA.

Human GH is a strong modulator of important physiological processes such as fuel homeostasis, cell differentiation and metabolic control. The GH/IGF-1 axis is essential for longitudinal skeletal growth. During growth, long bones increase in height through endochondral ossification, replacing a cartilage model by bone tissue. The main player in this process is the chondrocyte. 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 (5). Chondrocytes in OA cartilage share a fair amount of their expressed genes with those expressed in the terminal layer of the growth plate (3). Therefore, genes involved in skeletal morphogenesis early in life determining joint shape, might play a late-acting deleterious role towards OA. IGF-1 is associated with increased cartilage formation and laxity of peri-articular ligaments, and plays a role in osteophyte development (18). All these changes together contribute to an altered joint geometry, eventually resulting in an arthritic joint. The d3-GHR polymorphism is hypothesized to accelerate the OA process in susceptible patients by increasing GH responsiveness and, thereby, (local) IGF-1 levels.

Typically, the OR observed in the GARP discovery study was higher when compared to the replication studies but also generally higher than large scale GWA studies, such as of Zeggini *et al.*(19). This could be explained by the fact that for the GARP Study we have applied a family-based sampling scheme towards the extreme spectrum of the OA phenotype, consisting of sibling pairs with both symptomatic and radiographic OA at multiple sites. In general, such a study is tailored to find genetic variants in the low frequency range with moderate to large effect sizes. Here, the GARP phenotype may have been most efficient in detecting predisposition of the d3-GHR polymorphism, although the allele frequency is not rare. Moreover, in a sensitivity analysis excluding GARP, the association between the d3-GHR polymorphism and OA persisted, whereas the consistency of the effect sizes among the different cohorts and joint strata, adds to the credibility of d3-GHR.

Several potential limitations need to be addressed. Firstly, although the direction and effect sizes were similar in our replication cohorts, only the association in the PAPRIKA/RAAK cohort was significant. This is likely to be explained by the low number of cases of ACRO and Rotterdam Study, providing insufficient power. Secondly, inclusion of acromegaly patients might introduce a bias, since disease processes in acromegalic arthropathy may differ from those in primary OA. However, since the d3-GHR polymorphism is not predisposing to acromegaly itself, the inclusion of acromegalics with OA is not likely to influence our results. Merely, we expect that a general detrimental effect of GH excess on joint tissue homeostasis predisposes to OA. Finally, the unknown OA status in controls might have led to an underestimation of the reported effect.

In conclusion, we found an association between the d3-GHR polymorphism and symptomatic OA in women, especially in cases with hip OA. Being aware of the tendency of association studies to produce false-positive results, additional replication is necessary. Furthermore, studying the d3-GHR polymorphism in relation to GH profiles and IGF-1 levels could further elucidate the role of the GH/IGF-1 axis in OA.

REFERENCE LIST

1. Felson DT, Lawrence RC, Dieppe PA et al. Osteoarthritis: new insights. Part 1: the disease and its risk factors. Ann Intern Med 2000; 133(8):635-646.
2. Meulenbelt I. Osteoarthritis year 2011 in review: genetics. Osteoarthritis Cartilage 2012; 20(3):218-222.
3. Bos SD, Slagboom PE, Meulenbelt I. New insights into osteoarthritis: early developmental features of an ageing-related disease. Curr Opin Rheumatol 2008; 20(5):553-559.
4. Mackie EJ, Tatarczuch L, Mirams M. The skeleton: a multi-functional complex organ: the growth plate chondrocyte and endochondral ossification. J Endocrinol 2011; 211(2):109-121.
5. Pass C, MacRae VE, Ahmed SF et al. Inflammatory cytokines and the GH/IGF-I axis: novel actions on bone growth. Cell Biochem Funct 2009; 27(3):119-127.
6. Dos Santos C, Essioux L, Teinturier C et al. A common polymorphism of the growth hormone receptor is associated with increased responsiveness to growth hormone. Nat Genet 2004; 36(7):720-724.
7. Urbanek M, Russell JE, Cooke NE et al. Functional characterization of the alternatively spliced, placental human growth hormone receptor. J Biol Chem 1993; 268(25):19025-19032.
8. Riyazi N, Meulenbelt I, Kroon HM et al. Evidence for familial aggregation of hand, hip, and spine but not knee osteoarthritis in siblings with multiple joint involvement: the GARP study. Ann Rheum Dis 2005; 64(3):438-443.
9. Hofman A, van Duijn CM, Franco OH et al. The Rotterdam Study: 2012 objectives and design update. Eur J Epidemiol 2011; 26(8):657-686.
10. Keurentjes JC, Vliet Vlieland TPM, Fiocco M et al. A comparison of Health-related Quality of Life after Primary Total Hip and Knee Replacement. In preparation 2012.
11. Wassenaar MJ, Biermasz NR, van DN et al. High prevalence of arthropathy, according to the definitions of radiological and clinical osteoarthritis, in patients with long-term cure of acromegaly: a case-control study. Eur J Endocrinol 2009; 160(3):357-365.
12. Pantel J, Machinis K, Sobrier ML et al. Species-specific alternative splice mimicry at the growth hormone receptor locus revealed by the lineage of retroelements during primate evolution. J Biol Chem 2000; 275(25):18664-18669.
13. Panoutsopoulou K, Southam L, Elliott KS et al. Insights into the genetic architecture of osteoarthritis from stage 1 of the arcOGEN study. Ann Rheum Dis 2011; 70(5):864-867.
14. Glad CA, Johannsson G, Carlsson LM, Svensson PA. Rapid and high throughput genotyping of the growth hormone receptor exon 3 deleted/full-length polymorphism using a tagSNP. Growth Horm IGF Res 2010; 20(3):270-273.
15. Kerkhof HJ, Lories RJ, Meulenbelt I et al. A genome-wide association study identifies an osteoarthritis susceptibility locus on chromosome 7q22. Arthritis Rheum 2010; 62(2):499-510.
16. Diggle PJ, Liang KY, Zeger SL. General linear models for longitudinal data. Analysis of longitudinal data. Oxford University Press Oxford, 1994: 68-77.
17. Schwarzer G. Meta: an R package for meta-analysis. R News 2007; 7:40-45.
18. Okazaki K, Jingushi S, Ikenoue T et al. Expression of insulin-like growth factor I messenger ribonucleic acid in developing osteophytes in murine experimental osteoarthritis and in rats inoculated with growth hormone-secreting tumor. Endocrinology 1999; 140(10):4821-4830.
19. Identification of new susceptibility loci for osteoarthritis (arcOGEN): a genome-wide association study. Lancet 2012; 380(9844):815-823.
20. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002; 21(11):1539-1558.

SUPPLEMENTARY FILE 1.

Study populations & Phenotype definition

The discovery study is the GARP (Genetics, osteoARthritis and Progression) Study, which is a prospective cohort study aimed at identifying determinants of OA susceptibility and progression. This study consists of 191 sibling pairs (N=382) affected by symptomatic and radiographic OA at multiple joint sites. Radiographic OA of the knee, hip and hands was assessed on conventional radiographs, according to the Kellgren-Lawrence (KL) scale (1;2). Radiographic knee and hip OA were defined by the ROA score (ROA ≥1) (3), in which the specific ROA score (0-2) represents no, unilateral or bilateral involvement based on KL ≥2. For hand ROA we used the following definition to be comparable to the definitions used in the Rotterdam Study (*vide infra*): KL≥2 in at least 2 of the following 3 groups: distal interphalangeal (DIP), proximal interphalangeal (PIP), carpometacarpal (CMC) or trapezium scaphoid (TS) joints (4). Details of patient recruitment and phenotypic description are reported in Riyazi *et al.* (2). We used data from the baseline visit, excluding one patient for unsuccessful DNA analysis and eight for missing radiographs, resulting in the inclusion of 373 patients. The GARP Study was compared with 753 independent individuals (*i.e.* partners) from the Leiden Longevity Study (5). One patient was excluded for missing data on sex, resulting in 752 controls.

In the confirmation and replication studies, only female cases and controls were studied. The PAPRIKA (PAtients Prospectively Recruited In Knee and hip Arthroplasty) and RAAK (Research Articular osteoArthritis Cartilage) cohorts consist of patients with total knee prosthesis (TKP) or total hip prosthesis (THP) for end-stage OA (98% had a pre-operative KL score of ≥2), and were, therefore, likely to have significant symptomatic disease. Female patients aged ≤70 years were selected with a TKP or THP for primary OA (N=332). Controls comprise a random selection of unrelated female subjects (N=563) from the Rotterdam Study (6), that were not selected on the basis of phenotype.

The acromegaly cohort consists of long-term well-controlled patients (7), of which 41 female patients were successfully genotyped and therefore included. All patients had signs of arthropathy at any joint. In the acromegalics, radiographic OA was defined by the ROA score, based on KL ≥2. The controls comprise a random selection of another sample of unrelated subjects (N=361) from the Rotterdam Study (6), that were not selected on the basis of phenotype.

The Rotterdam Study, which comprises 7983 Caucasian participants, is a prospective, population-based cohort study of determinants and the prognosis of chronic diseases in the elderly (6). Radiographs of the knee, hip and hands were made; however, symptomatic data were not available. In order to select comparable female cases for the GARP and PAPRIKA/RAAK Studies, and in the absence of symptomatic data, 1497 Rotterdam cases were selected according to the following definitions: knee and hip OA as KL ≥3, and hand OA as KL ≥2 in at least 2 of the following 3 groups (DIP, PIP, CMC/TS joints). Subjects did not show overlap to the Rotterdam control subjects mentioned above. Rotterdam cases were compared to 1264 other female controls from the Rotterdam Study.

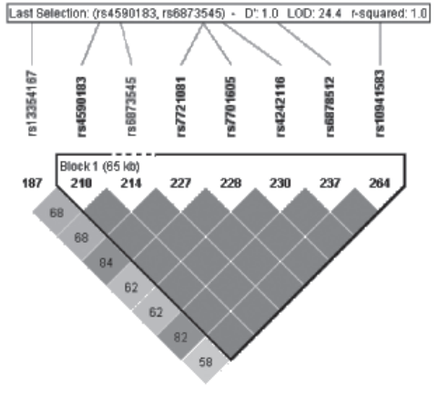
All patients and controls were from Dutch descent. Study protocols were approved by the local Medical Ethics Committee and written informed consent was obtained from all participants.

REFERENCE LIST

1. Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. Ann Rheum Dis 1957; 16(4):494-502.
2. Riyazi N, Meulenbelt I, Kroon HM et al. Evidence for familial aggregation of hand, hip, and spine but not knee osteoarthritis in siblings with multiple joint involvement: the GARP study. Ann Rheum Dis 2005; 64(3):438-443.
3. Meulenbelt I, Kloppenburg M, Kroon HM et al. Urinary CTX-II levels are associated with radiographic subtypes of osteoarthritis in hip, knee, hand, and facet joints in subject with familial osteoarthritis at multiple sites: the GARP study. Ann Rheum Dis 2006; 65(3):360-365.
4. Meulenbelt I, Min JL, Bos S et al. Identification of DIO2 as a new susceptibility locus for symptomatic osteoarthritis. Hum Mol Genet 2008; 17(12):1867-1875.
5. Schoenmaker M, de Craen AJ, de Meijer PH et al. Evidence of genetic enrichment for exceptional survival using a family approach: the Leiden Longevity Study. Eur J Hum Genet 2006; 14(1):79-84.
6. Hofman A, van Duijn CM, Franco OH et al. The Rotterdam Study: 2012 objectives and design update. Eur J Epidemiol 2011; 26(8):657-686.
7. Wassenaar MJ, Biermasz NR, Pereira AM et al. The exon-3 deleted growth hormone receptor polymorphism predisposes to long-term complications of acromegaly. J Clin Endocrinol Metab 2009; 94(12):4671-4678.

SUPPLEMENTARY FILE 2

Linkage disequilibrium patterns between the 7 SNPs selected from HAPMAP and the d3-GHR polymorphism.



SNP, single nucleotide polymorphism; GHR, growth hormone receptor.

SUPPLEMENTARY FILE 3.

Genotype frequencies of the d3-GHR polymorphism (using rs4590183) in the different case and control populations, for, respectively, the total cohort and female subjects only

Total cohort	**GARP**	**Controls**	**PAPRIKA/**	**Controls**	**ACRO**	**Controls**	**ROTTERDAM**	**Controls**
rs4590183	**(N = 373)**	**(N = 752)**	**RAAK**	**(N = 1000)**	**(N = 85)**	**(N = 668)**	**(N = 2198)**	**(N = 2645)**
N (freq)			**(N = 502)**					
GHR_{fl-fl}	202 (0.542)	442 (0.588)	268 (0.534)	579 (0.579)	50 (0.588)	374 (0.588)	1264 (0.575)	1585 (0.599)
GHR_{fl-d3}	154 (0.413)	260 (0.346)	206 (0.410)	354 (0.354)	29 (0.341)	255 (0.382)	817 (0.372)	936 (0.354)
GHR_{d3-d3}	17 (0.046)	50 (0.066)	28 (0.056)	67 (0.067)	6 (0.071)	39 (0.058)	117 (0.053)	124 (0.047)
Female subjects only	GARP	Controls	PAPRIKA/RAAK	Controls	ACRO	Controls	ROTTERDAM	Controls
rs4590183	(N = 305)	(N = 435)	(N = 332)	(N = 563)	(N = 41)	(N = 361)	(N = 1497)	(N = 1264)
N (freq)								
GHR_{fl-fl}	163 (0.534)	265 (0.609)	172 (0.518)	330 (0.586)	22 (0.537)	208 (0.576)	853 (0.570)	757 (0.599)
GHR_{fl-d3}	127 (0.416)	141 (0.324)	138 (0.416)	201 (0.357)	17 (0.415)	132 (0.366)	565 (0.377)	453 (0.358)
GHR_{d3-d3}	15 (0.049)	29 (0.067)	22 (0.066)	32 (0.057)	2 (0.049)	21 (0.058)	79 (0.053)	54 (0.043)

[a] *Females only.*

Data are shown as number (frequency). N, number of patients. GARP, Genetics osteoARthritis and Progression Study; PAPRIKA/RAAK, PAtients Prospectively Recruited In Knee and hip Arthroplasty/ Research Articular osteoArthritis Cartilage; ACRO, acromegaly patients; ROTTERDAM, cases of the Rotterdam Study.

SUPPLEMENTARY FILE 4A.

Study-specific and combined analyses by allele frequency data of rs4590183 for female cases with symptomatic OA

	GARP*		**PAPRIKA / RAAK***		**ACRO***		**RDAM***	
Joint site	OR (95%CI)**	p-value	OR (95%CI)**	p-value	OR (95%CI)**	p-value	OR (95%CI)**	p-value
Overall	1.17 (0.92-1.49)	0.205	1.28 (1.03-1.60)	0.025	1.08 (0.64-1.83)	0.763	1.07 (0.95-1.22)	0.280
Hip OA	1.29 (0.87-1.90)	0.203	1.31 (1.02-1.68)	0.038	1.30 (0.53-3.18)	0.569	1.17 (0.94-1.46)	0.160
Knee OA	1.40 (1.01-1.93)	0.042	1.24 (0.91-1.70)	0.172	1.05 (0.48-2.28)	0.930	0.87 (0.70-1.09)	0.230
Hand OA	1.30 (1.00-1.71)	0.052	NA	NA	1.23 (0.70-2.18)	0.473	1.09 (0.96-1.24)	0.190

	Combined analyses*		**Heterogeneity**	
Joint site	OR (95%CI)**	p-value	I^{2***}	p-value
Overall	1.13 (1.02-1.24)	0.016	0%	0.544
Hip OA	1.24 (1.06-1.44)	0.006	0%	0.924
Knee OA	1.11 (0.86-1.45)	0.414	56.2%	0.077
Hand OA	1.13 (1.01-1.27)	0.033	0%	0.472

**, Data were presented for female OA cases only.*
***, OR (95%CI) and p-values were calculated using the allelic model, and were presented with the minor allele (exon 3 deletion, d3) as risk allele.*
****, Heterogeneity across the OA studies quantified by the I2 statistic, whereas its statistical significance was determined by the X2 distributed Cochran Q statistic.*

OA, osteoarthritis; NA, not applicable; GARP, Genetics ARthrosis and Progression Study; PAPRIKA/ RAAK, PAtients Prospectively Recruited In Knee and hip Arthroplasty/Research Articular osteoArthritis Cartilage (primary OA); ACRO, acromegaly patients; RDAM, Rotterdam Study cases.

SUPPLEMENTARY FILE 4B.

Frequencies of the minor allele (exon 3 deletion, d3) across the different study cohorts with patients with symptomatic OA and corresponding controls

Joint site	GARP*	PAPRIKA / RAAK*	ACRO*	RDAM* #
Overall	0.257	0.283	0.256	0.245
Hip OA	0.276	0.287	0.292	0.262
Knee OA	0.293	0.277	0.250	0.210
Hand OA	0.278	NA	0.281	0.248
Controls	0.229**	0.235***	0.241***	0.226***

**, Minor allele frequencies (MAF), with the exon 3 deletion (d3) allele as minor allele, are presented for the different studies, stratified by joint site with symptomatic OA.*
***, Controls of the Leiden Longevity Study.*
****, Controls of the Rotterdam Study.*

#, The Rotterdam Study did not comprise cases with symptomatic OA. We chose a severe radiographic phenotype (see Method section) in order to be comparable to the other cohorts with symptomatic OA.

OA, osteoarthritis; NA, not applicable; GARP, Genetics ARthrosis and Progression Study; PAPRIKA/ RAAK, PAtients Prospectively Recruited In Knee and hip Arthroplasty/Research Articular osteoArthritis Cartilage (primary OA); ACRO, acromegaly patients; RDAM, Rotterdam cases.

Part C

Long-term outcome of recombinant human GH therapy in GH deficient adults

X.

Therapy of Endocrine Disease Long-term effects of recombinant human Growth Hormone (rhGH) replacement in adults with Growth Hormone Deficiency (GHD): a Systematic Review

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ABSTRACT

BACKGROUND: The beneficial effects of recombinant human growth hormone (rhGH) therapy in growth hormone deficient (GHD) adults are well-established in the short term. However, data documenting the effects during prolonged follow-up are relatively scarce.

OBJECTIVE: To evaluate the reported effects of rhGH replacement (≥5 years) in GHD adults on biochemical and anthropometric parameters, quality of life (QoL), bone metabolism, muscle strength, serious adverse events (SAEs) and mortality.

METHODS: We conducted a systematic literature search. Quality assessment of retrieved papers was performed using a quality assessment based on the modified STROBE statement.

RESULTS: We included 23 prospective studies with a rhGH treatment duration ranging from 5 to 15 years. Overall, beneficial effects were reported on QoL, body composition, lipid profile, carotid intima media thickness and bone mineral density. In contrast, the prevalence of the metabolic syndrome, glucose levels, BMI and muscle strength were not, or negatively influenced. Most of the studies were uncontrolled, lacked the presence of a control group (of non-treated GHD patients), and reported no data on lipid-lowering and anti-diabetic medication. Overall mortality was not increased.

CONCLUSION: rhGH treatment in adult GHD patients is well-tolerated and positively affects QoL in the long term. However, the metabolic and cardiovascular effects during long-term treatment are variable. The low numbers of long-term studies and studied patients and lack of control data hamper definite statements on the efficacy of prolonged treatment. Therefore, continuous monitoring of the effects of rhGH replacement to enable an adequate risk-benefit analysis that may justify prolonged, potentially life-long, treatment is advisable.

INTRODUCTION

Growth hormone deficiency (GHD) in adults has been associated with an adverse metabolic profile and increased cardiovascular mortality by inducing abdominal obesity, hypercholesterolaemia and hypertriglyceridaemia (1;2). Consistent beneficial effects of treatment with recombinant human growth hormone (rhGH) were reported on body composition and lipid profile, resulting in reduction of fat mass combined with an increase in lean body mass (LBM) and a reduction in total cholesterol (TC) levels (3). In addition, favorable effects were reported on bone turnover, bone mineral density (BMD), muscle strength, cognitive function and quality of life (QoL) (3-5). Most of these effects have been documented in short-term studies, sometimes placebo-controlled, with a follow-up duration ranging from 6 to 18 months.

The beneficial effects of rhGH therapy have been reported to be sustained for at least 5 years of treatment; however data with a longer follow-up duration are scarce. With respect to cardiovascular disease, a direct improvement of several cardiovascular risk factors was noted within the first treatment year, which was reported to be sustained during prolonged rhGH treatment (6;7). In a small study, sustained improvement of lipid spectrum and diastolic blood pressure (DBP) was reported over 7 and 10 years, suggesting ongoing beneficial effects even beyond 5 years (6;7). However, it is presently unknown whether rhGH therapy has favorable effects on 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 in BMD, reaching a plateau phase after 5 to 7 years of treatment (8-10).

In general, rhGH replacement therapy in adults is regarded as safe. Recently, Van Bunderen *et al.* reported no differences in overall or malignancy-related mortality rate between GHD-treated patients and the background population (11). However, an increased risk in cardiovascular death was observed in rhGH-treated women. This increased cardiovascular mortality, however, is not easily explained. These recent findings accentuate the necessity to critically evaluate long-term efficacy and safety data.

We performed a systematic review of all available papers on long-term rhGH replacement in adult GHD patients. We aimed to evaluate the effects of long-term rhGH replacement in GHD adults on cardiovascular parameters, bone metabolism, muscle strength, QoL, mortality and adverse events.

MATERIALS AND METHODS

SEARCH STRATEGY AND ELIGIBILITY CRITERIA: To identify studies that investigated the long-term effects of rhGH replacement in GHD adults, we searched the following databases, up to September 2012 (Date of initial search 9th March, 2012): Medline, EMBASE, Web of Science, and the Cochrane Library, in collaboration with a trained clinical librarian (J.S.). We composed a search strategy focusing on long-term rhGH treatment, GHD adults, bone metabolism, QoL, metabolic effects, muscle strength, mortality, and adverse events. Chronic stable replacement was arbitrarily defined as replacement for ≥5 years, since such a period will reflect a stable metabolic situation without confounding by ongoing metabolic changes induced by the start and titration of rhGH replacement (3;12). We used all relevant keyword variations, including free text words. Duplicated articles were excluded. The complete search strategy is provided in *Appendix 1*. References of relevant articles were checked for additional articles. Only original articles were included.

Studies were eligible when all of the following criteria were met: (1) they investigated effects on biochemical, metabolic/anthropometric parameters, bone metabolism, QoL, muscle strength, mortality, or adverse events, (2) in adult patients with either childhood-onset (CO) or adult-onset (AO) GHD and (3) with at least 5 years of rhGH treatment and (4) were written in English. Only human studies were evaluated.

DATA EXTRACTION AND ANALYSIS: Data extraction and eligibility were assessed by two independent investigators (K.M.J.A.C. and N.M.A.-D.). Inconsistencies were resolved by consensus. The following data were extracted: (1) study population (patient characteristics, population size, GHD assessment, control population); (2) exposure to rhGH (duration of rhGH treatment, rhGH dose), (3) outcome (biochemical and anthropometric parameters, QoL, bone metabolism, muscle strength, mortality, serious adverse events (SAEs)) (4) potential confounders (age, sex, CO/AO).

METHODOLOGICAL QUALITY ASSESSMENT: The quality of each included paper was assessed in consensus by two independent reviewers (K.M.J.A.C. and N.M.A.-D.), using a standardized set of criteria based on the STROBE statement (*Appendix 2*), which was modified to cover the topic of our review (12). A maximum score of 10 points could be obtained. According to the total quality score, articles were considered as having poor (0-2), intermediate (3-5), good (6-8), or excellent methodological quality (9-10).

RESULTS

IDENTIFICATION AND SELECTION OF THE LITERATURE: From the initial 841 potentially relevant studies identified, 803 were excluded on the basis of title and abstract. Thirty-eight papers were retrieved for detailed assessment: 9 were excluded because of rhGH treatment duration <5 years, 2 studies described pediatric patients only, 1 study did not report original data and 3 studies did not address the long-term effects of rhGH suppletion. Consequently, 23 studies were eligible for inclusion, several describing part of the same patient cohort (Göteborg) (4;6;9;10;13-16) (KIMS database, (5;17)) (*Figure 1 & Table 1*). Therefore, we also mention the specific study centre.

QUALITY ASSESSMENT: According to the predefined quality criteria, no studies were classified as being poor quality. Two studies scored intermediate (18;19), 15 studies were good quality (4-6;9;11;15;18;21-28) and 6 studies had an excellent methodological quality (7;10;13;15;16;20) (*Table 1*). Mean quality score was 7.3 (range 5.0-9.0), indicating that overall methodological quality was good. However, especially the items on potential bias, handling with missing data, reasons for non-participation at each study stage and the use of/handling with co-medication were not clearly described.

METHODOLOGY: All selected studies (n=23) were prospective follow-up studies, and eleven of these had a controlled design. Healthy controls were included in six studies (5;13;19-22), of which two studies used National Registries. Four other studies included GHD patients with intermittent rhGH treatment or untreated GHD patients as controls (11;22;24;25). Gibney *et al.* compared rhGH-treated patients with both untreated GHD patients and healthy controls (23). Four reports involved multi-center studies (5;11;17;24). No randomized controlled trials (RCT) were available.

ENDOCRINE EVALUATION: Different established stimulation tests were used for the diagnosis of severe GHD. The insulin tolerance test (ITT) was used as standard test in 16 studies and the combined GHRH-arginine test, glucagon stimulation test or stimulation with clonidine alone, in the case of contraindications for ITT. Eight studies did not specify which stimulation tests were used (5;11;14;17;22;24-26). Thirteen studies applied the generally used cut-off value for severe GHD of a peak GH<3µg/L (4;6;7;9;10;13;15;16;19;20;23;27;28), in seven studies no exact cut-off value was reported (5;11;14;17;22;24;26), and three studies used, respectively, <1.7µg/l (21), <5µg/l (18) and <10mU/l (25) as cut-off. In all studies, except for one (25), information on additional pituitary deficiencies was given. Most patients had multiple anterior deficiencies, especially gonadotrope deficiency, but thyreotrope and corticotrope deficiency was also frequently encountered.

Duration of rhGH treatment: Mean treatment duration with rhGH ranged from 5 years to 15 years: 8 studies reported 5-year effects, 2 studies had a 6-year follow-up (11;18), 3 studies investigated 7-year effects (7;21;28), 1 study investigated the 9-year effects (25), 8 studies reported 10-year effects (6;10;13-15;23;24;26), 1 study described 15 years of rhGH suppletion (4). In one study, treatment duration varied among the different countries from 4-8 years (5).

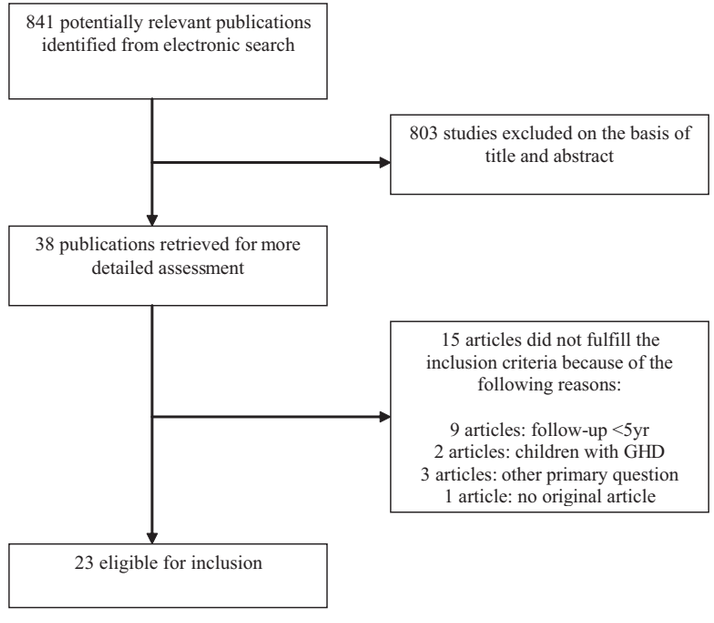

Figure 1: Flow diagram of study selection and exclusion stages.

Table 1. Included studies on the effects of long-term rhGH suppletion

Author (yr (ref))Centre	Total (M/F) AO/CO	Mean age (yr ± SD)	Controls	Mean rhGH dose (mg/day)	Duration of rhGH treatment (yr)
Gibney (1999(22))	10 (7/3) NR	38 (R 21-48)	Untreated GHD (N=11) Healthy controls, age, sex & BMI matched (N=12)	0.025IU/kg/d	10
Chrisoulidou (2000(20))	12 (6/6) 10/2	52 ± 10	GHD, 0.5-1.5yr rhGH (N=11) Untreated GHD (N=10)	NR	7
Clanget (2001(18))	12 (8/4) 9/3	42.5 (R 24-61)	-	µ 2.4IU (0.80mg)	6
Götherström (2001(9))** **Göteborg**	118 (70/48) 118/0	49.3 (R 22-74)	-	0.3 (median)	5
Svensson (2002(26))** **Göteborg**	11 (7/4) 9/2	48.0 (R 20-62)	Healthy controls, age & sex & BMI & WHR matched (N=11)	initial 1.10 end 0.61	10
Gilchrist (2002(23))	61 (27/34) 43/1	37.9 ± 11.8	Group A = continuously Group B = no Group C = intermittently	0.25IU/kg/wk	9
Svensson (2003(17))** **Göteborg**	109 (61/48) 109/0	50.0 (R 22-74)	-	initial 0.88 end 0.46	5

Author (yr (ref))Centre	Tested parameters							Quality score (points)
	Glucose	Cardio vascular#	Anthro-pometric/Body composition##	Muscle strength	Bone	QoL	SAE/ Mortality	
Gibney (1999(22)	x	x	x	x		x		7.5
Chrisoulidou (2000(20))	x	x	x					6.5
Clanget (2001(18))					x			5.0
Götherström (2001(9))** **Göteborg**	x	x	x		x			7.0
Svensson (2002(26))** **Göteborg**	x	x	x					8.0
Gilchrist (2002(23))						x		6.0
Svensson (2003(17))** **Göteborg**			x	x				9.0

Table 2. Outcome of long-term of rhGH therapy in GHD adults

Outcome parameters Nr of studies	Author (yr (ref)) Centre	Total (M/F) AO/CO	Mean age (yr ± SD)	Mean rhGH dose (mg/day)	Duration of rhGH treatment (yr)	Conclusion
Anthropometric parameters N=13	Chrisoulidou (2000(21))	12 (6/6) 10/2	52 ± 10	NR	7	↑ weight & BMI, = WHR
	Götherström (2001(9))** Göteborg	118 (70/48) 118/0	49.3 (R 22-74)	0.3 (median)	5	↑ BMI
	Svensson (2002(26))** Göteborg	11 (7/4) 9/2	48.0 (R 20-62)	initial 1.10 end 0.61	10	= weight & WHR & BMI
	Svensson (2003(17))** Göteborg	109 (61/48) 109/0	50.0 (R 22-74)	initial 0.88 end 0.46	5	= weight & BMI
	Giavoli (2004(28))	20 (11/9) 20/0	44 ± 14	initial 0.98 end 0.47	5	= BMI
	Götherström (2005(14))** Göteborg	26 (12/14) 26/0	65.0 (R 61-74)	initial 0.73 end 0.36	5	= weight & BMI
	Van der Klaauw (2006(7))*** Leiden	63 (30/33) 52/11	46.7 ± 14.3	initial 0.2 end 0.5	7	= WC & WHR ↑ BMI
	Van der Klaauw (2007(29))*** Leiden	50 (24/26) 50/0	45.2 ± 9.1	initial 0.2 end 0.5	5	= waist circ & WHR ↑ BMI
	Götherström (2007(6))** Göteborg	87 (52/35) 87/0	44.1 (R 22-74)	initial 0.98 end 0.47	7	= weight, ↑ BMI
	Cenci (2008(20))	14 (10/4) 14/0	R 33-62	initial 0.87 end 0.64	5	= weight & WC & WHR & BMI
	Götherström (2009(15))** Göteborg	109 (61/48) 109/0	50 R (22-74)	initial 0.88 end 0.47	10	= weight & BMI
	Spielhagen (2011(25))***** KIMS database	440 (224/216) 301/139	♀ 36.7 ± 8.4 ♂ 35.1 ± 8.1 (R 20-49)	♀ initial 0.27 end 0.46 ♂ initial 0.31 end 0.40	10	↑ BMI & WC & HC = WHR
	Elbornsson (2012(4))** Göteborg	126 (72/54) 126/0	49.4 (R 22-74)	initial 0.63 end 0.41	15	↑ weight & BMI
Body composition N=11	Gibney (1999(22))	10 (7/3) NR	38 (R 21-48)	0.025IU/kg/d	10	= BF ↑ LBM ↑ thigh muscle area
	Chrisoulidou (2000(21))	12 (6/6) 10/2	52 ± 10	NR	7	↓ BF ↑ LBM ↓ skinfold thickness (subscap)
	Götherström (2001(9))** Göteborg	118 (70/48) 118/0	49.3 (R 22-74)	initial 0.98 end 0.48	5	↓ BF ↑ LBM
	Svensson (2002(26))** Göteborg	11 (7/4) 9/2	48.0 (R 20-62)	initial 1.10 end 0.61	7	↓ BF ↑ LBM
	Svensson (2003(17))** Göteborg	109 (61/48) 109/0	50.0 (R 22-74)	initial 0.88 end 0.46	5	↓ BF ↑ LBM
	Giavoli (2004(28))	20 (11/9) 20/0	44 ± 14	initial 3-8 µg/kg/d end 0.3	5	↓ BF ↑ LBM
	Götherström (2005(14))** Göteborg	26 (12/14) 26/0	65.0 (R 61-74)	initial 0.73 end 0.36	5	↓ BF ↑ LBM
	Götherström (2007(6))** Göteborg	87 (52/35) 87/0	44.1 (R 22-74)	initial 0.98 end 0.47	10	↓ BF ↑ LBM
	Cenci (2008(20))	14 (10/4) 14/0	R 33-62	initial 0.87 end 0.64	5	↓ visceral fat
	Götherström (2009(15))** Göteborg	109 (61/48) 109/0	50 R (22-74)	initial 0.88 end 0.47	10	= BF ↑ LBM
	Roemmler (2010(24))*	22 (14/8) 16/6	51.5 (R 33-75)	0.3 (median)	10 (2-42)	= BF = LBM

Outcome parameters Nr of studies	Author (yr (ref)) Centre	Total (M/F) AO/CO	Mean age (yr ± SD)	Mean rhGH dose (mg/day)	Duration of rhGH treatment (yr)	Conclusion
Body composition **N=11**	Gibney (1999(22))	10 (7/3) NR	38 (R 21-48)	0.025IU/kg/d	10	= BF ↑ LBM ↑ thigh muscle area
	Chrisoulidou (2000(21))	12 (6/6) 10/2	52 ± 10	NR	7	↓ BF ↑ LBM ↓ skinfold thickness (subscap)
	Götherström (2001(9))** Göteborg	118 (70/48) 118/0	49.3 (R 22-74)	initial 0.98 end 0.48	5	↓ BF ↑ LBM
	Svensson (2002(26))** Göteborg	11 (7/4) 9/2	48.0 (R 20-62)	initial 1.10 end 0.61	7	↓ BF ↑ LBM
	Svensson (2003(17))** Göteborg	109 (61/48) 109/0	50.0 (R 22-74)	initial 0.88 end 0.46	5	↓ BF ↑ LBM
	Giavoli (2004(28))	20 (11/9) 20/0	44 ± 14	initial 3-8 µg/kg/d end 0.3	5	↓ BF ↑ LBM
	Götherström (2005(14))** Göteborg	26 (12/14) 26/0	65.0 (R 61-74)	initial 0.73 end 0.36	5	↓ BF ↑ LBM
	Götherström (2007(6))** Göteborg	87 (52/35) 87/0	44.1 (R 22-74)	initial 0.98 end 0.47	10	↓ BF ↑ LBM
	Cenci (2008(20))	14 (10/4) 14/0	R 33-62	initial 0.87 end 0.64	5	↓ visceral fat
	Götherström (2009(15))** Göteborg	109 (61/48) 109/0	50 R (22-74)	initial 0.88 end 0.47	10	= BF ↑ LBM
	Roemmler (2010(24))*	22 (14/8) 16/6	51.5 (R 33-75)	0.3 (median)	10 (2-42)	= BF = LBM

Outcome parameters Nr of studies	Author (yr (ref)) Centre	Total (M/F) AO/CO	Mean age (yr ± SD)	Mean rhGH dose (mg/day)	Duration of rhGH treatment (yr)	Conclusion TC HDL LDL TG
Lipid metabolism **N=10**	Gibney (1999(22))	10 (7/3) NR	38 (R 21-48)	0.025IU/kg/d	10	=
	Chrisoulidou (2000(21))	12 (6/6) 10/2	52 ± 10	NR	7	↓
	Götherström (2001(9))** Göteborg	118 (70/48) 118/0	49.3 (R 22-74)	initial 0.98 end 0.48	5	↓
	Svensson (2002(26))**** Göteborg	11 (7/4) 9/2	48.0 (R 20-62)	initial 1.10 end 0.61	7	=
	Van der Klaauw (2006(7))*** Leiden	63 (30/33) 52/11	46.7 ± 14.3	initial 0.2 end 0.5	7	↓
	Van der Klaauw (2007(29))*** Leiden	50 (24/26) 50/0	45.2 ± 9.1	initial 0.2 end 0.5	5	NR
	Götherström (2007(6))** Göteborg	87 (52/35) 87/0	44.1 (R 22-74)	initial 0.98 end 0.47	10	↓
	Cenci (2008(20))	14 (10/4) 14/0	R 33-62	initial 0.87 end 0.64	5	=
	Roemmler (2010(24))*	22 (14/8) 16/6	51.5 (R 33-75)	initial 1.10 end 0.61	10 (2-42)	=
	Spielhagen (2011(25))***** KIMS database	440 (224/216) 301/139	♀ 36.7 ± 8.4 ♂ 35.1 ± 8.1 (R 20-49)	♀ initial 0.27 end 0.46 ♂ initial 0.31 end 0.40	10	=

Outcome parameters Nr of studies	Author (yr (ref)) Centre	Total (M/F) AO/CO	Mean age (yr ± SD)	Mean rhGH dose (mg/day)	Duration of rhGH treatment (yr)	Conclusion
Glucose **N=11**	Gibney (1999(22))	10 (7/3) NR	38 (R 21-48)	0.025 IU/kg/d	10	= glucose, = insulin
	Chrisoulidou (2000(21))	12 (6/6) 10/2	52 ± 10	initial 0.98 end 0.48	7	= NEFA, = glucose, = insulin
	Götherström (2001(9))** Göteborg	118 (70/48) 118/0	49.3 (R 22-74)	0.3 (median)	5	↑ glucose, = insulin, ↓ HbA1c
	Svensson (2002(26))**** Göteborg	11 (7/4) 9/2	48.0 (R 20-62)	initial 3-8 µg/kg/d end 0.3	7	= glucose & insulin sensitivity
	Giavoli (2004(28))	20 (11/9) 20/0	44 ± 14	initial 0.98 end 0.47	5	↑ glucose & insulin = HOMA-IR & QUICKI
	Van der Klaauw (2006(7))*** Leiden	87 (52/35) 87/0	44.1 (R 22-74)	initial 0.2 end 0.5	7	= glucose
	Van der Klaauw (2007(29))*** Leiden	50 (24/26) 50/0	45.2 ± 9.1	initial 0.2 end 0.5	5	= glucose
	Götherström (2007(6))** Göteborg	63 (30/33) 52/11	46.7 ± 14.3	initial 0.2 end 0.5	10	↑ glucose, ↓ HbA1c
	Cenci (2008(20))	14 (10/4) 14/0	R 33-62	initial 0.87 end 0.6	5	= glucose & insulin & HOMA-IR
	Roemmler (2010(24))*	22 (14/8) 16/6	51.5 (R 33-75)	initial 1.10 end 0.61	10 (2-42)	= all glucose parameters (including glucose, insulin, HbA1c, insulin sensitivity, insulin resistance, β-cell-function, HOMA)
	Spielhagen (2011(25))***** KIMS database	440 (224/216) 301/139	♀ 36.7 ± 8.4 ♂ 35.1 ± 8.1 (R 20-49)	♀ initial 0.27 end 0.46 ♂ initial 0.31 end 0.40	10	= glucose

Outcome parameters Nr of studies	Author (yr (ref)) Centre	Total (M/F) AO/CO	Mean age (yr ± SD)	Mean rhGH dose (mg/day)	Duration of rhGH treatment (yr)	Conclusion
Cardiovascular parameters *Including Metabolic syndrome* **N=5**	Gibney (1999(22))	10 (7/3) NR	38 (R 21-48)	0.025 IU/kg/d	10	= SBP & DBP = LVM & ESM & EDM ↓ carotid IMT
	Chrisoulidou (2000(21))	12 (6/6) 10/2	52 ± 10	NR	5	= SBP & DBP = IVRT, = LVM = HR, ↓ resting DBP, = SBP = exercise time
	Van der Klaauw (2006(7))*** Leiden	63 (30/33) 52/11	46.7 ± 14.3	initial 0.2 end 0.5	7	= SBP & DBP
	Van der Klaauw (2007(29))*** Leiden	50 (24/26) 50/0	45.2 ± 9.1	initial 0.2 end 0.5	5	= SBP & DBP ↑ MS prevalence
	Cenci (2008(20))	14 (10/4) 14/0	R 33-62	initial 0.87 end 0.64	5	↓ carotid IMT
Neuromuscular function **N=5**	Gibney (1999(22))	10 (7/3) NR	38 (R 21-48)	0.025IU/kg/d	10	= muscle strength
	Svensson (2003(17))** Göteborg	109 (61/48) 109/0	50.0 (R 22-74)	initial 0.88 end 0.46	5	↑ knee flexor & extensor strength ↑ hand grip strength
	Götherström (2005(14))** Göteborg	26 (12/14) 26/0	65.0 (R 61-74)	initial 0.73 end 0.36	5	↑ knee flexor & extensor strength ↑ peak hand grip strength
	Götherström (2009(15))** Göteborg	109 (61/48) 109/0	50 R (22-74)	initial 0.88 end 0.47	10	↑ knee flexor & extensor strength ↑ hand grip strength
	Götherström (2010(16))** Göteborg	24 (11/13) 24/0	65.2 ± 3.4	initial 0.72 end 0.37	10	↑ knee flexor strength = hand grip strength = muscle endurance

Outcome parameters Nr of studies	Author (yr (ref)) Centre	Total (M/F) AO/CO	Mean age (yr ± SD)	Mean rhGH dose (mg/day)	Duration of rhGH treatment (yr)	Conclusion
Bone **N=5**	Clanget (2001(19))	12 (8/4) 9/3	42.5 (R 24-61)	µ 2.4IU (0.80mg)	6	↑ BMC & BMD (lumbar spine, trochanter, = femur neck)
	Götherström (2001(9))** Göteborg	118 (70/48) 118	49.3 (R 22-74)	initial 0.98 end 0.48	5	↑ BMC & BMD = osteocalcin & Ca2+ & PTH
	Götherström (2007(10))** Göteborg	87 (52/35) 87/0	44.1 (R 22-74)	initial 0.98 end 0.47	10	↑ BMC & BMD (total body, lumbar spine, prox femur) ↑ osteocalcin & Ca2+
	Roemmler (2010(24))*	22 (14/8) 16/6	51.5 (R 33-75)	0.3 (median)	10 (2-42)	= BMD
	Elbornsson (2012(4))** Göteborg	126 (72/54) 126/0	49.4 (R 22-74)	initial 0.63 end 0.41	15	↑ BMC & BMD (total body, lumbar spine, plateau) ↑ BMC, = BMD (prox femur, peak at 7yr, thereafter ↓) Fractures: 1 hip, 1 symp VF
Quality of life **N=4**	Gibney (1999(22))	10 (7/3) NR	38 (R 21-48)	0.025IU/kg/d	10	↑ overall psychological well-being, energy, emotional reaction
	Gilchrist (2002(23))	61 (27/34) 43/1	37.9 ± 11.8	0.25IU/kg/wk	9	**NHP**: ↑ energy levels, = sleep & emotional reaction & social isolation & physical mobility & pain **PGWB:** ↑ vitality score

Outcome parameters Nr of studies	Author (yr (ref)) Centre	Total (M/F) AO/CO	Mean age (yr ± SD)	Mean rhGH dose (mg/day)	Duration of rhGH treatment (yr)	Conclusion
	Koltowska-Häggström (2006(5))***** KIMS database	UK 758 (363/395) 704/54 NL 247 (123/124) 222/25 Spain 197 (75/122) 176/21 Sweden 484 (247/237) 453/31	UK 48.5±12.6 NL 48.2±13.3 Spain 45±11.1 Sweden 51.4±13.0	NR	UK 7 NL 6 Spain 4 Sweden 8	**QoL-AGHDA:** ↑ in 1st yr, after that regression to country-specific mean (normalization)
	Spielhagen (2011(25))***** KIMS database	440 (224/216) 301/139	♀ 36.7 ± 8.4 ♂ 35.1 ± 8.1 (R 20-49)	♀ initial 0.27 end 0.46 ♂ initial 0.31 end 0.40	10	**QoL-AGHDA:** ↑ in 1st yr, sustained during longer follow-up
Mortality/SAE N=3	**Svensson (2004(27))**** Göteborg	289 (186/103) 225/64	47.6 ± 14.8	N=80 11.9µg/kg/d N=209 indiv dose titration based on IGF1	5	= overall mortality & malignancy rate ↓ MI rate, ↑ CVA rate
	Van Bunderen (2011(11))	2229 (1160/1069) 1718/511	42.6 ± 16.3	µ 0.48	5.7 (median)	= mortality compared to background pop (after exclusion high-risk) = malignancy mortality ↑ CVD mortality in ♀
	Gaillard (2012(18))***** KIMS database	13983 (7174/6809) 10767/3216	CO 26.9 ± 9.9 AO 48.8 ± 13.0	NR	4.9	↑ overall mortality in ♀ (moderate) = malignancy & CVD mortality ↑ CVA & infection mortality ↑ progression primary tumour & endocrine complications

* *3 patients treated <5yr*

** *Overlapping patient cohorts Götherström 2010, Götherström 2005, Götherström 2001, Götherström 2007, Götherström 2007, Götherström 2009, Elbornsson 2012, Svensson 2003, Svensson 2004*

*** *Overlapping patient cohorts Van der Klaauw 2006 & Van der Klaauw 2007*

**** *Control group at baseline differed from controls at 10 year*

***** *Overlapping patient cohorts KIMS database Koltowska-Häggström 2006, Gaillard 2012, Spielhagen 2011*

rhGH, recombinant human growth hormone; GHD, growth hormone deficiency; AO, adult-onset GHD; CO, childhood-onset GHD; R, range; BF, body fat; LBM, lean body mass; ITT, insulin-tolerance test; GHRH, growth hormone releasing hormone; BMD, bone mineral density; BMC, bone mineral content; TG, triglycerides; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; BP, blood pressure; MS, metabolic syndrome; WC, waist circumference; HC, hip circumference; WHR, waist-hip ratio; HOMA-IR, homeostasis model assessment of insulin resistance; QUICKI, quantitative insulin check index, Carotid IMT, intima media thickness of carotid arteries; QoL, quality of life; NHP, Nottingham Health Profile; PGWB, psychological general well-being schedule; QoL-AGHDA, quality of life-assessment of GH deficiency in adults; LVM, left ventricular mass; ESM, end systolic diameter; EDM, end diastolic diameter; Ca2+, calcium; NEFA, nonesterified fatty acids; Lp (a), lipoprotein (a); APU, apparent phalangeal ultrasound transmission velocity; CVD, cardiovascular disease; IVRT, isovolumic relaxation time; LVM, left ventricular mass; VF; vertebral fracture; UK, United Kingdom; NL, Netherlands; MI, myocardial infarction; CVA, cerebrovascular attack; SAE, serious adverse event; NR, not reported.

DOSE OF RHGH TREATMENT & IGF-1 LEVELS: Fourteen studies (nine from Göteborg) calculated the GH starting dose based on body weight (4;6;9;10;13-16;19;21-23;25;27). Of the remaining nine studies, three studies used dose titration of rhGH on an individual basis in the beginning of treatment, with the aim to reach IGF-1 levels within the normal age- and sex-related range, and adjusted the rhGH dose accordingly (7;20;26). Six studies did not report their rhGH dosage regimen (5;11;17;18;24;28). GH start dose ranged from 0.2mg/day (7;20) to 1.1mg/day (21). Twelve studies reported mean GH dose after dose titration (at 1 year) (6;9;10;13-17;19;21;24;27), ranging from 0.3mg/day (19) to 0.85mg/day (21) (median dose 0.54 mg/day).

Sixteen studies reported mean IGF-1 levels (4;7;9;10;13-16;18;19;21-24;26;28), and 13 studies described IGF-1 SDS to evaluate treatment response (4;6;9;10;13-17;20-22;24) (*Appendix 3)*. Based on available IGF-1 values, we can conclude that all patients were physiologically substituted at least after 5 to 7 years treatment with rhGH, independently of the applied starting dosage regimen.

Outcome of long-term rhGH replacement (*Table 2*)

ANTHROPOMETRIC PARAMETERS: Thirteen studies (N=1185) assessed the effects on body weight, body mass index (BMI) and/or waist-hip ratio (WHR) (4;6;7;9;10;13;14;20;21;24;26-28). rhGH suppletion did not affect WHR or waist circumference (7;20;21;26-28), except for one study reporting an increased waist circumference (24). Seven studies reporting BMI found a significant increase during rhGH suppletion ≥5years, while the other six studies reported no change (4;6;7;9;13;14;16;19-21;24;27;28). Two of the eight studies reporting body weight found an increase in weight during rhGH suppletion; the other studies reported no change (4;10;13;14;16;21;27;28).

In conclusion, results on long-term effects on BMI appear to be inconclusive with seven studies reporting an increase, and six reporting no change. In addition, there is little evidence for an increase in body weight.

BODY COMPOSITION: Eleven studies (N=538) (6;9;13;14;16;19;21;23;26-28) assessed the effects of rhGH suppletion on body composition using dual-energy X-ray absorptiometry (DEXA) scan (6;9;13;14;16;26;28), bioelectrical impedance analysis (BIA) (19;21) or a helicoidal CT scan (27). Gibney et al. measured body composition with radioisotope potassium (23). The majority of studies (n=9) reported consistent effects with an increase in LBM and a decrease in total body fat (BF) (6;9;13;14;16;19;21;23;28). One study could not detect any effect of rhGH on body composition (26). Cenci et al. reported only a decrease in visceral fat (27).

In conclusion, rhGH suppletion had favourable effects on body composition, with an increase in LBM and a decrease in total BF. Several methods are available to evaluate body composition, which may account for differences between studies. These differences might have influenced the results significantly. In literature, DEXA is reported to be the best currently available technique for measuring body composition (29); however, in this respect, it has to be noted that DEXA cannot distinguish between body cell mass (BCM) and water. Therefore, the LBM data may not be accurate, since rhGH replacement is associated with an increase in the intracellular water component (30-33). However, also when using other modalities to evaluate body composition, positive long-term effects of rhGH replacement were found.

LIPID METABOLISM: Ten studies (N=827) assessed the effects of rhGH suppletion on fasting plasma lipid profiles (6;7;9;20;21;23;24;26-28). Three studies did not find any effect of rhGH therapy on plasma lipids (24;26;27). The seven remaining studies reported generally similar results, *i.e.* reduction of total cholesterol (TC) and low-density lipoprotein (LDL) cholesterol, an increase in high-density lipoprotein (HDL) cholesterol and no effect on triglycerides (6;7;9;20;21;23;28). In 6 patients, new-onset hyperlipidaemia was reported (Göteborg). However, only four studies reported the number of patients on lipid-lowering medication, especially statins, (6;7;20;26) but without excluding these patients from analysis.

In summary, there are ongoing beneficial effects of rhGH suppletion on plasma lipids. However, a strong limitation is the lack of data concerning lipid-lowering treatment, which has a considerable influence on the outcome parameter. Due to the lack of information on lipid-lowering drugs and the overlap in patient groups, meta-analysis was not an option.

GLUCOSE METABOLISM: Eleven studies (N=847) assessed the effects of rhGH suppletion on glucose and insulin levels (6;7;9;19-21;23;24;26-28). Seven studies showed no effect on glucose (7;20;23;24;26-28), three an increase (6;9;19) and one study found a transient increase of glucose levels only during the first year of rhGH therapy (21). Insulin levels increased only in one study (19). HbA1c was evaluated in three studies, two of which observed a decrease (6;9); the other found no change (26). Three studies investigated insulin resistance by the homeostasis model assessment (HOMA) (19;26;27), reporting no significant effects of rhGH suppletion. Insulin sensitivity was not affected in two studies (21;26), while another study reported a transient decrease, using the quantitative insulin check index (QUICKI), showing a trend to return towards basal values afterwards (19). In at least ten patients, new-onset diabetes mellitus type 2 (DM2) was diagnosed (4 Göteborg, 6 KIMS (24)). Five studies reported the number of patients starting anti-diabetic medication during follow-up (6;9;21;26;27). Only one study excluded diabetic patients from analysis (9), while the other studies did not report the handling with anti-diabetic medication in their statistical analysis.

In conclusion, there is moderate evidence for an increase in mean glucose levels after chronic rhGH treatment; however, insulin sensitivity appeared not to be affected in the long term. Due to the lack of information on anti-diabetic medication and the overlap in patient cohorts, we were not able to perform a meta-analysis on this parameter.

CARDIOVASCULAR PARAMETERS: Four studies (N=135) investigated the effects of rhGH suppletion on blood pressure (7;9;20;23;28). No effect was found on systolic blood pressure (SBP); one study reported a decrease in resting DBP (28). Only two studies described the number of patients on antihypertensive medication (7;20), without excluding them from analysis. Prevalence of the metabolic syndrome (MS) was assessed in one study, reporting a strong increase when compared to healthy controls, despite 5 years of rhGH treatment (38.0% *vs* 15.7%) (20).

Two studies (N=22) evaluated the long-term effects on cardiac function (23;28) using echocardiography; Chrisoulidou *et al.* also performed an exercise test (28). Left ventricular mass (LVM) was not affected. In addition, no effect on end diastolic or systolic diameter (23) nor on isovolumetric relaxation time (IVRT) or LVM was demonstrated (23;28). Two studies (N=24) evaluated carotid intima media thickness (IMT), reporting a decrease in IMT up to 18% after long-term rhGH therapy (23;27).

In conclusion, no effect on SBP of LVM was reported. In addition, conclusions on the prevalence of MS cannot be made, since this parameter was only evaluated in one study. Long-term rhGH suppletion had positive effects on IMT.

MUSCLE STRENGTH: Five studies (N=278, all AO-GHD) investigated the effects of rhGH suppletion on muscle strength (13-16;23). In general, 5 years of rhGH improved knee flexor and extensor strength and hand-grip strength. However, these effects were not sustained after 10 years. One study also investigated muscle endurance; no significant effects of rhGH suppletion were demonstrated (15). In addition, rhGH suppletion did not affect muscle strength in the elbow, shoulder or hip (23). Compared with population-based controls, rhGH was found to be protective to the normal age-related decline in muscle performance and neuromuscular function (13;14).

In general, rhGH suppletion improved muscle strength during the first 5 years of treatment; however, these effects were not sustained after prolonged follow-up. Since almost all studies described one patient group, we were not able to perform a meta-analysis on this parameter.

BONE PARAMETERS: Four studies (N=343) evaluated the long-term effects of rhGH on bone (4;9;10;18). After an initial decline, all studies reported an increase in both BMD and BMC within the first 5 years of treatment. After 5 years, all studies reported a plateau phase, which lasted for the entire study period (*i.e.* sustained effects) (4;9;10;18). Only

one study investigated (symptomatic) fracture prevalence, reporting 1 hip fracture and 1 symptomatic vertebral fracture (4). None of the studies described the use of calcium or Vitamin D suppletion, or the use of bisphosphonates, selective estrogen receptor modulator (SERM), teriparatide or strontium ranelate. Again, most patients were from the same study centre, prohibiting the performance of a meta-analysis.

QOL: Two large cohort studies (both KIMS database, N=1686 and N=440, respectively) and two smaller studies (N=71) addressed the effects on QoL and neuropsychological well-being, by QoL-Assessment of GHD in Adults (QoL-AGHDA), Nottingham Health Profile (NHP), and Psychological General Well-Being (PGWB) questionnaires (5;23-25). Within the first year of treatment, a direct beneficial response on well-being was noticed, as assessed by QoL-AGHDA, with marginal changes after the dose-titrating phase (5;24). Improvement was related to the degree of QoL impairment at baseline (5). Scores on NHP and PGWB improved in the long-term, especially with respect to energy level and emotional reaction (23;25).

In conclusion, QoL seemed to improve during long-term rhGH replacement, but positive effects on QoL were especially seen within the first year of treatment. Since studies are scarce and none of them had a placebo-controlled randomized controlled design, which is of paramount importance for the interpretation of QoL, no firm conclusions can be drawn on the effects of long-term rhGH replacement on QoL.

EFFECTS OF RHGH ON MORTALITY: Three cohort studies (N=16501) reported data on mortality after long-term rhGH suppletion (11;17;22), of which one study reports KIMS data (N=13983). In Gaillard *et al.*, overall mortality rate was moderately increased, especially in females, young attained age, patients with aggressive tumours and lower IGF-1 SDS during therapy (17). The other studies did not report differences in overall mortality or malignancy-related death. In two studies (17;22), the rate of cerebrovascular accidents (CVAs) was increased, but the incidence of myocardial infarction was decreased (risk ratio (RR) 0.27, 95%CI 0.03-0.99) when compared with the background population (22). In addition, rates of death due to infections (standardized mortality ratio (SMR) 4.97, 95%CI 3.98-6.14), primary tumour progression (SMR=6.96, 95%CI 4.45-10.3) and endocrine complications (SMR=2.79, 95%CI 1.30-5.23) were increased (17). Van Bunderen *et al.* (N=2229; median rhGH treatment of 5.7 yr) reported an increase in cardiovascular-related deaths in GHD-treated women (SMR 2.36) (11). This was not supported by the other long-term studies (17;22).

In summary, there is evidence for a moderate increased mortality rate despite long-term rhGH replacement, especially in females. Differences in pituitary disease, hormone replacement- and radiation therapies may account for differences between studies. However, since long-term studies on mortality are scarce, at this moment, no firm conclusions on the safety of long-term rhGH replacement can be drawn.

SERIOUS ADVERSE EVENTS (SAES): Twelve studies reported SAEs during long-term rhGH treatment (n=9 from Göteborg) (4;6;7;9;13-16;21;24;28). Since several studies described the same patient cohort, the total number of SAEs could not be extracted.

Malignancies: A total of 6 malignancy cases were reported in the GHD cohort of Göteborg: colonic carcinoma (N=1), prostate carcinoma (N=1), chronic lymphatic leukaemia (N=1), renal carcinoma (N=1), bladder carcinoma (N=1), and pulmonary carcinoma (N=1). In addition, 12 cases of *de novo* malignancies, not further specified, were reported (7;24).

Cardiovascular events: Three cases of myocardial infarction (Göteborg, Van der Klaauw *et al.* (7)) were reported, of which two with fatal outcome. Five patients suffered from a CVA (Göteborg, Van der Klaauw *et al.* (7)), resulting in death in four cases, and one patient died from a subarachnoid hemorrhage (Göteborg). In addition, angina pectoris was reported (N=3) (Göteborg), and 1 patient died from a pulmonary embolism (Göteborg). In at least ten patients, new-onset DM2 was diagnosed (4 Göteborg, 6 KIMS (24)). In, respectively, 6 and 4 patients new-onset hyperlipidaemia and hypertension were reported (Göteborg, (28)).

Other SAEs: Two studies reported primary tumour growth (N=11), either recurrence of the pituitary or central nervous system tumour (7;24).

In conclusion, half of the studies reported SAEs during rhGH therapy. Due to the lack of a control group including non-treated GHD patients, no firm conclusions can be drawn.

DISCUSSION

The short-term effects of rhGH treatment are well-established, showing favourable effects of rhGH therapy within the first 3 years on body composition, lipid profile, bone turnover, BMD, muscle strength, cognitive function, and QoL. This is the first systematic review focusing on the long-term effects of rhGH treatment (beyond ≥5 years of treatment). Overall, long-term rhGH therapy beneficially affected QoL and BMD. In addition, sustained beneficial effects were reported on lipid spectrum and on carotid IMT after 10 years of treatment. Consistent effects on body composition were reported with an increased LBM and a decreased total BF. Muscle strength increased after 5 years of rhGH suppletion, but these effects were not sustained during longer follow-up. Negative effects were reported on mean glucose levels; however, insulin sensitivity appeared not to be affected by long-term rhGH treatment.

The effects of rhGH replacement on bone turnover, and thus on BMD, are dynamic over time. Initially, BMD decreases due to increased bone resorption and formation, followed by an increase within the first years of treatment, finally resulting in a plateau phase. The five studies addressing this topic showed a significant increase within the first 5 years of treatment. Thereafter, BMD was conserved but did not further increase (4;9;10). Only one study described the number of symptomatic fractures (4), however, without radiographic assessment of the vertebral column. As a consequence, the reported fracture rate is most probably underestimated, since 21% of the fractures in patients with osteopenia or osteoporosis are asymptomatic (34). Fracture incidence was also studied by Holmer *et al.* (35), but a considerable proportion of patients were treated <5 years. In CO-GHD women, not in AO-GHD women or CO-GHD men, fracture risk was doubled, and in men with AO-GHD fracture incidence was even decreased (35). Further research has to establish the efficacy of rhGH treatment with respect to fracture risk.

Presently, in clinical practice, QoL is the most frequently addressed outcome parameter, but in the long-term, QoL was studied only in four studies (5;23-25). The two largest studies (KIMS database) showed improvement within the first year of treatment, with thereafter steady progress towards the country-specific mean (*i.e.* normalization) (5;24). The other two studies found ongoing beneficial effects on energy level and emotional reaction (23;25). Unfortunately, none of these studies had a placebo-controlled randomized controlled design. Thus, based on current literature, long-term effects on QoL are inconclusive, and therefore, in daily clinical practice, QoL should be individually evaluated in each patient.

With respect to plasma lipids, seven out of ten studies investigating the long-term effects of rhGH replacement on the lipid profile report ongoing beneficial effects, with a reduction of TC and LDL-C levels, an increase in HDL-C and no effect on triglycerides. However, in this respect, a strong limitation is the lack of data concerning lipid-lowering treatment, prohibiting to draw firm conclusions on this outcome parameter. When comparing these long-term results to short-term studies on plasma lipids, it has be noted that there were also several (randomized controlled) short-term studies reporting no change or minimal effects on lipid levels, and that even these effects were limited to men (36;37). Overall, however, as shown in a meta-analysis of Maison *et al.* investigating 37 placebo-controlled randomized controlled studies (follow-up up to 18 months), rhGH replacement was shown to improve LDL-C and TC levels in the short term (3).

Concerning the safety of rhGH suppletion, in children, long-term safety data have shown conflicting data, raising the question whether long-term use of rhGH is safe. Several large studies did not find an increased mortality in paediatric GHD patients after rhGH replacement, especially not due to new-onset malignancies or recurrence of central nervous system tumors. Cause of death was assessed as unrelated to rhGH replacement in the majority of patients (38-42). In contrast, a recent study of Carel *et al.* showed an increased incidence in bone tumor-related mortality and mortality due to cardiovascular diseases in a large cohort of French children, especially in those with highest GH doses (>50µg/kg/day) (43). Cancer mortality was also increased in a large paediatric UK cohort, especially in high-risk patients (44). Moreover, Bell *et al.* reported an increase in the occurrence of second tumors in children with a prior history of malignancy, especially in case of prior exposure to radiotherapy (38). These discrepant results could be possibly explained by the different dosage regime, with higher GH doses in the French study. A recent meta-analysis underlines this hypothesis, establishing that both low and high IGF-1 levels are associated with increased mortality (hazard ratio (HR) 1.18, 95%CI 1.04-1.34) (45). Adult studies did not show an increased mortality or malignancy rate (11;22), except for one study showing moderate increase in overall mortality in females (17). However, the studies do not represent unselected patients, as initiation of rhGH replacement is not likely in patients with an a priori increased cancer risk, thus creating a clear selection bias. With respect to cardiovascular outcomes, an increase in cardiovascular deaths and number of CVAs was reported in women, whereas the myocardial infarction rate decreased (11;17;22). The increased incidence in cardiovascular deaths in

women might partly be attributed to the more atypical presentation of cardiovascular diseases in women (46). Other factors such as inadequately substituted hypogonadism causing preterm menopause or hypocortisolism may also have influenced this risk. In this respect, it has to be noted that available data suggest that both mortality risk and the causes for mortality are related in both patients receiving and those not receiving rhGH replacement. However, it is of paramount importance to accentuate that all observed increased mortality rates reported to date have been associated only with the presence of hypopituitarism *per se*, but that it is still uncertain if, and to what extent, this can be attributed to GHD.

We assessed the methodological quality of all studies using a modified STROBE list (12), classifying 21 of the 23 included studies as having a good or excellent methodological quality. Several quality criteria were not clearly described; specifically, information on potential bias, handling with missing data and reasons for dropout was lacking in most studies. In addition, the use of co-medication, especially lipid-lowering and anti-diabetic drugs, was not clearly reported. It is of importance to emphasize this limitation, because the effects of rhGH therapy on lipid profile, glucose levels and other cardiovascular parameters could not be interpreted without this information. In addition, no RCTs were identified with a follow-up duration >5 years and only a limited number of centres have reported their data, with half of the long-term studies describing (part of) the same patient cohort (Göteborg). Other studies were relatively small and might have been, therefore, underpowered to allow firm conclusions. Due to these limitations, we were not able to perform a meta-analysis.

Another limitation is the report of outcomes only for patients that had completed 5 or 10-years of follow-up, instead of describing the original cohort. This may introduce a significant selection bias, since patients discontinued rhGH therapy for specific reasons, and, therefore, are likely to differ from patients with complete follow-up. Furthermore, a major limitation of most studies is the lack of a control group and thereby the lack of control for ageing. This limitation is important to notice when interpreting the long-term effects of rhGH replacement on for example body weight, BMI, blood pressure, lipid profile, body composition and BMD (47-49). Consequently, it is very difficult to discriminate between effects as a consequence of natural ageing processes and effects by rhGH replacement *per se*. Firm conclusions on the effects of rhGH *per se* can therefore not be drawn from present results. Another feature that needs to be addressed is the change in patient characteristics and approach to rhGH suppletion over time, which may represent an important confounder for any analysis (50). This emphasizes the need of ongoing monitoring during rhGH treatment.

In summary, 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 and lacked a control group (of non-treated GHD patients) enabling adjustment for subjective changes or changes due to ageing. Second, only a limited number of centres have reported their data, resulting in a low number of evaluable patients with a follow-up duration of ≥5 years of rhGH replacement 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 the long-term for body composition, lipid profile, carotid IMT and BMD, but overall cardiovascular risk, as assessed by the prevalence of the metabolic syndrome, glucose levels, BMI appeared not to be influenced or were even negatively affected. Therefore, 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.

REFERENCE LIST

1. Rosen T, Bengtsson BA. Premature mortality due to cardiovascular disease in hypopituitarism. Lancet 1990; 336(8710):285-288.
2. Tomlinson JW, Holden N, Hills RK et al. Association between premature mortality and hypopituitarism. West Midlands Prospective Hypopituitary Study Group. Lancet 2001; 357(9254):425-431.
3. Maison P, Griffin S, Nicoue-Beglah M, Haddad N, Balkau B, Chanson P. Impact of growth hormone (GH) treatment on cardiovascular risk factors in GH-deficient adults: a Metaanalysis of Blinded, Randomized, Placebo-Controlled Trials. J Clin Endocrinol Metab 2004; 89(5):2192-2199.
4. Elbornsson M, Gotherstrom G, Bosaeus I, Bengtsson BA, Johannsson G, Svensson J. Fifteen years of GH replacement increases bone mineral density in hypopituitary patients with adult-onset GH deficiency. Eur J Endocrinol 2012; 166(5):787-795.
5. Koltowska-Haggstrom M, Mattsson AF, Monson JP et al. Does long-term GH replacement therapy in hypopituitary adults with GH deficiency normalise quality of life? Eur J Endocrinol 2006; 155(1):109-119.
6. Gotherstrom G, Bengtsson BA, Bosaeus I, Johannsson G, Svensson J. A 10-year, prospective study of the metabolic effects of growth hormone replacement in adults. J Clin Endocrinol Metab 2007; 92(4):1442-1445.
7. van der Klaauw AA, Romijn JA, Biermasz NR et al. Sustained effects of recombinant GH replacement after 7 years of treatment in adults with GH deficiency. Eur J Endocrinol 2006; 155(5):701-708.
8. Biermasz NR, Hamdy NA, Pereira AM, Romijn JA, Roelfsema F. Long-term skeletal effects of recombinant human growth hormone (rhGH) alone and rhGH combined with alendronate in GH-deficient adults: a seven-year follow-up study. Clin Endocrinol (Oxf) 2004; 60(5):568-575.
9. Gotherstrom G, Svensson J, Koranyi J et al. A prospective study of 5 years of GH replacement therapy in GH-deficient adults: sustained effects on body composition, bone mass, and metabolic indices. J Clin Endocrinol Metab 2001; 86(10):4657-4665.
10. Gotherstrom G, Bengtsson BA, Bosaeus I, Johannsson G, Svensson J. Ten-year GH replacement increases bone mineral density in hypopituitary patients with adult onset GH deficiency. Eur J Endocrinol 2007; 156(1):55-64.
11. van Bunderen CC, van N, I, Arwert LI et al. Does growth hormone replacement therapy reduce mortality in adults with growth hormone deficiency? Data from the Dutch National Registry of Growth Hormone Treatment in adults. J Clin Endocrinol Metab 2011; 96(10):3151-3159.
12. von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. J Clin Epidemiol 2008; 61(4):344-349.
13. Gotherstrom G, Bengtsson BA, Sunnerhagen KS, Johannsson G, Svensson J. The effects of five-year growth hormone replacement therapy on muscle strength in elderly hypopituitary patients. Clin Endocrinol (Oxf) 2005; 62(1):105-113.
14. Gotherstrom G, Elbornsson M, Stibrant-Sunnerhagen K, Bengtsson BA, Johannsson G, Svensson J. Ten years of growth hormone (GH) replacement normalizes muscle strength in GH-deficient adults. J Clin Endocrinol Metab 2009; 94(3):809-816.
15. Gotherstrom G, Elbornsson M, Stibrant-Sunnerhagen K, Bengtsson BA, Johannsson G, Svensson J. Muscle strength in elderly adults with GH deficiency after 10 years of GH replacement. Eur J Endocrinol 2010; 163(2):207-215.
16. Svensson J, Sunnerhagen KS, Johannsson G. Five years of growth hormone replacement therapy in adults: age- and gender-related changes in isometric and isokinetic muscle strength. J Clin Endocrinol Metab 2003; 88(5):2061-2069.
17. Gaillard RC, Mattsson AF, Akerblad AC et al. Overall and cause-specific mortality in GH-deficient adults on GH replacement. Eur J Endocrinol 2012; 166(6):1069-1077.
18. Clanget C, Seck T, Hinke V, Wuster C, Ziegler R, Pfeilschifter J. Effects of 6 years of growth hormone (GH) treatment on bone mineral density in GH-deficient adults. Clin Endocrinol (Oxf) 2001; 55(1):93-99.
19. Giavoli C, Porretti S, Ronchi CL et al. Long-term monitoring of insulin sensitivity in growth hormone-deficient adults on substitutive recombinant human growth hormone therapy. Metabolism 2004; 53(6):740-743.
20. van der Klaauw AA, Biermasz NR, Feskens EJ et al. The prevalence of the metabolic syndrome is increased in patients with GH deficiency, irrespective of long-term substitution with recombinant human GH. Eur J Endocrinol 2007; 156(4):455-462.
21. Svensson J, Fowelin J, Landin K, Bengtsson BA, Johansson JO. Effects of seven years of GH-replacement therapy on insulin sensitivity in GH-deficient adults. J Clin Endocrinol Metab 2002; 87(5):2121-2127.
22. Svensson J, Bengtsson BA, Rosen T, Oden A, Johannsson G. Malignant disease and cardiovascular morbidity in hypopituitary adults with or without growth hormone replacement therapy. J Clin Endocrinol Metab 2004; 89(7):3306-3312.
23. Gibney J, Wallace JD, Spinks T et al. The effects of 10 years of recombinant human growth hormone (GH) in adult GH-deficient patients. J Clin Endocrinol Metab 1999; 84(8):2596-2602.
24. Spielhagen C, Schwahn C, Moller K et al. The benefit of long-term growth hormone (GH) replacement therapy in hypopituitary adults with GH deficiency: results of the German KIMS database. Growth Horm IGF Res 2011; 21(1):1-10.
25. Gilchrist FJ, Murray RD, Shalet SM. The effect of long-term untreated growth hormone deficiency (GHD) and 9 years of GH replacement on the quality of life (QoL) of GH-deficient adults. Clin Endocrinol (Oxf) 2002; 57(3):363-370.
26. Roemmler J, Kuenkler M, Schneider HJ, Dieterle C, Schopohl J. Comparison of glucose and lipid metabolism and bone mineralization in patients with growth hormone deficiency with and without long-term growth hormone replacement. Metabolism 2010; 59(3):350-358.
27. Cenci MC, Conceicao FL, Soares DV et al. Impact of 5 years of growth hormone replacement therapy on cardiovascular risk factors in growth hormone-deficient adults. Metabolism 2008; 57(1):121-129.
28. Chrisoulidou A, Beshyah SA, Rutherford O et al. Effects of 7 years of growth hormone replacement therapy in hypopituitary adults. J Clin Endocrinol Metab 2000; 85(10):3762-3769.
29. Henche SA, Torres RR, Pellico LG. An evaluation of patterns of change in total and regional body fat mass in healthy Spanish subjects using dual-energy X-ray absorptiometry (DXA). Eur J Clin Nutr 2008; 62(12):1440-1448.
30. Salomon F, Cuneo RC, Hesp R, Sonksen PH. The effects of treatment with recombinant human growth hormone on body composition and metabolism in adults with growth hormone deficiency. N Engl J Med 1989; 321(26):1797-1803.
31. Moller J, Jorgensen JO, Moller N, Hansen KW, Pedersen EB, Christiansen JS. Expansion of extracellular volume and suppression of atrial natriuretic peptide after growth hormone administration in normal man. J Clin Endocrinol Metab 1991; 72(4):768-772.
32. Moller J, Moller N, Frandsen E, Wolthers T, Jorgensen JO, Christiansen JS. Blockade of the renin-angiotensin-aldosterone system prevents growth hormone-induced fluid retention in humans. Am J Physiol 1997; 272(5 Pt 1):E803-E808.
33. Janssen YJ, Deurenberg P, Roelfsema F. Using dilution techniques and multifrequency bioelectrical impedance to assess both total body water and extracellular water at baseline and during recombinant human growth hormone (GH) treatment in GH-deficient adults. J Clin Endocrinol Metab 1997; 82(10):3349-3355.
34. Netelenbos JC, Lems WF, Geusens PP et al. Spine radiographs to improve the identification of women at high risk for fractures. Osteoporos Int 2009; 20(8):1347-1352.
35. Holmer H, Svensson J, Rylander L et al. Fracture incidence in GH-deficient patients on complete hormone replacement including GH. J Bone Miner Res 2007; 22(12):1842-1850.
36. Sesmilo G, Biller BM, Llevadot J et al. Effects of growth hormone administration on inflammatory and other cardiovascular risk markers in men with growth hormone deficiency. A randomized, controlled clinical trial. Ann Intern Med 2000; 133(2):111-122.
37. Hoffman AR, Biller BM, Cook D et al. Efficacy of a long-acting growth hormone (GH) preparation in patients with adult GH deficiency. J Clin Endocrinol Metab 2005; 90(12):6431-6440.
38. Bell J, Parker KL, Swinford RD, Hoffman AR, Maneatis T, Lippe B. Long-term safety of recombinant human growth hormone in children. J Clin Endocrinol Metab 2010; 95(1):167-177.
39. Ogilvy-Stuart AL, Gleeson H. Cancer risk following growth hormone use in childhood: implications for current practice. Drug Saf 2004; 27(6):369-382.

40. Savendahl L, Maes M, bertsson-Wikland K et al. Long-term mortality and causes of death in isolated GHD, ISS, and SGA patients treated with recombinant growth hormone during childhood in Belgium, The Netherlands, and Sweden: preliminary report of 3 countries participating in the EU SAGhE study. J Clin Endocrinol Metab 2012; 97(2):E213-E217.
41. Sklar CA, Mertens AC, Mitby P et al. Risk of disease recurrence and second neoplasms in survivors of childhood cancer treated with growth hormone: a report from the Childhood Cancer Survivor Study. J Clin Endocrinol Metab 2002; 87(7):3136-3141.
42. Taback SP, Dean HJ. Mortality in Canadian children with growth hormone (GH) deficiency receiving GH therapy 1967-1992. The Canadian Growth Hormone Advisory Committee. J Clin Endocrinol Metab 1996; 81(5):1693-1696.
43. Carel JC, Ecosse E, Landier F et al. Long-term mortality after recombinant growth hormone treatment for isolated growth hormone deficiency or childhood short stature: preliminary report of the French SAGhE study. J Clin Endocrinol Metab 2012; 97(2):416-425.
44. Swerdlow AJ, Higgins CD, Adlard P, Preece MA. Risk of cancer in patients treated with human pituitary growth hormone in the UK, 1959-85: a cohort study. Lancet 2002; 360(9329):273-277.
45. Burgers AM, Biermasz NR, Schoones JW et al. Meta-analysis and dose-response metaregression: circulating insulin-like growth factor I (IGF-I) and mortality. J Clin Endocrinol Metab 2011; 96(9):2912-2920.
46. Dey S, Flather MD, Devlin G et al. Sex-related differences in the presentation, treatment and outcomes among patients with acute coronary syndromes: the Global Registry of Acute Coronary Events. Heart 2009; 95(1):20-26.
47. Ettinger WH, Wahl PW, Kuller LH et al. Lipoprotein lipids in older people. Results from the Cardiovascular Health Study. The CHS Collaborative Research Group. Circulation 1992; 86(3):858-869.
48. Nooyens AC, Visscher TL, Verschuren WM et al. Age, period and cohort effects on body weight and body mass index in adults: The Doetinchem Cohort Study. Public Health Nutr 2009; 12(6):862-870.
49. Franklin SS, Gustin W, Wong ND et al. Hemodynamic patterns of age-related changes in blood pressure. The Framingham Heart Study. Circulation 1997; 96(1):308-315.
50. Kreitschmann-Andermahr I, Siegel S, Francis F et al. Variation of the baseline characteristics and treatment parameters over time: an analysis of 15 years of growth hormone replacement in adults in the German KIMS database. Pituitary 2012.

APPENDIX 1.

Search strategy used for systematic literature search

The following combined key words were used: *((“recombinant growth hormone” OR “recombinant growth hormones” OR “recombinant human growth hormone” OR “recombinant human growth hormones” OR “r-hGH” OR ((“Growth Hormone”[mesh] OR “growth hormone” OR “growth hormones” OR Somatotropin OR Somatotropins OR Somatropin OR hGH OR Serostim OR Zomacton OR “Cryo-Tropin” OR “Cryo Tropin” OR CryoTropin OR “r-hGH-M” OR Humatrope OR Umatrope OR Maxomat OR Norditropin OR Norditropine OR Nutropin OR Omnitrope OR Saizen OR Genotropin OR Genotonorm) AND (“Recombinant Proteins”[mesh] OR recombinant[tw]))) AND (“long term effects” OR “long term effect” OR “longterm effects” OR “longterm effect” OR longterm OR “long term” OR ((“Twenty years” OR “Twenty year” OR “20 years” OR “20 year” OR “Nineteen years” OR “Nineteen year” OR “19 years” OR “19 year” OR “Eighteen years” OR “Eighteen year” OR “18 years” OR “18 year” OR “Seventeen years” OR “Seventeen year” OR “17 years” OR “17 year” OR “Sixteen years” OR “Sixteen year” OR “16 years” OR “16 year” OR “Fifteen years” OR “Fifteen year” OR “15 years” OR “15 year” OR “Fourteen years” OR “Fourteen year” OR “14 years” OR “14 year” OR “Thirteen years” OR “Thirteen year” OR “13 years” OR “13 year” OR “Twelve years” OR “Twelve year” OR “12 years” OR “12 year” OR “Eleven years” OR “Eleven year” OR “11 years” OR “11 year” OR “Ten years” OR “Ten year” OR “10 years” OR “10 year” OR “Nine years” OR “Nine year” OR “9 years” OR “9 year” OR “Eight years” OR “Eight year” OR “8 years” OR “8 year” OR “Seven years” OR “Seven year” OR “7 years” OR “7 year”) AND (effect[tiab] OR effects[tiab] OR outcome OR outcomes))) AND (adult OR adults)) OR ((“recombinant growth hormone” OR “recombinant growth hormones” OR “recombinant human growth hormone” OR “recombinant human growth hormones” OR “r-hGH” OR ((“Growth Hormone”[mesh] OR “growth hormone” OR “growth hormones” OR Somatotropin OR Somatotropins OR Somatropin OR hGH OR Serostim OR Zomacton OR “Cryo-Tropin” OR “Cryo Tropin” OR CryoTropin OR “r-hGH-M” OR Humatrope OR Umatrope OR Maxomat OR Norditropin OR Norditropine OR Nutropin OR Omnitrope OR Saizen OR Genotropin OR Genotonorm) AND (“Recombinant Proteins”[mesh] OR recombinant[tw]))) AND (“long term effects” OR “long term effect” OR “longterm effects” OR “longterm effect” OR longterm OR “long term” OR ((“Twenty years” OR “Twenty year” OR “20 years” OR “20 year” OR “Nineteen years” OR “Nineteen year” OR “19 years” OR “19 year” OR “Eighteen years” OR “Eighteen year” OR “18 years” OR “18 year” OR “Seventeen years” OR “Seventeen year” OR “17 years” OR “17 year” OR “Sixteen years” OR “Sixteen year” OR “16 years” OR “16 year” OR “Fifteen years” OR “Fifteen year” OR “15 years” OR “15 year” OR “Fourteen years” OR “Fourteen year” OR “14 years” OR “14 year” OR “Thirteen years” OR “Thirteen year” OR “13 years” OR “13 year” OR “Twelve years” OR “Twelve year” OR “12 years” OR “12 year” OR “Eleven years” OR “Eleven year” OR “11 years” OR “11 year” OR “Ten years” OR “Ten year” OR “10 years” OR “10 year” OR “Nine years” OR “Nine year” OR “9 years” OR “9 year” OR “Eight years” OR “Eight year” OR “8 years” OR “8 year” OR “Seven years” OR “Seven year” OR “7 years” OR “7 year”) AND (effect[tiab] OR effects[tiab] OR outcome OR outcomes))) NOT (“child”[mesh] NOT “Adult”[mesh])) NOT (“Animals”[mesh] NOT “Humans”[mesh]).*

APPENDIX 2.

List of criteria used for methodological quality assessment, modified STROBE statement

Item	Criterion
1.	Clear presentation of key elements of study design.
2.	Description of setting, locations, relevant dates including periods of recruitment, exposure, follow-up, and data collection.
3.	Description of eligibility criteria, and the sources and methods of selection of participants. i) Description of methods of follow-up (cohort study) ii) Rationale for the choice of cases and controls (case-control study)
4.	Clear definition of all outcomes, exposures, predictors, potential confounders.
5.	Description of any effects to address potential sources of bias.
6.	Clear description of statistical methods and handling with missing data.
7.	Report of reasons of non-participation at each stage of the study (for example, flow diagram).
8.	Clear description of characteristics of study participants.
9.	Clear description of follow-up time.
10.	Presentation of unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (*i.e.* 95% confidence interval).

APPENDIX 3.

Mean serum IGF-1 levels and IGF-1 SDS at start of rhGH suppletion and during follow-up, specified per study

Study(ref)	Baseline	1yr	5yr	7yr	10yr	15yr
Götherström 2010(16)						
IGF-1 ug/l						
IGF-1 SDS	87.0 (54.0)	245.0 (123.0)	200.0 (98.0)	208.0 (98.0)	170.0 (83.0)	NR
	-1.10 (1.08)	2.05 (2.40)	1.17 (2.06)	1.33 (1.76)	1.17 (1.52)	NR
Svensson 2003(17)						
IGF-1 ug/l	109.0 (6.0)	300.0 (13.0)	256.0 (11.0)	NR	NR	NR
IGF-1 SDS	-1.54 (0.12)	2.35 (0.27)	1.53 (0.21)	NR	NR	NR
Götherström 2005(14)						
IGF-1 ug/l	88.0 (10.0)	246.0 (13.0)	202.0 (18.0)	NR	NR	NR
IGF-1 SDS	-1.10 (0.20)	2.06 (0.46)	1.21 (0.39)	NR	NR	NR
Götherström 2009(15)						
IGF-1 ug/l	109.6 (62.4)	300.0 (135.2)	256.0 (114.0)	256.0 (114.0)	202.0 (94.0)	NR
IGF-1 SDS	-1.54 (1.25)	2.35 (2.81)	1.53 (2.18)	1.34 (1.70)	1.12 (2.18)	NR
Roemmler(25)						
IGF-1 ug/l	NR	NR	NR	NR	167.0 (R74.0-319.0)	NR
IGF-1 SDS	NR	NR	NR	NR	NR	NR
Van der Klaauw 2007(29)						
IGF-1	NR	NR	NR	NR	NR	NR
IGF-1 SDS	-2.00 (0.8)	NR	0.80 (1.9)	NR	NR	NR
Van der Klaauw 2006(7)						
IGF-1 nmol/l	9.1 (4.6)	NR	NR	25.5 (9.9)	NR	NR
IGF-1 SDS	NR	NR	NR	NR	NR	NR
Giavoli(20)						
IGF-1 nmol/l	8.4 (4.4)	24.8 (9.6)	22.2 (8.0)	NR	NR	NR
IGF-1 SDS	NR	NR	NR	NR	NR	NR
Götherström 2007(6)						
IGF-1 ug/l	NR	NR	NR	NR	NR	NR
IGF-1 SDS	-1.80 (0.12)	3.10 (0.28)	1.88 (0.24)	1.51 (0.19)	1.29 (0.25)	NR
Gibney(23)						
IGF-1 nmol/l	12.3 (3.0)	NR	NR	NR	26.9 (4.0)	NR
IGF-1 SDS	NR	NR	NR	NR	NR	NR

Study(ref)	Baseline	1yr	5yr	7yr	10yr	15yr
Götherström 2001(9)						
IGF-1 ug/l	102.0 (6.0)	313.0 (13.0)	268.0 (11.0)	NR	NR	NR
IGF-1 SDS	-1.73 (0.11)	2.55 (0.25)	1.66 (0.20)	NR	NR	NR
Chrisoulidou(22)						
IGF-1 nmol/l	12.4 (R9.2-18.1)	NR	NR	29.1 (R16.5-41.9)	NR	NR
IGF-1 SDS	NR	NR	NR	NR	NR	NR
Cenci(21)						
IGF-1	NR	NR	NR	NR	NR	NR
IGF-1 SDS	NR	NR	NR	NR	NR	NR
Svensson 2002(27)						
IGF-1 ug/l	74.0 (14.0)	361.0 (35.0)	NR	260.0 (47.0)	NR	NR
IGF-1 SDS	-2.94 (0.36)	2.90 (0.78)	NR	1.89 (0.89)	NR	NR
Götherström 2007(10)						
IGF-1 ug/l	99.5 (6.6)	341.6 (14.1)	281.0 (13.0)	279.6 (14.5)	223.3 (9.8)	NR
IGF-1 SDS	-1.81 (0.12)	3.10 (0.28)	1.88 (0.24)	1.51 (0.19)	1.29 (0.25)	NR
Spielhagen 2010(26)						
IGF-1 ng/ml (M)	144.3 (11.8)	248.0 (12.3)	224.9 (11.6)	203.1 (12.4)	177.3 (11.7)	NR
IGF-1 ng/ml (F)	114.6 (9.0)	187.6 (10.1)	160.2 (9.2)	155.2 (9.5)	153.4 (NR)	NR
IGF-1 SDS (M)	-1.9 (0.3)	0.2 (0.2)	0.2 (0.2)	0.2 (0.2)	0.1 (0.2)	NR
IGF-1 SDS (F)	-2.4 (0.2)	-0.6 (0.2)	-0.6 (0.2)	-0.3 (0.2)	0.0 (0.2)	NR
Elbornsson 2012(4)						
IGF-1 ug/l	103.0 (6.0)	NR	273.0 (10.0)	246.0 (8.0)	206.0 (8.0)	183.0 (7.0)
IGF-1 SDS	-1.69 (0.11)	NR	1.89 (0.21)	1.47 (0.18)	0.84 (0.17)	0.62 (0.16)
Clanget(19)						
IGF-1 ug/l	95.4 (16.2)	304.3 (49.6)	235.0 (24.0)	NR	NR	NR
IGF-1 SDS	NR	NR	NR	NR	NR	NR
Koltowska(5)						
IGF-1	NR	NR	NR	NR	NR	NR
IGF-1 SDS	NR	NR	NR	NR	NR	NR
Gilchrist(24)						
IGF-1	NR	NR	NR	NR	NR	NR
IGF-1 SDS	NR	NR	NR	NR	NR	NR
Svensson 2004(28)						
IGF-1 ug/l (M)	125.0 (6.0)	322.0 (11.0)	283.0 (11.0)	275.0 (16.0)	NR	NR
IGF-1 ug/l (F)	83.0 (6.0)	224.0 (11.0)	229.0 (15.0)	231.0 (21.0)	NR	NR
IGF-1 SDS (M)	-1.39 (0.12)	2.74 (0.21)	2.06 (0.22)	1.88 (0.34)	NR	NR
IGF-1 SDS (F)	-2.12 (0.13)	0.51 (0.22)	0.83 (0.29)	0.87 (0.43)	NR	NR
Van Bunderen(11)						
IGF-1	NR	NR	NR	NR	NR	NR
IGF-1 SDS	NR	NR	NR	NR	NR	NR
Gaillard(18)						
IGF-1	NR	NR	NR	NR	NR	NR
IGF-1 SDS (M)	NR	0.54 (1.77)	NR	NR	NR	NR
IGF-1 SDS (F)	NR	-0.16 (1.67)	NR	NR	NR	NR

Data are presented as mean (SD), unless specified otherwise.
IGF-1, insulin-like growth; NR, not reported; R, range; M, males; F, females

XI.

Metabolic profile in Growth Hormone Deficient (GHD) adults after long-term recombinant human Growth Hormone (rhGH) therapy

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ABSTRACT

BACKGROUND: The metabolic effects of recombinant human GH (rhGH) therapy in adults are well-documented in the short term. The effects of long-term rhGH therapy beyond 5 years on metabolic parameters are presently unknown.

OBJECTIVE: The aim of the study was to evaluate the long-term effects of rhGH treatment on biochemical and anthropometric parameters in a large cohort of GH-deficient adults.

METHODS: Ninety-eight adult GH-deficient patients treated with rhGH for at least 10 years were included (mean age 59.4 years, 50% female). Total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, anthropometric parameters, IGF-1, and glucose were evaluated at baseline, and after 5, 10 and 15 years of treatment. In addition, the prevalence of the metabolic syndrome (MS) and the incidence of cardiovascular events were assessed.

RESULTS: Total cholesterol and low-density lipoprotein cholesterol concentrations were lower, and high-density lipoprotein cholesterol levels were significantly higher during long-term rhGH replacement when compared to baseline (all $p<0.001$). Both waist circumference ($p<0.001$) and BMI ($p=0.018$) were significantly higher after 10 years, as were fasting plasma glucose levels ($p<0.001$). No significant changes were observed in triglycerides, waist-to-hip ratio and blood pressure during follow-up. In the subset of patients with 15-yr rhGH treatment (N=43), generally similar metabolic effects were found. MS prevalence was increased after 10 years of rhGH treatment (57.1% vs 32.7%, $p<0.001$), especially in males (69.4% vs 32.7%, $p<0.001$).

CONCLUSION: Despite improvement of several cardiovascular risk factors, MS prevalence increased significantly during rhGH treatment. The effect of long-term rhGH treatment on overall cardiovascular risk profile needs to be established in a larger cohort.

INTRODUCTION

Recombinant human GH (rhGH) replacement therapy has been a regular treatment option for adult GH-deficient (GHD) patients since the nineties. Adult GHD is hypothesized to be a cardiovascular risk factor associated with increased mortality, by inducing abdominal obesity, hypercholesterolemia, and hypertriglyceridemia (1;2). In short-term studies, GH replacement reduces some, but not all of these cardiovascular risk factors (3). Consistent 'short-term' effects on body composition and lipid metabolism were documented, resulting in reduction of body fat combined with an increase of fat-free mass, and a reduction of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels (3). In addition, favorable effects on bone mineral density (BMD) and quality of life (QoL) have been reported (3-6).

Sustained improvement of lipid spectrum and diastolic blood pressure (DBP) has been reported over 7-10 years (7-9). However, despite a significant improvement of some, but not all, individual cardiovascular risk factors, overall cardiovascular risk profile, as reflected by the prevalence of the metabolic syndrome (MS), was still increased when compared with the general population and was not affected by rhGH after 5 years of treatment (10). Moreover, a recent study reported an increased risk of cardiovascular death in GHD women treated with rhGH when compared with background population (11).

A recent review addressing the effects of rhGH replacement in elderly patients (>60 years old) with GHD, revealed that rhGH replacement decreases LDL-C levels and improves QoL, but the effects on other parameters were not unequivocal (12). However, sufficient data concerning the use of long-term rhGH therapy in elderly are currently unavailable, as is the case in younger patients. In view of the overall scarce documentation of cardiovascular effects over 10 years (8;13;14), the efficacy of ongoing rhGH therapy in reducing cardiovascular risk in adult GHD still has to be established.

Therefore, the aim of this study was to evaluate the long-term effects of rhGH treatment on biochemical and anthropometric parameters in a large cohort of GHD adults that were treated with rhGH for at least 10 years.

PATIENTS AND METHODS

PATIENTS: Since 1994, consecutive patients diagnosed with GHD at the Endocrinology Department of the Leiden University Medical Center were collected in a database, including both adult-onset (AO) and childhood-onset (CO) GHD. Severe GHD had been defined prior to start of treatment by a GH peak response to the Insulin Tolerance Test (ITT) <3μg/l (glucose nadir<2.2mmol/l) or Growth Hormone Releasing Hormone/Arginine-test (GHRH/Arg) (with body mass index (BMI)-adjusted GH cut-offs) in case of contraindications for ITT, according to guidelines (10;15). All patients with rhGH treatment during childhood were retested at time of transition to the adult outpatient clinic, after treatment cessation for more than 3 months. After dose titration, aiming at an IGF-1 level in the normal range, patients were evaluated at least yearly at the outpatient clinic according to a standard protocol.

For the present analysis, we selected patients who started with rhGH treatment in 2002 or before (N=184). Exclusion criteria were: 1) rhGH treatment duration less than 10 years; 2) cessation for at least 2 years; 3) more than 3 missing visits. Part of this GHD cohort used for present analysis was previously described (9;10;16;17).

TREATMENT PROTOCOL: All patients were treated with s.c. injections of rhGH (Genotropin Pharmacia/Pfizer, Zomacton Ferring, or Norditropin NovoNordisk) injected in the evening. The initial dose of rhGH was 0.2mg/day, which was individually adjusted each month in the first half year to achieve serum IGF-1 concentrations within the age-dependent laboratory reference range, aimed at an SD score (SDS) between 0 and +2. When stable plasma concentrations were reached, this individualized dose was continued and adjusted as necessary. ACTH deficiency was defined as an insufficient increase in cortisol levels (absolute value 0.55mmol/l) after a CRH stimulation test or ITT. When secondary amenorrhea was present for more than 1year, premenopausal women were classified as gonadotropin deficient. In men, gonadotropin deficiency was defined as a testosterone level below the reference range (8.0nmol/l). TSH deficiency was defined as total T4 or free T4 level below the reference range (<10pmol/l). Hypopituitarism was supplemented by hydrocortisone, l-T4, testosterone in men, and estrogen in combination with prostagens in premenopausal women only. Dosages were monitored and adjusted as required. Thyroid hormone replacement, lipid-lowering medication and antihypertensive medication were started according to the discretion of the attending physicians.

EFFICACY PARAMETERS: The following efficacy parameters were assessed at baseline and at the yearly visits at the outpatient clinic:

I. Biochemical parameters: levels of glucose, TC, high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) after an overnight fast. LDL-C concentrations were calculated using the Friedewald formula.
II. Anthropometric parameters: body weight and height, waist circumference, hip circumference, systolic and diastolic blood pressures (SBP and DBP, respectively) were measured. BMI and waist-to-hip (WH) ratio were calculated. Body weight was measured to the nearest 0.1kg, and body height was measured barefoot to the nearest 0.001m. The BMI was calculated as weight in kilograms divided by the square of height in meters.
III. Additional information with respect to medication use, co-morbidity and possible side effects and adverse events was gathered from patient records.

For the present study, we analyzed the efficacy parameters at baseline and after 5, 10 and 15 years of rhGH therapy.

METABOLIC SYNDROME: The MS was defined according to the updated third report of the 2006 National Cholesterol Education Program's Adult Treatment Panel (NCEP-ATP III) criteria, which required at least three of the following conditions (18;19):

1. Fasting plasma glucose concentration of at least 100mg/dl or on anti-diabetic drug treatment;
2. TG concentration of at least 150mg/dl or on drug treatment;
3. HDL-C concentration below 40mg/dl in men and below 50mg/dl in women, or on drug treatment;
4. Blood pressure of at least 130/85mmHg or on antihypertensive treatment
5. Waist circumference greater than 102cm in men and greater than 88cm in women.

ASSAYS: Serum GH was measured with a sensitive IFMA (Wallac, Turku, Finland), specific for the 22 kDA GH protein (detection limit: 0.01µg/l,interassay coefficient of variation (CV): 1.6-8.4% of 0.01-15.4µg/l) from 1992 onwards. For the conversion of µg/l to mU/l, multiply by 2.6. Before 1992, GH was measured by RIA (Biolab, Serona, Coissins, Switzerland), detection limit: 0.5mU/l, with an interassay CV<5%; for the conversion of µg/l to mU/l, multiply by 2.

From 1986 to 2005, serum IGF-1 concentrations were determined by RIA (Incstar, Stillwater, MN) with a detection limit of 1.5nmol/l and an interassay CV less than 11%. IGF-1 is expressed as SD score for age- and sex-related normal levels determined in the same laboratory (20). Since 2005, serum IGF-1 concentrations (nmol/l) were measured using an immunometric technique on an Immulite 2500 system (Siemens Healthcare Diagnostics, Deerfield, IL, USA). The intra-assay variations at mean plasma levels of 8 and 75nmol/l were 5.0 and 7.5%. IGF-1 levels were expressed as SDS, using lambda-mu-sigma smoothed reference curves based on 906 controls (21;22).

A Hitachi 747 autoanalyzer (Roche) was used to quantify serum concentrations of glucose, TC, and TG. HDL-C was measured with a homogenous enzymatic assay (Hitachi 911,Roche). In 2003, the Hitachi 747 was replaced by a modular P800 with no change in the chemistry components.

STATISTICS: SPSS for Windows, Version 17.0 (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. Results are presented as mean±SD, unless stated otherwise. ANOVA repeated measurements with Bonferroni correction for multiple comparisons were used to compare biochemical and anthropometric parameters between baseline and after rhGH treatment. We incorporated age, sex, CO *vs* AO GHD, hydrocortisone use, radiotherapy and GH dose in a linear regression model to identify factors influencing the metabolic effects of long-term rhGH suppletion. The Friedman test for related fractions was used to assess the effect of rhGH treatment on the MS prevalence. Furthermore, we calculated the incidence rate of cardiovascular events (*i.e.* cardiovascular death, myocardial infarction, cerebrovascular attack, intermittent claudication, (progressive) angina pectoris or coronary bypass surgery, pulmonary embolism) during rhGH therapy.

RESULTS

A total of 184 adult patients with GHD started rhGH suppletion in 2002 or before (*Figure 1*). Forty-two patients are on current rhGH treatment, but did not complete 10 years of treatment yet. Fourty-four patients discontinued rhGH treatment for various reasons (*Figure 1*). Reasons for preliminary discontinuation of rhGH treatment were: no subjective beneficial effect (N=10), death (N=9), tumor growth (N=7), malignancy (N=12), high age (N=1), new-onset diabetes mellitus type 2 (N=1), other (N=4). Consequently, we included 98 patients (50% female, mean age 59.4yr) for the present analysis, of which 43 patients completed 15 years of rhGH therapy (44% female, mean age 61.3yr). Baseline characteristics are shown in *Table 1*.

No differences were found between the patients with and without complete 10-yr ore more rhGH treatment, with respect to sex, BMI, age of start rhGH therapy, etiological diagnosis, surgery, radiotherapy, pituitary deficiencies, and use of lipid-lowering or antihypertensive medication. Only the number of patients with CO GHD was higher among non-completers (p=0.009) (*data not shown*).

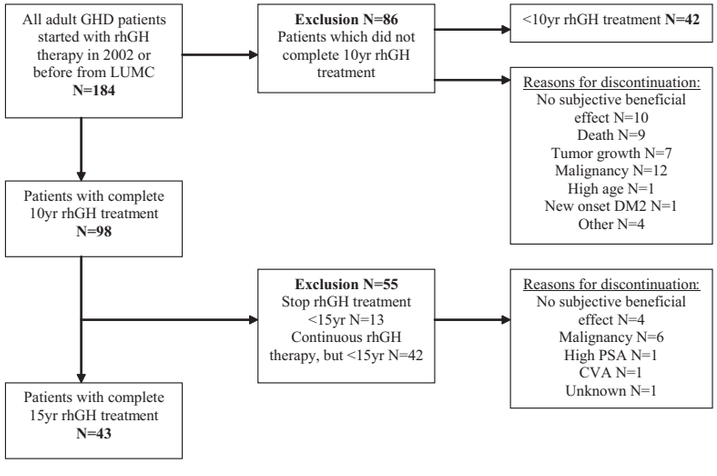


Figure 1. Flow chart of patient selection and follow-up

GH DOSE AND IGF-1 CONCENTRATION: Mean GH dose after dose titration (after 1yr) was 0.46±0.20mg/day (range 0.20-1.50mg), 0.48±0.25mg/day (range 0.15-1.50mg) at 5yr. At 10 and 15 years mean GH doses were 0.44±0.26mg/day (range 0.10-1.50mg) and 0.39±0.21mg/day (range 0.10-1.50mg), respectively (5-, 10- and 15-yr GH doses were not significantly different from the GH dose after dose titration). Serum IGF-1 levels remained significantly higher during rhGH replacement for the duration of the study up to 15 years compared with baseline (*Table 2*). During the entire study period, mean IGF-1 SDS was within the normal range, increased from -0.68±2.27 at baseline to 0.20±2.25 at 15 years of rhGH suppletion (*Figure 2*). In males, mean IGF-1 SDS was -0.24±2.26 at baseline and increased to 0.21±2.31 at 15 years (p=0.007); in females, mean IGF-1 SDS increased from -1.13±2.17 at baseline to 0.19±2.25 after 15 years of rhGH treatment (p<0.001).

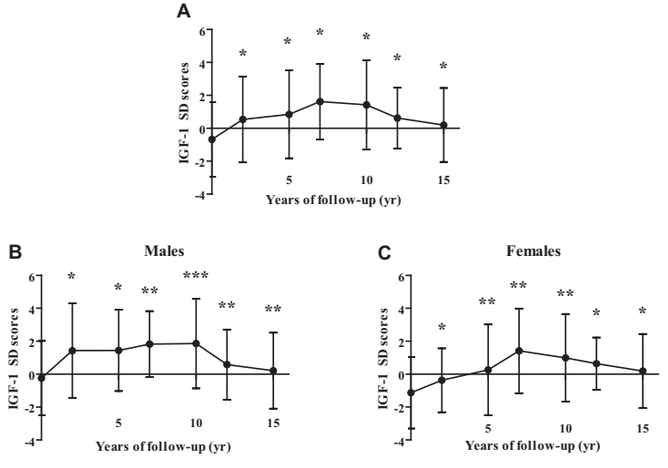


Figure 2. Mean IGF-1 SD scores during 15 years of rhGH therapy, for the total cohort of GHD patients (A, N=98) and separated for males (B) and females (C), respectively

Mean IGF-1 SD scores ± SD during rhGH treatment are presented for the total GHD cohort (N=98), and separately for male (N=49) and female patients (N=49). IGF-1 SD scores were evaluated at start of rhGH therapy, and after 2, 5, 7, 10, 12, and 15 years of rhGH suppletion.

**, p<0.001; **, p<0.01; ***, p<0.05 vs baseline*

Table 1. Baseline characteristics of 98 patients with GHD, which completed minimal 10 years of rhGH replacement therapy

Patient characteristics	**GHD patients with complete 10yr follow-up (N=98)**
Sex, female / male (n)	49 / 49
Age at start rhGH therapy, yr (range)	44.9 ± 13.6 (17 – 84)
AO / CO (n)	87 / 11
BMI, kg/m^2 (range)	28.2 ± 5.9 (16.9 – 45.4)
Etiological diagnosis of GHD (n)	
NFA	30
Functioning adenoma	25
Craniopharyngeoma	14
Cerebral malignancy	5
Congenital	9
Other causes	15
Surgery, TS / TC (n)	53 / 24
Radiotherapy (n)	37
Pituitary deficiencies (n)	
TSH	89
ACTH	88
FSH/LH	85
ADH	28
Isolated GHD	1
Lipid-lowering drugs (n)	10
Antihypertensive medication (n)	10

Data are presented as mean ± SD, unless specified otherwise. n, number of patients.

GHD, growth hormone deficiency; rhGH, recombinant human growth hormone; AO, adult-onset GHD; CO, childhood-onset GHD; NFA, non-functioning adenoma; TSH, thyroid stimulating hormone; ACTH, adrenocorticotropic hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; ADH, anti-diuretic hormone

Table 2. Effects of 10 years of rhGH replacement in 98 adults with GHD on biochemical and anthropometric parameters

	Baseline†	**5 years of rhGH replacement††**	**10 years of rhGH replacement†††**
IGF-1, nmol/l	14.8 ± 10.3	25.4 ± 13.3 *	22.2 ± 10.7 *
Fasting glucose, mmol/l	4.7 ± 0.8	5.0 ± 1.1 **	5.1 ± 1.0 *
TC, mmol/l	6.2 ± 1.4	5.5 ± 1.0 *	5.2 ± 1.0 *, b
LDL-C, mmol/l	4.5 ± 1.4	3.7 ± 0.9 *	3.3 ± 0.8 *, a
HDL-C, mmol/l	1.3 ± 0.5	1.5 ± 0.5 *	1.6 ± 0.6 *
TG, mmol/l	1.8 ± 1.1	1.8 ± 1.0	1.8 ± 1.1
Waist circumference, cm	94.6 ± 13.7	97.0 ± 14.3	98.9 ± 14.2 *, a
WH ratio	0.95 ± 0.07	0.97 ± 0.12	0.96 ± 0.06
BMI, kg/m^2	28.2 ± 5.9	28.8 ± 6.3	29.9 ± 6.9 ***, c
SBP, mmHg	129.9 ± 14.7	130.4 ± 17.8	130.9 ± 16.6
DBP, mmHg	83.5 ± 9.7	80.4 ± 9.8 **	80.4 ± 9.1 ***

Data are presented as mean ± SD. IGF-1, insulin-like growth factor-1; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglycerides; WH ratio, waist-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure.

†, statins N=10 (10.2%), antihypertensive medication N=10 (10.2%); ††, statins N=32 (32.7%), antihypertensive medication N=32 (32.7%); †††, statins N=34 (34.7%), antihypertensive medication N=33 (33.7%)

**, p<0.001; **, p<0.01; ***, p<0.05 vs baseline*
a, p<0.001; b, p<0.01; c, p<0.05 10 vs 5 years of rhGH treatment

EFFECTS ON GLUCOSE, LIPID PROFILE, ANTHROPOMETRIC PARAMETERS AND BLOOD PRESSURE: Fasting plasma glucose levels significantly increased from 4.7±0.8mmol/l at baseline to 5.0±1.1mmol/l at 5 years (6% increase), thereafter remaining stable at 5.1±1.0mmol/l after 10 years rhGH treatment (*Table 2*) (p<0.001 *vs* baseline). TC and LDL-C concentrations decreased, and HDL-C levels increased significantly after 5 years when compared with baseline (all p<0.001), further improving after 10 years of rhGH treatment. Both waist circumference and BMI increased after 10 years of rhGH suppletion (p<0.001 and p=0.018, respectively). No significant changes were observed in TG, WH ratio and SBP; only DBP decreased slightly after 5 and 10 years (p<0.01 and p=0.016, resp.).

Among the subgroup of patients who completed 15 years of rhGH treatment (N=43), comparable significant effects of rhGH suppletion were found on fasting glucose levels, lipids and waist circumference (*Table 3*). The effects on TC, HDL-C, LDL-C and TG were not affected when patients using lipid-lowering medication at any time point during follow-up were excluded (N=41, 42%). TG, WH ratio, BMI and DBP did not change when compared to baseline; SBP was significantly higher (p=0.001). Within the 15-yr treated group, there were, in general, no differences between 10 and 15 years of rhGH therapy; except for SBP, being significantly higher after 15 years. Excluding patients on antihypertensive medication at any time point (N=41, 42%) revealed no changes in DBP; however, the observed increase in SBP between 10 and 15 years of treatment was not significant any longer.

INFLUENCE OF SEX: The dose of rhGH was significantly higher in women in comparison to men at all time points, resulting in a higher GH dose/IGF-1 SDS ratio in female GHD patients. After 10 years of rhGH treatment, the GH dose was 0.53±0.30mg (range 0.20-1.50mg) and 0.36±0.18mg (range 0.10-1.20mg) in females and males, respectively (p=0.001); at 15 years 0.47±0.23mg (range 0.20-1.20mg) and 0.30±0.14mg (range 0.10-0.60mg), respectively (p=0.009). We found no differences in the metabolic response to rhGH treatment between both sexes.

OTHER POTENTIAL INFLUENCING FACTORS: Forty-nine patients (50%) were less than 60 years old, 49 patients (50%) were at least 60 years of age at baseline. The individualized rhGH dose used in older patients did not differ from the dose in younger patients. In addition, there were no significant differences in the rhGH response between younger and older patients with respect to IGF-1, glucose, lipid profile, WH-ratio, BMI or blood pressure. Furthermore, the number of pituitary insufficiencies did not affect the response to rhGH treatment. Mean hydrocortisone doses were 25.6±7.3mg/day, 23.1±5.8mg/day, 21.4±4.1mg/day, and 21.1±4.3mg/day at baseline and after 5, 10 and 15 years of rhGH suppletion, respectively. However, patients with hydrocortisone substitution did not differ from hydrocortisone-independent patients, except for a greater decrease in LDL-C levels after 10 years (-1.35 *vs* -0.70mmol/l, p=0.021). Patients with CO-GHD did not differ from AO-GHD patients in their response to any of the metabolic parameters studied. In addition, there was no difference between patients with or without cranial irradiation, except for a higher waist circumference (p=0.024) among irradiated patients.

When incorporating all factors in a linear regression model, neither age, sex, CO- *vs* AO-GHD, nor hydrocortisone use significantly influenced any of the metabolic parameters. Radiotherapy, however, influenced waist circumference and DBP negatively. In addition, higher GH dose was associated with higher BMI and waist circumference after 10 years of rhGH suppletion.

PREVALENCE OF THE MS: The prevalence of the MS increased from 32.7% at baseline to 46.9% after 5 years of rhGH therapy (p=0.040), further increasing to 57.1% after 10 years of rhGH treatment (p<0.001 *vs* baseline). As shown in *Figure 3*, this was mainly due to a gradual increase in abdominal obesity, hypertriglyceridemia, and hyperglycaemia.

At baseline, the MS was equally prevalent in men and women (32.7% *vs* 32.7%). After 5 years of rhGH suppletion, 49.0% of the males and 44.9% of the female GHD patients had MS (p=0.687). After 10 years of rhGH suppletion, 69.4% of men fulfilled the criteria of MS *vs* 44.9% of the women, p=0.015. This indicates that males especially drove the increase in MS prevalence over time, and that MS prevalence stabilized in females after 5 years. In a logistic regression model incorporating age, sex, hydrocortisone use, radiotherapy and GH dose, only higher GH dose negatively influenced the MS prevalence after 10 years of rhGH suppletion.

CARDIOVASCULAR EVENTS: We assessed the number of major cardiovascular events in the patients who completed at least 10year of rhGH treatment (N=98). In total, 25 events were reported: myocardial infarction (N=2), progressive angina pectoris and/or coronary bypass surgery (N=7), cerebrovascular attack (N=2), intermittent claudication (N=1), pulmonary embolism (N=1). No cardiovascular death was reported. Consequently, the incidence rate of major cardiovascular

events in our GHD cohort was 25/16552 (mean duration of rhGH therapy 168.9 months x 98 patients) = 1.5/1000py.
In addition, new-onset diabetes mellitus (N=3), new-onset hypertension or start of antihypertensive medication (N=24), new-onset hypercholesterolaemia or start of lipid-lowering treatment (N=23) was reported during rhGH therapy.

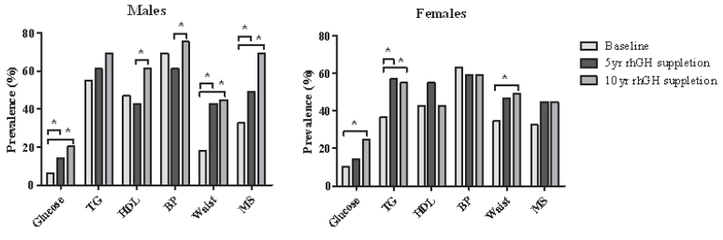


Figure 3. Prevalence of (individual components of) the MS in 98 GHD adults at baseline, and after 5 and 10 years of rhGH suppletion, respectively, separated for males and females

The bars denote the prevalence of the (individual components of) the MS, at baseline and after 5 and 10 years of rhGH treatment, respectively.

*TG, triglycerides; HDL, high-density lipoprotein cholesterol; BP, blood pressure; waist, waist circumference; MS, metabolic syndrome. *, p<0.05*

Table 3. Effects of 15 years of rhGH treatment in 43 adults with GHD on biochemical and anthropometric parameters

	Baseline†	**15 years of rhGH replacement††**
IGF-1, nmol/l	9.6 ± 5.5	19.6 ± 8.2 ^{*,a}
Fasting glucose, mmol/l	4.5 ± 0.7	5.0 ± 0.5 **
TC, mmol/l	6.6 ± 1.5	5.4 ± 1.1 *
LDL-C, mmol/l	4.9 ± 1.4	3.4 ± 0.9 *
HDL-C, mmol/l	1.4 ± 0.5	1.7 ± 0.6 *
TG, mmol/l	1.7 ± 1.0	1.5 ± 0.6
Waist circumference, cm	91.2 ± 10.5	99.5 ± 11.9 *
WH ratio	0.96 ± 0.06	0.95 ± 0.07
BMI, kg/m^2	25.9 ± 3.6	29.2 ± 8.3 ***
SBP, mmHg	128.2 ± 14.0	134.6 ± 15.3 ^{**,b}
DBP, mmHg	83.5 ± 9.1	80.8 ± 9.1

Data are presented as mean ± SD. IGF-1, insulin-like growth factor-1; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglycerides; WH ratio, waist-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure.

†, statins N=4 (9.3%), antihypertensive medication N=5 (11.6%); ††, statins N=20 (46.5%), antihypertensive medication N=20 (46.5%)

**, p<0.001; **, p<0.01; ***, p<0.05 vs baseline*
[a], p<0.001; [b], p<0.01; [c], p<0.05 15 vs 10 years of rhGH treatment

DISCUSSION

The present study demonstrates ongoing beneficial effects of rhGH treatment on lipids in patients with GHD after 10 and 15 years in the presence of an increase in anthropometric parameters such as BMI, waist circumference and SBP. As a consequence, overall cardiovascular risk, as assessed by the prevalence of the MS increased significantly after 10 years of rhGH replacement. This increase in MS prevalence was higher than expected as a consequence of ageing alone in non-GHD adults (23).

The short-term effects of rhGH therapy on cardiovascular risk factors are well documented. A meta-analysis of placebo-controlled studies in GHD adults showed favorable effects of short-term (up to 1.5yr) rhGH replacement therapy on TC and HDL-C levels, DBP, as well as on lean body and fat mass, but unfavorable effects on glucose and insulin concentrations (3). Data on the long-term effects of adult rhGH replacement therapy are limited and are based on observational studies. The metabolic changes after at least 10 years of rhGH substitution have been reported in only three studies (8;13;14), including a total of 119 patients, and their conclusions were inconclusive. The first study, Gibney *et al.* (13), reported favorable effects on HDL-C and LDL-C, but no changes in TC, TG, insulin, or blood pressure, whereas the second study, Götherström *et al.* (8), reported a decrease in TC and LDL-C, an increase in HDL-C levels, and an increase in BMI and glucose levels. The third study, Roemmler *et al.* (14), did not demonstrate any effect of treatment on lipids, glucose or anthropometric parameters.

In this study, we documented a decrease in TC and LDL-C levels, and an increase in HDL-C, in accordance with the findings reported by Gibney *et al.* and Götherström *et al.*(8;13). TG levels, however, did not change during rhGH therapy. The pattern of observed changes in lipid concentrations did not change after exclusion of patients using lipid-lowering drugs. The observed mean decrease in TC and LDL-C levels after 15 years of rhGH therapy was 1.3 (20%) and 1.6mmol/l (32%), respectively. In the general population, every 10% decrease in cholesterol levels by statins reduces cardiovascular mortality risk in patients with hypercholesterolemia by 15% (24). Whether lowering of TC levels by rhGH replacement is associated with the same magnitude of reduction in cardiovascular mortality remains to be established. Furthermore, it is crucial not only to ascertain whether the beneficial effects of rhGH suppletion on lipid profile are comparable to the effects obtained with conventional lipid-lowering drugs, but also whether these effects can be superimposed. From a cost-effectiveness point of view, the latter is of paramount importance, but to date, only one study, including 61 GHD patients on statin treatment, has demonstrated such an additional beneficial effect of rhGH treatment on lipid profile (25). In addition, Schneider *et al.* reported a cardiovascular risk reduction of approximately 50% after 2 years of rhGH treatment, using Framingham and Procam risk scores (26). Because hypopituitarism *per se* is also associated with increased cardiovascular mortality (12,13), any additional effect of rhGH substitution next to conventional lipid-lowering drugs may be beneficial.

Despite improvement of lipid spectrum, the MS prevalence increased after 10 years of rhGH replacement when compared with baseline, especially in males. Previously, we demonstrated an increased MS prevalence in untreated GHD adults, defined by the NCEP-ATP III criteria (27), in comparison to a Dutch historical reference population (data collected between 1993 and 1997) (38.0% *vs* 15.7%) (10). Subsequently, age-adjusted prevalence rates of 29% for the MS were reported by Attanasio *et al.* before start of rhGH treatment in 1420 European adults with AO and CO-GHD (Hypopituitary Control and Complications Study, HypoCCS) (28), and of 41% before start of rhGH suppletion by Verhelst and colleagues among 2479 patients with severe AO-GHD, using the same updated NCEP-ATP III criteria (27). Two studies have shown a persistently high MS prevalence after 3 and 5 years of rhGH treatment, respectively (10;28). In our study, we found a further increase in MS prevalence to 57% after 10 years of rhGH substitution, mainly due to an increase in abdominal obesity, hypertriglyceridaemia and hyperglycaemia.

A limitation of the present study, and a general drawback of long-term follow-up studies, is the lack of a non-treated control group. Since the beneficial effects of rhGH therapy are well-established in the short term, it is unethical to withhold patients with GHD receive rhGH in case of no contra-indications. This makes it difficult, if not impossible, to perform long-term randomized, controlled follow-up studies including GHD patients without rhGH treatment. However, several Dutch population-based studies reported the MS prevalence in the general population. In a Dutch survey among 4000 subjects, conducted in 2009-2010, an overall MS prevalence of 34% and 24% was reported, in males and females, respectively. When stratified by age (per decade), MS prevalence for males and females, respectively, were 20% and 10% (30-39yr), 29% and 17% (40-49yr), 41% and 29% (50-59yr), and 48% and 44% (60-69yr) (23). Another study involving a cohort of Dutch adults aged 65 years or older (Longitudinal Aging Study Amsterdam, LASA) reported an MS prevalence of 37.1% (29). Based on these literature data, the MS prevalence of 57.1%

in our cohort of GHD patients (mean age 59yr) is strongly increased, despite 10 years of rhGH replacement.

As demonstrated previously (8;30;31), we observed a significant increase in fasting glucose levels during rhGH replacement. This finding is in accordance with the well-known negative effects of GH on peripheral insulin sensitivity, thereby impairing peripheral glucose uptake. These increased fasting glucose levels may have significantly affected cardiovascular morbidity or mortality, because previous reports have indicated that even when glucose levels were below the diabetic threshold, there was a positive correlation with the occurrence of cardiovascular events (32;33).

Previous radiotherapy was shown to negatively influence abdominal obesity and blood pressure in GHD adults. This finding is consistent with earlier studies among long-term survivors of childhood cancer, reporting a higher MS prevalence after cranial irradiation (34;35). A possible explanation might be that, in addition to pituitary damage, cranial irradiation induces damage to the hypothalamic area. Therefore, cranial radiotherapy might be an independent risk factor for cardiovascular disease.

Due to the presence of multiple pituitary hormone deficiencies in almost all patients, it is difficult to examine to what extent the reported effects can be attributed to rhGH treatment, or whether they are the consequence of suboptimal or excessive replacement therapy of other hormones. Isolated GHD provides the ideal model to characterize GHD without interference from other pituitary deficiencies or their treatment. Abs *et al.* showed generally similar clinical presentation and rhGH treatment response in IGHD patients and patients with multiple deficiencies, especially in AO-GHD (36), supporting the concept that GH *per se*, at least in part, affects the metabolic phenotype of substituted patients with multiple pituitary hormone deficiencies. In accordance with these findings, in a recent meta-analysis, both low and high IGF-1 levels increased mortality in the general population (hazard ratio (HR) 1.18, 95%CI 1.04-1.34) (37).

Paradoxically, there are also human studies linking reduced IGF-1 levels/signaling to a reduced cancer risk as well as improved longevity (38). In addition, functional mutations of the IGF-1R gene resulting in altered IGF-1 signaling are more common in centenarians than in younger controls. The activity of the GH/IGF-1 axis decreased with ageing, yet smaller individuals within a species usually live longer (39). Life span was also expanded in mice lacking GHR, resulting in lower IGF-1 levels. Although these mutant mice lack other hormones, their extended

longevity is thought to be primarily due to GHD, as restoration of GH levels reverted their longevity to that of non-mutants (40;41). Collectively, these data suggest that optimizing the GH/IGF-1 axis to promote healthy ageing in humans is more complex than originally appreciated and will require a greater understanding of its array of interactions and tissue specificity.

Another feature that needs to be addressed is the presence of interactions between glucocorticoids and rhGH during substitution. In the past, hydrocortisone doses used in hypopituitarism resulted in supraphysiological cortisol levels, accounting for at least part of the adverse metabolic profile. Decreasing the glucocorticoid dose from 20-30mg/day to 15mg/day had beneficial effects (42). In our study, mean hydrocortisone dose was lowered during 15 years of follow-up from 25.6±7.3 to 21.1±4.3mg/day. When hydrocortisone use was incorporated in a regression model, no relation to MS presence/worsening was found. Possible explanations for the absence of an effect may be the lower hydrocortisone substitution scheme when compared to Danilowicz *et al.*, in addition to less variation in the hydrocortisone doses used. Furthermore, it has been reported that GH accelerates cortisol metabolism by inhibiting 11β-hydroxysteroid dehydrogenase 1 resulting in decreased tissue exposure to cortisol (43;44), thereby reducing hydrocortisone bioavailability.

IGF-1 SD levels were closely monitored and were titrated on a physiological level, adjusted for age during the entire study period. This is one of the most important differences between our GHD cohort and other GHD cohorts, in which an initial high dose resulted high IGF-1 SDS (over +2SDS) and excessive changes in body composition, especially in men. Our data showed no obvious differences in IGF-1 levels or metabolic effects of rhGH suppletion between men and women, taken into account the need of a higher rhGH substitution dose in women to maintain stable plasma IGF-1 concentrations. In addition, we did not find differences between younger and older GHD patients with respect to response to long-term rhGH treatment. However, we cannot exclude lack of power to detect any differences due to the relatively small number of patients within each age group.

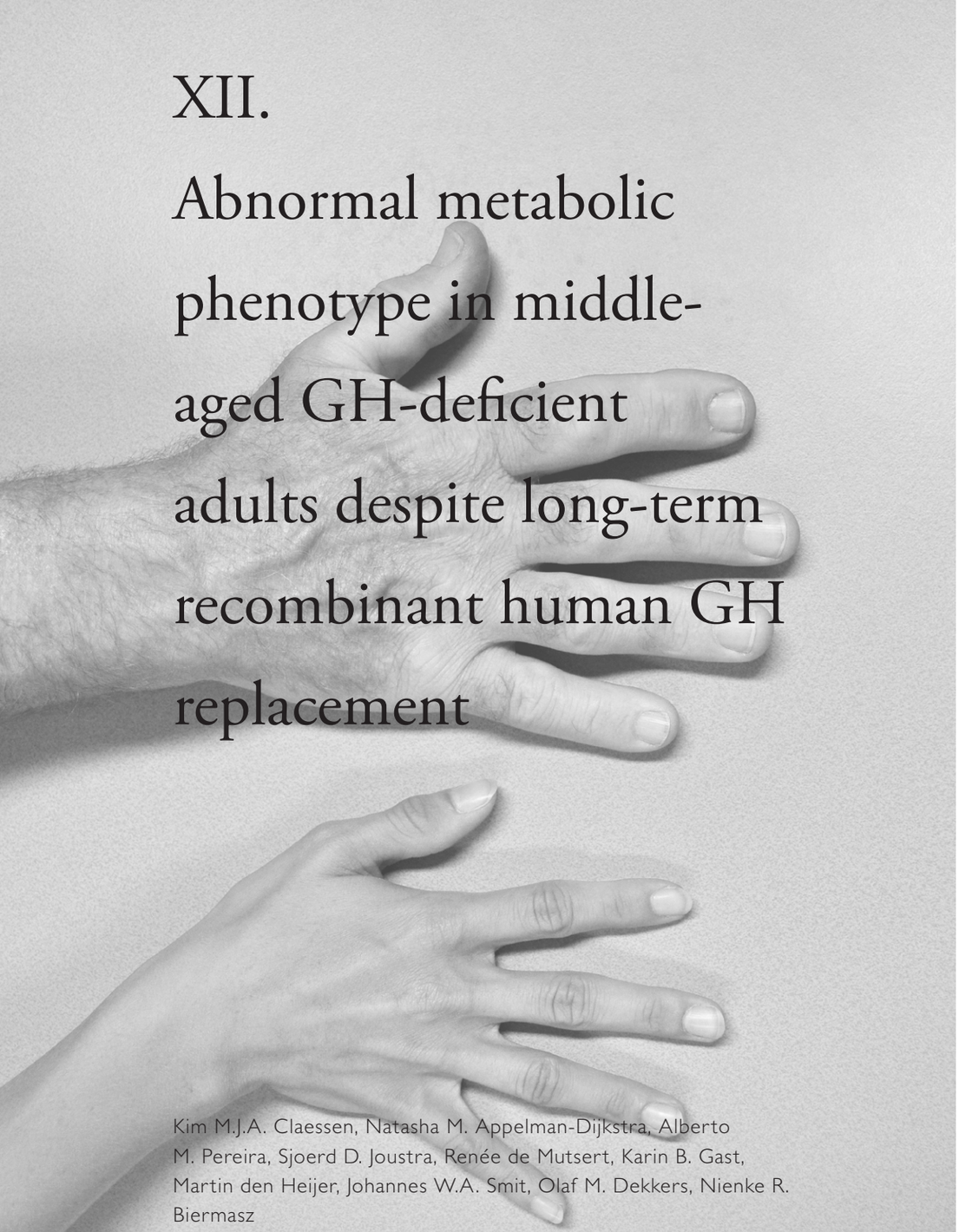
In conclusion, the present study showed ongoing beneficial effects of rhGH therapy in GHD adults on lipid profile, whereas other cardiovascular risk factors continued to deteriorate after long-term rhGH treatment. The increases in glucose levels and BMI were striking and negatively affected the prevalence of the MS in the long-term. Therefore, the net beneficial effects of long-term rhGH treatment on overall cardiovascular risk still need to be established in further studies that adequately address and balance all these factors involved, including cost-effectiveness and QoL.

REFERENCE LIST

1. Rosen T, Bengtsson BA. Premature mortality due to cardiovascular disease in hypopituitarism. Lancet 1990; 336(8710):285-288.
2. Tomlinson JW, Holden N, Hills RK et al. Association between premature mortality and hypopituitarism. West Midlands Prospective Hypopituitary Study Group. Lancet 2001; 357(9254):425-431.
3. Maison P, Griffin S, Nicoue-Beglah M, Haddad N, Balkau B, Chanson P. Impact of growth hormone (GH) treatment on cardiovascular risk factors in GH-deficient adults: a Metaanalysis of Blinded, Randomized, Placebo-Controlled Trials. J Clin Endocrinol Metab 2004; 89(5):2192-2199.
4. Elbornsson M, Gotherstrom G, Bosaeus I, Bengtsson BA, Johannsson G, Svensson J. Fifteen years of GH replacement increases bone mineral density in hypopituitary patients with adult-onset GH deficiency. Eur J Endocrinol 2012; 166(5):787-795.
5. Gotherstrom G, Bengtsson BA, Bosaeus I, Johannsson G, Svensson J. Ten-year GH replacement increases bone mineral density in hypopituitary patients with adult onset GH deficiency. Eur J Endocrinol 2007; 156(1):55-64.
6. Koltowska-Haggstrom M, Mattsson AF, Monson JP et al. Does long-term GH replacement therapy in hypopituitary adults with GH deficiency normalise quality of life? Eur J Endocrinol 2006; 155(1):109-119.
7. Chrisoulidou A, Beshyah SA, Rutherford O et al. Effects of 7 years of growth hormone replacement therapy in hypopituitary adults. J Clin Endocrinol Metab 2000; 85(10):3762-3769.
8. Gotherstrom G, Bengtsson BA, Bosaeus I, Johannsson G, Svensson J. A 10-year, prospective study of the metabolic effects of growth hormone replacement in adults. J Clin Endocrinol Metab 2007; 92(4):1442-1445.
9. van der Klaauw AA, Romijn JA, Biermasz NR et al. Sustained effects of recombinant GH replacement after 7 years of treatment in adults with GH deficiency. Eur J Endocrinol 2006; 155(5):701-708.
10. van der Klaauw AA, Biermasz NR, Feskens EJ et al. The prevalence of the metabolic syndrome is increased in patients with GH deficiency, irrespective of long-term substitution with recombinant human GH. Eur J Endocrinol 2007; 156(4):455-462.
11. van Bunderen CC, van N, I, Arwert LI et al. Does growth hormone replacement therapy reduce mortality in adults with growth hormone deficiency? Data from the Dutch National Registry of Growth Hormone Treatment in adults. J Clin Endocrinol Metab 2011; 96(10):3151-3159.
12. Kokshoorn NE, Biermasz NR, Roelfsema F, Smit JW, Pereira AM, Romijn JA. GH replacement therapy in elderly GH-deficient patients: a systematic review. Eur J Endocrinol 2011; 164(5):657-665.
13. Gibney J, Wallace JD, Spinks T et al. The effects of 10 years of recombinant human growth hormone (GH) in adult GH-deficient patients. J Clin Endocrinol Metab 1999; 84(8):2596-2602.
14. Roemmler J, Kuenkler M, Schneider HJ, Dieterle C, Schopohl J. Comparison of glucose and lipid metabolism and bone mineralization in patients with growth hormone deficiency with and without long-term growth hormone replacement. Metabolism 2010; 59(3):350-358.
15. Ghigo E, Aimaretti G, Corneli G. Diagnosis of adult GH deficiency. Growth Horm IGF Res 2008; 18(1):1-16.
16. Janssen YJ, Frolich M, Roelfsema F. A low starting dose of genotropin in growth hormone-deficient adults. J Clin Endocrinol Metab 1997; 82(1):129-135.
17. Janssen YJ, Hamdy NA, Frolich M, Roelfsema F. Skeletal effects of two years of treatment with low physiological doses of recombinant human growth hormone (GH) in patients with adult-onset GH deficiency. J Clin Endocrinol Metab 1998; 83(6):2143-2148.
18. Grundy SM, Brewer HB, Jr., Cleeman JI, Smith SC, Jr., Lenfant C. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/ American Heart Association conference on scientific issues related to definition. Circulation 2004; 109(3):433-438.
19. Grundy SM, Cleeman JI, Daniels SR et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation 2005; 112(17):2735-2752.
20. Walker BR, Andrew R, MacLeod KM, Padfield PL. Growth hormone replacement inhibits renal and hepatic 11B-hydroxysteroid dehydrogenases in ACTH-deficienti patients. Clin Endocrinol (Oxf) 1998; 49(2):257-263.
21. Cole TJ. The LMS method for constructing normalized growth standards. Eur J Clin Nutr 1990; 44(1):45-60.
22. Rikken B, van DJ, Ringeling A, Van den Brande JL, Massa G, Wit JM. Plasma levels of insulin-like growth factor (IGF)-I, IGF-II and IGF-binding protein-3 in the evaluation of childhood growth hormone deficiency. Horm Res 1998; 50(3):166-176.
23. www.rivm.nl/nldemaat. RIVM. 2012. Ref Type: Electronic Citation
24. Gould AL, Rossouw JE, Santanello NC, Heyse JF, Furberg CD. Cholesterol reduction yields clinical benefit: impact of statin trials. Circulation 1998; 97(10):946-952.
25. Monson JP, Jonsson P, Koltowska-Haggstrom M, Kourides I. Growth hormone (GH) replacement decreases serum total and LDL-cholesterol in hypopituitary patients on maintenance HMG CoA reductase inhibitor (statin) therapy. Clin Endocrinol (Oxf) 2007; 67(4):623-628.
26. Schneider HJ, Klotsche J, Wittchen HU et al. Effects of growth hormone replacement within the KIMS survey on estimated cardiovascular risk and predictors of risk reduction in patients with growth hormone deficiency. Clin Endocrinol (Oxf) 2011; 75(6):825-830.
27. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA 2001; 285(19):2486-2497.
28. Attanasio AF, Mo D, Erfurth EM et al. Prevalence of metabolic syndrome in adult hypopituitary growth hormone (GH)-deficient patients before and after GH replacement. J Clin Endocrinol Metab 2010; 95(1):74-81.
29. Oosterwerff MM, van Schoor NM, Lips P, Eekhoff EM. Osteocalcin as a predictor of the metabolic syndrome in older persons: a population-based study. Clin Endocrinol (Oxf) 2012.
30. Giavoli C, Porretti S, Ronchi CL et al. Long-term monitoring of insulin sensitivity in growth hormone-deficient adults on substitutive recombinant human growth hormone therapy. Metabolism 2004; 53(6):740-743.
31. Gotherstrom G, Svensson J, Koranyi J et al. A prospective study of 5 years of GH replacement therapy in GH-deficient adults: sustained effects on body composition, bone mass, and metabolic indices. J Clin Endocrinol Metab 2001; 86(10):4657-4665.
32. Coutinho M, Gerstein HC, Wang Y, Yusuf S. The relationship between glucose and incident cardiovascular events. A metaregression analysis of published data from 20 studies of 95,783 individuals followed for 12.4 years. Diabetes Care 1999; 22(2):233-240.
33. Gerstein HC. Dysglycemia, not just diabetes, is a continuous risk factor for cardiovascular disease. Evid Based Cardiovasc Med 1997; 1(4):87-88.
34. Heikens J, Ubbink MC, van der Pal HP et al. Long term survivors of childhood brain cancer have an increased risk for cardiovascular disease. Cancer 2000; 88(9):2116-2121.
35. van Waas M, Neggers SJ, Pieters R, van den Heuvel-Eibrink MM. Components of the metabolic syndrome in 500 adult long-term survivors of childhood cancer. Ann Oncol 2010; 21(5):1121-1126.
36. Abs R, Mattsson AF, Bengtsson BA et al. Isolated growth hormone (GH) deficiency in adult patients: baseline clinical characteristics and responses to GH replacement in comparison with hypopituitary patients. A sub-analysis of the KIMS database. Growth Horm IGF Res 2005; 15(5):349-359.
37. Burgers AM, Biermasz NR, Schoones JW et al. Meta-analysis and dose-response metaregression: circulating insulin-like growth factor I (IGF-I) and mortality. J Clin Endocrinol Metab 2011; 96(9):2912-2920.
38. Guevara-Aguirre J, Balasubramanian P, Guevara-Aguirre M et al. Growth hormone receptor deficiency is associated with a major reduction in pro-aging signaling, cancer, and diabetes in humans. Sci Transl Med 2011; 3(70):70ra13.
39. Barzilai N, Huffman DM, Muzumdar RH, Bartke A. The critical role of metabolic pathways in aging. Diabetes 2012; 61(6):1315-1322.
40. Russell SJ, Kahn CR. Endocrine regulation of ageing. Nat Rev Mol Cell Biol 2007; 8(9):681-691.Tatar M, Bartke A, Antebi A. The endocrine regulation of aging by insulin-like signals. Science 2003; 299(5611):1346-1351.
41. Danilowicz K, Bruno OD, Manavela M, Gomez RM, Barkan A. Correction of cortisol overreplacement ameliorates morbidities in patients with hypopituitarism: a pilot study. Pituitary 2008; 11(3):279-285.
42. Filipsson H, Johannsson G. GH replacement in adults: interactions with other pituitary hormone deficiencies and replacement therapies. Eur J Endocrinol 2009; 161 Suppl 1:S85-S95.

XII.

Abnormal metabolic phenotype in middle-aged GH-deficient adults despite long-term recombinant human GH replacement



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ABSTRACT

BACKGROUND: Adult GH deficiency (GHD) is associated with increased cardiovascular mortality. Recombinant human GH (rhGH) replacement has beneficial short-term metabolic effects. Although these positive effects sustain during longer follow-up, the prevalence of the metabolic syndrome (MS) remains increased in comparison with population data not adjusted for the higher mean BMI in GHD adults.

OBJECTIVE: To explore whether middle-aged patients with proposed physiological rhGH replacement have been normalized with respect to MS and its individual components in comparison with the general population, adjusted for age, sex and BMI.

METHODS: One-hundred and sixty-one GHD patients (aged 40-70yr) were studied before the start and after 5 years of rhGH replacement, and were compared with 1671 subjects (aged 45-66yr) from the general population (NEO Study).

RESULTS: MS proportion in GHD patients was 41.0% before the start of rhGH suppletion, increasing to 53.4% after 5 years (p=0.007). Despite chronic rhGH replacement, GHD patients had a 1.3-times higher MS proportion than the general population, independently of age, sex and BMI (95%CI 1.1-1.5, p=0.008). The GHD population showed a different metabolic profile than the general population with similar BMI: an increased risk of hypertriglyceridaemia (adjusted prevalence ratio (PR) 2.0, 95%CI 1.7-2.3) and low HDL-C (adjusted PR 1.8, 95%CI 1.5-2.2), but less hyperglycaemia (adjusted PR 0.5, 95%CI 0.4-0.7).

CONCLUSIONS: Despite 5 years of rhGH replacement, GHD patients still have a different metabolic profile and more frequently MS than the general population. These differences were independent of BMI, and resemble the unfavorable metabolic profile of untreated GHD patients, pointing to question the long-term benefits of rhGH replacement.

INTRODUCTION

Growth Hormone Deficiency (GHD) in adults is associated with an adverse metabolic profile, including abdominal obesity, dyslipidemia, and an increased mortality risk (1;2). Short-term follow-up studies have shown that recombinant human GH (rhGH) replacement reduced some, but not all of these cardiovascular risk factors (3). 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 (3). Based on these positive short-term metabolic effects, as well as an improvement in quality of life (QoL), rhGH replacement has become widely accepted as chronic therapy in adult GHD.

Despite improvement of individual components of the metabolic syndrome (MS), the overall prevalence of the MS was not normalized in GHD patients after several years of physiological rhGH replacement (4;5). At present, data on long-term efficacy and safety of rhGH replacement are limited and uncontrolled for the effect of aging (5-18). Recently, we have reported a further increase in body mass index (BMI) and MS prevalence despite 10 years of rhGH replacement (19). Thus, it can be questioned whether in the long term rhGH replacement is able to improve or even normalize the adverse metabolic profile present in hypopituitary patients. As an ideal unselected GHD control group without rhGH replacement is not available, we selected the Leiderdorp cohort of the Netherlands Epidemiology of Obesity (NEO) Study as representation of the general population for comparison. The NEO Study is a population-based cohort study of middle-aged Dutch adults from the same geographical area as the GHD patients.

The primary aim of the present study was to compare the metabolic profile, as reflected by proportion of the MS and its individual components, between GHD patients after chronic rhGH replacement and the general population, adjusted for age and sex, to assess the ability of current rhGH replacement strategies to normalize MS features. Second, we critically compared metabolic parameters between treated GHD patients and the general population, while additionally adjusting for BMI. Finally, we compared the MS proportion in GHD patients before start and after chronic rhGH replacement.

PATIENTS AND METHODS

STUDY DESIGN: In the present study, we compared the proportion of the MS and its individual components between middle-aged GHD patients and the general population. First, adult GHD patients were studied before the start of rhGH suppletion and after 5 years of rhGH replacement, to study the metabolic effects of chronic rhGH suppletion. Second, we compared chronically treated GHD patients (defined as 5 years) with the general population, using data from the Leiderdorp cohort of the NEO Study (*vide infra*) (20), adjusting for age and sex and subsequently with additional adjustments for BMI, to assess the ability of current rhGH replacement strategies to completely normalize MS features.

PATIENT SELECTION: This study includes all consecutive patients diagnosed with adult-onset (AO) and childhood-onset (CO) GHD at the Endocrinology Department of the LUMC from 1994 onwards. Data have been prospectively collected in a database. We investigated the effects of chronic stable rhGH replacement in middle-aged GHD patients. Chronic stable replacement was arbitrarily defined as replacement for ≥5 years, because in such a period metabolic changes induced by rhGH therapy start will have been stabilized (3;21). We selected all GHD patients aged 40-70 years, who started rhGH replacement ≤2007, to enable a 5-year follow-up period. The age criterion used was chosen to match the GHD patients with middle-aged persons from the general population from the Leiderdorp cohort (*vide infra*) and to avoid nonpositivity. This resulted in a total inclusion of 161 eligible GHD patients (AO 155/6 CO). The 6 patients with CO-GHD received rhGH replacement during childhood, and restarted with rhGH replacement as this therapy became widely accepted for adult GHD patients.

TREATMENT PROTOCOL: GHD had been defined before start of rhGH replacement by a GH peak response to the Insulin Tolerance Test (ITT) <3µg/l (glucose nadir <2.2mmol/l) according to current guidelines or Growth Hormone Releasing Hormone/Arginine-test (GHRH/Arg) with BMI-adjusted GH cut-offs, in case of contraindications for ITT (5;22). All patients receiving rhGH replacement during childhood were retested at the time of transition to the adult outpatient clinic, after treatment cessation for >3 months. All patients were treated with rhGH (Genotropin Pharmacia/Pfizer, Skokie, IL; Zomacton Ferring, Troy Hills, NJ; or Norditropin NovoNordisk, Princeton, NJ), injected subcutaneously in the evening. In all patients, GH starting dose was 0.2mg/day, which was

individually adjusted each month in the first half year to achieve serum IGF-1 concentrations within the age-dependent laboratory reference range, aimed at SDS between 0 and +2. After reaching stable plasma concentrations, this individualized dose was continued and adjusted according to the IGF-1 SDS.

Adrenocorticotropic hormone (ACTH) deficiency was defined as an insufficient increase in cortisol levels (absolute value 0.55mmol/l) after an ITT or corticotropin-releasing hormone stimulation test in case of contraindications for ITT. When secondary amenorrhea was present for >1 year, premenopausal women were classified as gonadotropin-deficient. In men, gonadotropin deficiency was defined as a testosterone level <8.0nmol/l. Thyroid-stimulating hormone (TSH) deficiency was defined as total thyroxine (T_4) or free T_4 level <10pmol/l in addition to ≥2 deficient pituitary axes. Hypopituitarism was adequately supplemented by hydrocortisone, L-thyroxine, testosterone in men, and/or estrogen in combination with progestagens (in premenopausal women only). Dosages of the hormonal replacement therapy were monitored and adjusted as required.

Patients were treated with lipid-lowering medication and antihypertensive medication according to the discretion of their attending physicians. Efficacy and safety parameters were assessed yearly, next to a routine assessment of pituitary function.

GENERAL POPULATION: The NEO Study is currently performed at the Leiden University Medical Center (LUMC) to investigate pathways that lead to obesity-related diseases. The NEO Study is a population-based prospective cohort study of individuals aged 45-65 years, with an oversampling of individuals with overweight or obesity (BMI ≥27kg/m^2) (20). Within the NEO Study, as reference group, all inhabitants aged between 45-65 years from one municipality (Leiderdorp) were invited, irrespective of their BMI. Baseline measurements have been performed between 2008 and 2012. The study was approved by the Medical Ethics Committee of the LUMC and all participants gave informed consent.

We used the Leiderdorp cohort (N=1671) for comparison with our chronically rhGH-treated GHD patients. GHD patients and subjects from the general population were from the same geographic area.

STUDY PARAMETERS: The following efficacy parameters were studied:

1. Biochemical parameters: fasting glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) levels. LDL-C concentrations were calculated by the Friedewald formula. Blood samples were taken after an overnight fast.
2. Anthropometric parameters: body weight and height, waist circumference, hip circumference, systolic and diastolic blood pressures (SBP and DBP, respectively) were measured. BMI (in kg/m^2) and waist-hip ratio were calculated.
3. Additional information on medication use and comorbidity was gathered from patient records.

METABOLIC SYNDROME (MS): The MS was defined according to the updated third report of the 2006 National Cholesterol Education Program's Adult Treatment Panel (NCEP-ATP III) criteria, which required the presence of ≥3 of the following conditions (23;24):

1. Fasting plasma glucose concentration ≥100mg/dl or on anti-diabetic drug treatment;
2. TG concentration ≥150mg/dl or on lipid-lowering drug treatment (statins and/or fibrates);
3. HDL-C concentration<40mg/dl in men and<50mg/dl in women, or on lipid-lowering drug treatment (statins and/or fibrates);
4. BP ≥130/85mmHg or on anti-hypertensive treatment;
5. Waist circumference ≥102cm in men and ≥88cm in women.

ASSAYS: From 1986 to 2005, serum IGF-1 concentrations were determined by RIA (Incstar, Stillwater, MN) with a detection limit of 1.5nmol/l and an interassay CV <11%. IGF-1 is expressed as SDS for age- and gender-related normal levels determined in the same laboratory (21). Since 2005, serum IGF-1 concentrations (nmol/l) were measured using an immunometric technique on an Immulite 2500 system (Siemens Healthcare Diagnostics, Deerfield, IL, USA). The intra-assay variations at mean plasma levels of 8 and 75nmol/l were 5.0 and 7.5%, respectively. IGF-1 levels were expressed as SDS, using lambda-mu-sigma smoothed reference curves based on 906 controls (25;26).

In GHD patients, a Hitachi 747 autoanalyzer (Roche) was used to quantify serum concentrations of glucose, TC, and TG. HDL-C was measured with a homogenous enzymatic assay (Hitachi 911, Roche). In 2003, a Roche Modular Analytics P800 replaced the Hitachi 747 with no change in the chemistry components. In the general population, glucose, TC, HDL-C and TG levels were measured by Roche Modular Analytics

P800. Samples in patients and controls were analyzed in the same laboratory. Samples of patients were obtained between 1994 and 2013; samples of controls between 2008 and 2012.

STATISTICAL ANALYSIS: SPSS for Windows, Version 20.0 (SPSS,Inc.,Chicago,IL,USA) was used for statistical analysis. Results are presented as mean±SD, unless stated otherwise. For comparison of the MS proportion in GHD patients before start and after 5 years of rhGH suppletion, we performed the Friedman Test for related fractions. Although 19 patients did not complete 5 years of rhGH replacement for various reasons (*vide infra*), these patients were included using the last observation carried forward method (intention-to-treat approach), thereby including the last available measurements during rhGH replacement in the analyses. Independent *T* tests were used to compare crude metabolic data between 5-year rhGH-treated GHD patients and the general population. The relationships in MS (components) proportion between GHD patients after 5 years of rhGH replacement and the general population are reported as prevalence ratios (PRs), using the log-binominal regression model using STATA Statistical Software version 12.1(Statacorp,College Station,TX), allowing direct estimation of PRs (27;28), which is, because of the high incidence of the MS, more appropriate than logistic models that only allow estimation of odds ratios. Analyses were adjusted for age and sex, and subsequently with additional adjustments for BMI. P<0.05 is considered statistically significant.

RESULTS

CHARACTERISTICS OF PATIENTS AND CONTROLS: We studied 161 adult GHD patients, aged 40-70 years at the time of GHD diagnosis (*Table 1*). Pituitary deficiency was mainly caused by pituitary tumors or their treatment. Patients had been treated with surgery (transsphenoidal N=109/ transcranial N=24) and radiotherapy (N=76). Etiological diagnoses of GHD were non-functioning adenoma (N=67), functioning adenoma (N=52), craniopharyngeoma (N=12), cerebral malignancy (N=2), congenital (N=6) and other causes (N=22). Most patients had multiple pituitary hormone deficiencies (ACTH deficiency N=142, TSH deficiency N=132, FSH/LH deficiency N=120, ADH deficiency N=27), whereas only 6 patients had isolated GHD. Sex steroids were supplemented in 21% of female patients (N=15) and 87% of male patients (N=77).

One-hundred and forty-two patients completed 5 years of rhGH replacement (*Figure 1*). Reasons for preliminary discontinuation in 19 patients (11.8%) wer: death (N=5), malignancy (N=2), growth of primary tumor (N=2), increase in prostate-specific antigen (PSA) level (N=1), lack of subjective benefit (N=4), side-effects: carpal tunnel syndrome (N=2), weight increase (N=1), and follow-up in other centers (N=2).

At 5 years of rhGH replacement, GHD patients were compared with 1671 subjects from the general population (*Table 1*). Proportion of men and mean BMI were higher in patients than in the general population (p=0.006 and p<0.001, respectively).

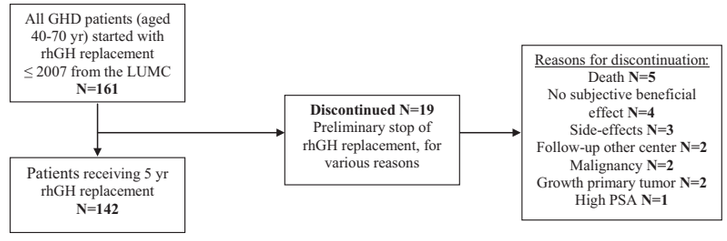

Figure 1. Flow chart of selection and follow-up of our middle-aged GHD cohort

GH DOSE, IGF-1 SDS AND HYDROCORTISONE DOSE DURING RHGH REPLACEMENT: Mean GH doses after dose titration (1 year) and 5 years of rhGH replacement were, respectively, 0.35±0.16mg/day and 0.37±0.19mg/day (p=0.399). After 5 years of rhGH replacement, females received higher mean GH doses than males (0.40±0.23mg/day and 0.34±0.17mg/day, respectively, p=0.05).

During the entire period of rhGH suppletion, median of IGF-1 SDS was within the normal range. Median IGF-1 SDS increased significantly from the untreated state from -1.30 (interquartile range (IQR) -2.43, +0.16) to 0.47 (IQR -0.50, +1.93) after 5 years of rhGH suppletion (p<0.001). In males, median IGF-1 SDS increased from -1.17 (IQR -2.17, +0.92) before treatment to 0.42 (IQR -0.43, +2.03) after 5 years (p<0.001); in females, median IGF-1 SDS increased from -1.63 (IQR -3.04, +-0.47) before treatment to 0.51 (IQR -0.57, +1.71) after 5 years (p<0.001).

Mean hydrocortisone doses were 17.43±12.40mg/day and 18.18±10.81mg/day at the start and after 5 years of rhGH suppletion, respectively, indicating no significant change during follow-up. Mean fT4 levels did not significantly change during 5 years of rhGH replacement (16.2±3.7pmol/l at the start and 16.8±3.7pmol/l after 5 years of rhGH).

MS PROPORTION IN UNTREATED GHD PATIENTS: Before the initiation of rhGH replacement, 66 out of 161 patients fulfilled the NCEP-ATP III criteria, resulting in an MS proportion of 41.0% in untreated GHD (37.1% in males, 45.8% in females). Hyperglycaemia, hypertriglyceridaemia, low HDL-C levels, hypertension and abdominal obesity were present in, respectively, 13.0%, 59.0%, 47.8%, 68.3% and 35.4% of the patients.

EFFECTS OF 5 YEARS RHGH REPLACEMENT ON THE MS PROPORTION IN GHD ADULTS: The MS proportion increased significantly from 41.0% before to 53.4% after 5 years of rhGH replacement (p=0.007), especially due to an increased proportion of hyperglycaemia (p=0.02). In males, HDL-C levels decreased significantly (p<0.001). As indicated in *Figure 2*, the increase in MS proportion was most prominent in males (from 37.1% at the start of rhGH replacement to 53.9% after 5 years, p=0.005). Exclusion of CO-GHD patients (N=6) did not significantly change these results. BMI did not significantly change over time, from 27.5±4.5 kg/m^2 before rhGH replacement to 28.0±4.5 kg/m^2 after 5 years (p=0.772).

COMPARISON OF MS PROPORTION BETWEEN GHD PATIENTS ON CHRONIC RHGH REPLACEMENT AND THE GENERAL POPULATION, ADJUSTED FOR AGE AND SEX (*TABLE 2*): In the general population, we observed an MS proportion of 30.3%. The MS proportion in chronically treated GHD patients was increased when compared to controls, taking into account age and sex (53.4% *vs.* 30.3%) (adjusted PR (95%CI) = 1.4(1.1-1.6), p=0.001) (*Table 2*). For clinical interpretation, mean laboratory values were also presented (*Table 3*).

COMPARISON OF MS PROPORTION BETWEEN GHD PATIENTS AFTER 5 YEARS OF RHGH REPLACEMENT AND THE GENERAL POPULATION, ADDITIONALLY ADJUSTED FOR BMI: After additional correction for BMI, besides age and sex, GHD patients still had a 1.3-times increased MS proportion when compared with the general population, despite 5 years of rhGH replacement (adjusted PR (95%CI) = 1.3(1.1-1.5), p=0.008) (*Table 2*). Both in male and female GHD patients, an significant increased MS proportion was seen when compared with the general population (*Figure 2*). When looking at the individual MS components, treated GHD patients differed from BMI-matched controls with respect to glucose, triglycerides and HDL-C levels (*Table 2*).

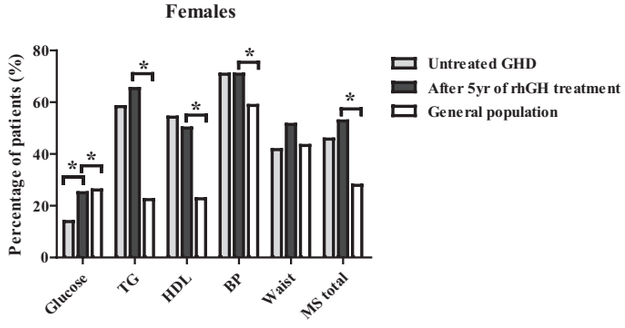

Figure 2A.

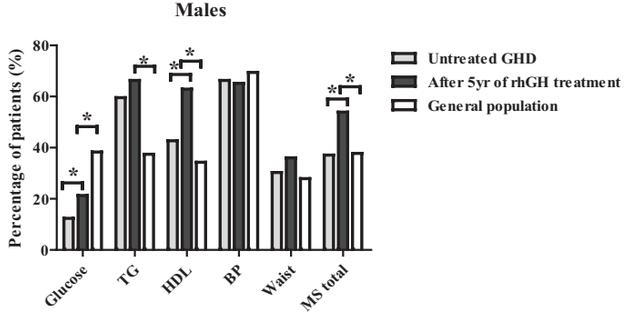

Figure 2B.

Proportion of MS (components) in adult GHD patients, before the initiation of rhGH replacement and after 5 years, in comparison to the general population, separated for females (A) and males (B)

We studied the MS proportion and its individual components in: (1) 5-year treated GHD patients in comparison to situation before the start of rhGH replacement, using the Friedman test for related fractions; and (2) between GHD patients after 5 years of rhGH replacement compared to the general population, using a log-binominal regression model with adjustments for age and BMI. Analyses were stratified for sex. MS was defined according to the NCEP-ATP III criteria (23;24).

**, p<0.05*

TG, triglycerides; HDL, high-density lipoprotein cholesterol; BP, blood pressure; waist, waist circumference; MS, metabolic syndrome; rhGH, recombinant human Growth Hormone.

Table 1. Characteristics of 161 middle-aged GHD patients before the start and after 5 yr of rhGH replacement and 1671 controls representing the general population

Patient characteristics	GHD patients Before start of rhGH (N = 161)	GHD patients After 5 years of rhGH (N = 161)	General population# (N = 1671)
Sex, female (N (%))	72 (44.7%)	72 (44.7%)	937(56.1%)*
Age, years	54.7 ± 8.5 (40 – 70)	59.7 ± 8.5 (45 – 75)	56.0 ± 6.0 (45 – 66)*
BMI, kg/m^2 (range)	27.5 ± 4.5 (19.6 – 57.1)	28.0 ± 4.5 (18.5 – 53.8)	26.3 ± 4.5 (17.2 – 57.1)*
Lipid-lowering drugs (N (%))	36 (22.3%)	78 (48.4%)	182 (10.9%)*
Antihypertensive medication (N (%))	36 (22.3%)	77 (47.8%)	402 (24.1%)*
Anti-diabetic medication (N (%))	8 (5.0%)	14 (8.7%)	52 (3.1%)*

Data are presented as mean ± SD, unless specified otherwise.
#, The Leiderdorp cohort of the NEO Study was used as control group representing the general population (20).
**, p<0.05 (Patients after 5 years of rhGH replacement vs general population).*

N, number of patients; GHD, Growth Hormone Deficiency; rhGH, recombinant human Growth Hormone replacement; BMI, body mass index.

Table 2. Comparison of MS proportion, according to the NCEP-ATP III criteria, between middle-aged GHD patients on chronic rhGH replacement and the general population

	GHD patients (N = 161)	General population (N = 1671)	PR (95%CI)[1]	PR (95%CI)[2]
Fasting glucose	37 / 161 (23.0%)	539 / 1663 (32.4%)	0.5 (0.4 – 0.7)*	0.5 (0.4 – 0.7)*
Triglycerides	106 / 161 (65.8%)	427 / 1666 (25.6%)	2.0 (1.7 – 2.4)*	2.0 (1.7 – 2.3)*
HDL-C	92 / 161 (57.1%)	418 / 1666 (25.1%)	2.0 (1.7 – 2.3)*	1.8 (1.5 – 2.2)*
Blood pressure	109 / 161 (67.7%)	1054 / 1668 (63.2%)	0.9 (0.8 – 1.0)	0.9 (0.8 – 1.1)
Waist	69 / 161 (42.9%)	597 / 1669 (35.8%)	1.1 (0.9 – 1.4)	1.0 (0.8 – 1.3)
Metabolic syndrome	86 / 161 (53.4%)	503 / 1660 (30.3%)	1.4 (1.1 – 1.6)*	1.3 (1.1 – 1.5)*

Values are expressed as number (percentage). Chronic rhGH replacement was defined as 5 years. MS was defined according to the NCEP-ATP III criteria (23;24). Log-binominal regression models were applied with robust standard errors, allowing direct estimations of PRs adjusted for age and sex, and, subsequently, with additional adjustments for BMI.

N, number of patients; rhGH, recombinant human Growth Hormone replacement; GHD, Growth Hormone Deficiency; HDL-C, high-density lipoprotein cholesterol; waist, waist circumference; BMI, body mass index; PR, prevalence ratio; 95%CI, 95% confidence interval.

RR1, adjusted for age and sex.
RR2, adjusted for age, sex and BMI.
**, p<0.01.*

Table 3. Mean values of the metabolic parameters of the MS as included in the NCEP-ATP III criteria, in adult GHD patients before the start and after 5 years of rhGH replacement, and the general population

	GHD patients Before start of rhGH (N = 161)	GHD patients After 5 yr of rhGH (N = 161)	General population (N = 1671)
Fasting glucose (mmol/l)	4.8 ± 0.8**	5.2 ± 1.6*	5.5 ± 1.1
Triglycerides (mmol/l)	2.0 ± 1.2	1.9 ± 0.9*	1.2 ± 0.8
HDL-C (mmol/l)	1.4 ± 0.4**	1.5 ± 0.5#	1.6 ± 0.5
Systolic blood pressure (mmHg)	134.0 ± 17.8	135.7 ± 17.6*	130.4 ± 16.9
Diastolic blood pressure (mmHg)	83.9 ± 9.3	83.4 ± 9.5	83.4 ± 10.1
Waist circumference (cm)	97.2 ± 11.8	98.6 ± 11.2*	91.2 ± 12.8

Values are expressed as mean ± SD.

N, number of patients; rhGH, recombinant human Growth Hormone replacement; GHD, Growth Hormone Deficiency; MS, metabolic syndrome; HDL-C, high-density lipoprotein cholesterol.

**, p<0.01; #, p<0.05 (GHD after 5 years rhGH replacement vs the general population).*
***, p<0.001 (GHD after 5 years of rhGH replacement vs GHD before the start of rhGH suppletion).*

DISCUSSION

This study demonstrates a high MS proportion of 41.0% in middle-aged GHD patients before the start of treatment, which further increases to 53.4% after 5 years of rhGH replacement. Especially a higher proportion of hyperglycaemia contributed to this increase, whereas the lipid profile was not affected. Despite chronic rhGH replacement, GHD patients still have a 1.3-times increased MS proportion when compared to the general population, independently of age, sex and BMI. GHD patients still have a different metabolic profile, characterized by hypertriglyceridaemia and low HDL-C, whereas the proportion of hyperglycaemia is lower than that observed in the general population.

In middle-aged GHD patients, the increased cardiovascular risk profile is a main justification of rhGH replacement. Important components of this risk are dyslipidemia, manifesting as raised levels of LDL-C and TGs, and reduced HDL-C levels (29). In addition, increased fat mass, reduced insulin sensitivity, and increases in inflammatory markers are often present (29;30). Mortality is substantially higher in GHD adults than in general population, particularly among women (1;2). RhGH replacement has beneficial metabolic effects in the short-term, including improvement in the lipid profile, body composition and cardiac function (3), which were reported to be sustained during longer follow-up (14;18), although glucose and insulin levels increase. However, despite improvement of several cardiovascular risk factors, MS prevalence remains increased (4;5;19). Obvious limitations of the few available long-term studies are the lack of an untreated GHD control population and difficulties with physiological rhGH replacement.

This study is the first to investigate the metabolic profile of chronically treated GHD patients in a controlled manner, taking into account not only age and sex, but also BMI. In this respect, the Leiderdorp cohort represents the general population with comparable BMI distribution to that observed in other Dutch population-based studies (http://www.rivm.nl/nldemaat.rivm.2012). We demonstrated that, independent of BMI, chronically treated GHD patients have a persistently different metabolic profile than the general population, with a lower prevalence of insulin resistance, but an adverse lipid profile. It is remarkable that although this GHD cohort is thought to be adequately substituted, as reflected by mean IGF-1 SDS within the physiological range as well as no hydrocortisone oversubstitution, the metabolic profile after chronic rhGH replacement resembles the profile of an untreated GHD patient, with predominantly lipid abnormalities. Additional analyses in which we compared the GHD patients before starting rhGH replacement with general population show similar results, suggesting that the metabolic profile is not significantly influenced by long-term rhGH replacement (*data not shown*).

These findings give rise to several questions. First, there is an increasing awareness of intrinsic imperfections of endocrine replacement therapy in general (31), that may also well apply to GHD patients. It is questioned whether circulating total IGF-1 levels truly reflect peripheral IGF-1 activity, as several earlier reports indicate that measuring free unbound IGF-1 levels and IGF-1 bioactivity better reflect the GH/IGF-1 status (32). Further study is needed to identify sensitive biomarkers to monitor rhGH replacement therapy. Second, it is previously shown that there is an optimum for GH dosing with respect to insulin resistance (33). In this respect, further lowering of the GH dose is probably needed to minimize insulin resistance. On the other hand, the MS proportion in GHD patients remained increased despite chronic rhGH replacement and resembles the metabolic profile of untreated GHD, suggesting that GHD may have irreversible effects on the cardiovascular system. In the context of increasing evidence for, on the one hand, a limited or even negative role of GH and IGF-1 in cancer, longevity and cardiovascular disease (34-36), and, on the other hand, the limited evidence for benefit of rhGH substitution in the elderly GHD population and long-term positive effects on QoL (37)27), long-term rhGH use in GHD adults should be critically re-evaluated. Third, we cannot exclude coexisting hypothalamic damage, especially in case of a history of large tumors with suprasellar extension (38). Obesity is a well-recognized complication in patients treated for tumors in the hypothalamic-pituitary region, which is attributed to compression of surrounding tissues (39). This hypothalamic damage could be an independent factor influencing the metabolic profile in GHD patients. Finally, most patients had multiple pituitary hormone deficiencies, making distinction between effects of rhGH replacement or effects of suboptimal or excessive replacement therapy of other hormones difficult.

Several validated definitions can be applied for the definition of the MS, precluding direct comparisons between studies (40). We used the NCEP-ATP III criteria (23;24) to be comparable to our previous studies on MS (5;19). In the NCEP-ATP III criteria, the use of antihypertensive, anti-diabetic and lipid-lowering medication is incorporated. In the original description of the NCEP-ATP III criteria, statins are not specifically mentioned. However, the lipid-lowering effects of statins are well established in the general population, and thereby significantly influencing the lipid criteria of the MS (41).

We found sex-specific differences in MS proportion and the metabolic response to rhGH replacement. In untreated GHD females, MS proportion was very high compared with the general population. This might be in line with a recent report of Van Bunderen *et al.*, describing an increased risk of cardiovascular death in GHD females, despite rhGH substitution (42). The increased incidence in cardiovascular deaths in females might partly be attributed to the more atypical presentation of cardiovascular diseases (43) or inadequately substituted hypogonadism causing preterm menopause or hypocortisolism. The most predominant increase in the MS proportion during rhGH replacement in males further supports that the cardiovascular risk pattern in GHD patients is sex-specific.

When compared with other population-based control cohorts, such as the MORGEN cohort (1993-1997) or the Tromsø Study (1994-1995) (44), the MS proportion found in the Leiderdorp cohort was nearly doubled (30.3% in Leiderdorp cohort *vs* 15.7% in MORGEN cohort and 19.2% (males) and 17.8% (females) in the Tromsø Study) (5). As the same criteria for MS were applied, we expect that this increase in MS proportion most likely reflects the increase in BMI and an increased use of antihypertensive medication in the general population over 15 years and emphasizes the importance for a contemporary comparison group from the general population.

The strength of the present study is the availability of large recent population-based control data and the inclusion of analysis on the crude metabolic laboratory data in addition to the age-, sex-, and BMI-adjusted analysis using the NCEP-ATP III criteria of MS. Our relatively large middle-aged GHD cohort reflects the best-case scenario in which hormonal suppletion of all pituitary axes is optimalized and adequately monitored. A potential limitation could be the fact that we evaluated the MS proportion in the untreated GHD situation only in patients in whom rhGH replacement was actually initiated. This could introduce a selection bias, because GHD patients without receiving rhGH replacement are likely to differ from patients that actually received rhGH replacement. Second, it has to be noted that the patients in our GHD cohort are very heterogeneous with respect to the cause of GHD, failure of other pituitary hormone axes and history of cranial irradiation and/or surgery, which may have influenced the results. Third, we only reported the metabolic parameters included in the MS according to the NCEP-ATP III criteria, not for example LDL-C or body fat percentages, which are also GH-dependent parameters. Another feature that needs to be addressed is the presence of interactions between glucocorticoids and rhGH during substitution. In the past, hydrocortisone suppletion in hypopituitary patients frequently resulted in supraphysiological cortisol levels, which accounted for at least part of the adverse metabolic profile in these patients. However, as most patients received hydrocortisone doses of 20 mg/day or less during the entire follow-up period and cortisol levels were highly monitored, we expect that in our cohort hydrocortisone oversuppletion is not likely to have negatively influenced the results. In this respect, it has also be noted that minor thyroid dysfunction and insufficient thyroid replacement has a significant independent negative impact on cardiovascular risk factors in hypopituitary adults (45), further emphasizing the importance of adequate hormonal suppletion of all deficient pituitary axes. In addition, it would be ideally preferred to include a longitudinal follow-up control group for comparison; however, such a control group was not available in the Netherlands. Finally, it has to be noted that a priori GHD patients are more likely to receive anti-diabetic and/or lipid-lowering medication than controls in case of lipid and/or glucose abnormalities, since these patients are regularly followed up at the Deptartment of Endocrinology, which is not the case for controls without a history of pituitary disease.

In conclusion, despite 5 years of rhGH replacement, GHD patients still have a different metabolic profile and a higher MS proportion than the general population. These differences were independent of BMI, and resemble the metabolic profile of untreated GHD patients. This emphasizes the need for further study to establish whether rhGH replacement is actually beneficial in the long-term, adequately incorporating the cost-effectiveness, QoL, and the potential negative effects of GH/IGF-1 on cancer, longevity and cardiovascular risk.

REFERENCE LIST

1. Rosen T, Bengtsson BA. Premature mortality due to cardiovascular disease in hypopituitarism. *Lancet* 1990;336:285-288.
2. Tomlinson JW, Holden N, Hills RK, Wheatley K, Clayton RN, Bates AS, Sheppard MC, Stewart PM. Association between premature mortality and hypopituitarism. West Midlands Prospective Hypopituitary Study Group. *Lancet* 2001;357:425-431.
3. Maison P, Griffin S, Nicoue-Beglah M, Haddad N, Balkau B, Chanson P. Impact of growth hormone (GH) treatment on cardiovascular risk factors in GH-deficient adults: a Metaanalysis of Blinded, Randomized, Placebo-Controlled Trials. *J Clin Endocrinol Metab* 2004;89:2192-2199.
4. Attanasio AF, Mo D, Erfurth EM, Tan M, Ho KY, Kleinberg D, Zimmermann AG, Chanson P. Prevalence of metabolic syndrome in adult hypopituitary growth hormone (GH)-deficient patients before and after GH replacement. *J Clin Endocrinol Metab* 2010;95:74-81.
5. van der Klaauw AA, Biermasz NR, Feskens EJ, Bos MB, Smit JW, Roelfsema F, Corssmit EP, Pijl H, Romijn JA, Pereira AM. The prevalence of the metabolic syndrome is increased in patients with GH deficiency, irrespective of long-term substitution with recombinant human GH. *Eur J Endocrinol* 2007;156:455-462.
6. Cenci MC, Conceicao FL, Soares DV, Spina LD, Brasil RR, Lobo PM, Michmacher E, Vaisman M. Impact of 5 years of growth hormone replacement therapy on cardiovascular risk factors in growth hormone-deficient adults. *Metabolism* 2008;57:121-129.
7. Chrisoulidou A, Beshyah SA, Rutherford O, Spinks TJ, Mayet J, Kyd P, Anyaoku V, Haida A, Ariff B, Murphy M, Thomas E, Robinson S, Foale R, Johnston DG. Effects of 7 years of growth hormone replacement therapy in hypopituitary adults. *J Clin Endocrinol Metab* 2000;85:3762-3769.
8. Elbornsson M, Gotherstrom G, Bosaeus I, Bengtsson BA, Johannsson G, Svensson J. Fifteen years of GH replacement increases bone mineral density in hypopituitary patients with adult-onset GH deficiency. *Eur J Endocrinol* 2012;166:787-795.
9. Giavoli C, Porretti S, Ronchi CL, Cappiello V, Ferrante E, Orsi E, Arosio M, Beck-Peccoz P. Long-term monitoring of insulin sensitivity in growth hormone-deficient adults on substitutive recombinant human growth hormone therapy. *Metabolism* 2004;53:740-743.
10. Gibney J, Wallace JD, Spinks T, Schnorr L, Ranicar A, Cuneo RC, Lockhart S, Burnand KG, Salomon F, Sonksen PH, Russell-Jones D. The effects of 10 years of recombinant human growth hormone (GH) in adult GH-deficient patients. *J Clin Endocrinol Metab* 1999;84:2596-2602.
11. Gotherstrom G, Svensson J, Koranyi J, Alpsten M, Bosaeus I, Bengtsson B, Johannsson G. A prospective study of 5 years of GH replacement therapy in GH-deficient adults: sustained effects on body composition, bone mass, and metabolic indices. *J Clin Endocrinol Metab* 2001;86:4657-4665.
12. Gotherstrom G, Bengtsson BA, Sunnerhagen KS, Johannsson G, Svensson J. The effects of five-year growth hormone replacement therapy on muscle strength in elderly hypopituitary patients. *Clin Endocrinol (Oxf)* 2005;62:105-113.
13. Gotherstrom G, Bengtsson BA, Bosaeus I, Johannsson G, Svensson J. Ten-year GH replacement increases bone mineral density in hypopituitary patients with adult onset GH deficiency. *Eur J Endocrinol* 2007;156:55-64.
14. Gotherstrom G, Bengtsson BA, Bosaeus I, Johannsson G, Svensson J. A 10-year, prospective study of the metabolic effects of growth hormone replacement in adults. *J Clin Endocrinol Metab* 2007;92:1442-1445.
15. Gotherstrom G, Elbornsson M, Stibrant-Sunnerhagen K, Bengtsson BA, Johannsson G, Svensson J. Ten years of growth hormone (GH) replacement normalizes muscle strength in GH-deficient adults. *J Clin Endocrinol Metab* 2009;94:809-816.
16. Roemmler J, Kuenkler M, Schneider HJ, Dieterle C, Schopohl J. Comparison of glucose and lipid metabolism and bone mineralization in patients with growth hormone deficiency with and without long-term growth hormone replacement. *Metabolism* 2010;59:350-358.
17. Svensson J, Fowelin J, Landin K, Bengtsson BA, Johansson JO. Effects of seven years of GH-replacement therapy on insulin sensitivity in GH-deficient adults. *J Clin Endocrinol Metab* 2002;87:2121-2127.
18. van der Klaauw AA, Romijn JA, Biermasz NR, Smit JW, van DJ, Dekkers OM, Roelfsema F, Pereira AM. Sustained effects of recombinant GH replacement after 7 years of treatment in adults with GH deficiency. *Eur J Endocrinol* 2006;155:701-708.
19. Claessen KM, Appelman-Dijkstra NM, Adoptie DM, Roelfsema F, Smit JW, Biermasz NR, Pereira AM. Metabolic profile in growth hormone-deficient (GHD) adults after long-term recombinant human growth hormone (rhGH) therapy. *J Clin Endocrinol Metab* 2013;98:352-361. doi:jc.2012-2940 [pii];10.1210/jc.2012-2940 [doi].
20. de Mutsert R, den Heijer M, Rabelink AJ, Smit JWA, Romijn JA, Rabe KF, Jukema JW, de Roos A, le Cessie S, Middeldorp, Rosendaal. The Netherlands Epidemiology of Obesity (NEO) Study: study design and data collection.
21. Janssen YJ, Frolich M, Roelfsema F. A low starting dose of genotropin in growth hormone-deficient adults. *J Clin Endocrinol Metab* 1997;82:129-135.
22. Ghigo E, Aimaretti G, Corneli G. Diagnosis of adult GH deficiency. *Growth Horm IGF Res* 2008;18:1-16.
23. Grundy SM, Brewer HB, Jr., Cleeman JI, Smith SC, Jr., Lenfant C. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/ American Heart Association conference on scientific issues related to definition. *Circulation* 2004;109:433-438.
24. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC, Jr., Spertus JA, Costa F. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 2005;112:2735-2752.
25. Cole TJ. The LMS method for constructing normalized growth standards. *Eur J Clin Nutr* 1990;44:45-60.
26. Rikken B, van DJ, Ringeling A, Van den Brande JL, Massa G, Wit JM. Plasma levels of insulin-like growth factor (IGF)-I, IGF-II and IGF-binding protein-3 in the evaluation of childhood growth hormone deficiency. *Horm Res* 1998;50:166-176.
27. Zou G. A modified poisson regression approach to prospective studies with binary data. *Am J Epidemiol* 2004;159:702-706.
28. Thompson ML, Myers JE, Kriebel D. Prevalence odds ratio or prevalence ratio in the analysis of cross sectional data: what is to be done? *Occup Environ Med* 1998;55:272-277.
29. Rosen T, Eden S, Larson G, Wilhelmsen L, Bengtsson BA. Cardiovascular risk factors in adult patients with growth hormone deficiency. *Acta Endocrinol (Copenh)* 1993;129:195-200.
30. Johansson JO, Landin K, Tengborn L, Rosen T, Bengtsson BA. High fibrinogen and plasminogen activator inhibitor activity in growth hormone-deficient adults. *Arterioscler Thromb* 1994;14:434-437.
31. Romijn JA, Smit JW, Lamberts SW. Intrinsic imperfections of endocrine replacement therapy. *Eur J Endocrinol* 2003;149:91-97.
32. Varewijck AJ, Lamberts SW, Uitterlinden P, Hofland LJ, Janssen JA. IGF-I bioactivity better reflects growth hormone deficiency than total IGF-I. *J Clin Endocrinol Metab* 2011;96:2248-2254. doi:jc.2011-0051 [pii];10.1210/jc.2011-0051 [doi].
33. Brugts MP, van Duijn CM, Hofland LJ, Witteman JC, Lamberts SW, Janssen JA. Igf-I bioactivity in an elderly population: relation to insulin sensitivity, insulin levels, and the metabolic syndrome. *Diabetes* 2010;59:505-508. doi:db09-0583 [pii];10.2337/db09-0583 [doi].
34. Barzilai N, Huffman DM, Muzumdar RH, Bartke A. The critical role of metabolic pathways in aging. *Diabetes* 2012;61:1315-1322.
35. Hartman ML, Xu R, Crowe BJ, Robison LL, Erfurth EM, Kleinberg DL, Zimmermann AG, Woodmansee WW, Cutler GB, Jr., Chipman JJ, Melmed S. Prospective safety surveillance of GH-deficient adults: comparison of GH-treated vs untreated patients. *J Clin Endocrinol Metab* 2013;98:980-988. doi:jc.2012-2684 [pii];10.1210/jc.2012-2684 [doi].
36. Rozing MP, Westendorp RG, Frolich M, de Craen AJ, Beekman M, Heijmans BT, Mooijaart SP, Blauw GJ, Slagboom PE, van HD. Human insulin/IGF-1 and familial longevity at middle age. *Aging (Albany NY)* 2009;1:714-722.
37. Kokshoorn NE, Biermasz NR, Roelfsema F, Smit JW, Pereira AM, Romijn JA. GH replacement therapy in elderly GH-deficient patients: a systematic review. *Eur J Endocrinol* 2011;164:657-665.
38. Karavitaki N, Cudlip S, Adams CB, Wass JA. Craniopharyngiomas. *Endocr Rev* 2006;27:371-397. doi:er.2006-0002 [pii];10.1210/er.2006-0002 [doi].
39. Iughetti L, Bruzzi P. Obesity and craniopharyngioma. *Ital J Pediatr* 2011;37:38. doi:1824-7288-37-38 [pii];10.1186/1824-7288-37-38 [doi].
40. Nilsson PM, Engstrom G, Hedblad B. The metabolic syndrome and incidence of cardiovascular disease in non-diabetic subjects--a population-

based study comparing three different definitions. *Diabet Med* 2007;24:464-472. doi:DME2142 [pii];10.1111/j.1464-5491.2007.02142.x [doi].
41. Gould AL, Rossouw JE, Santanello NC, Heyse JF, Furberg CD. Cholesterol reduction yields clinical benefit: impact of statin trials. *Circulation* 1998;97:946-952.
42. van Bunderen CC, van N, I, Arwert LI, Heymans MW, Franken AA, Koppeschaar HP, van der Lely AJ, Drent ML. Does growth hormone replacement therapy reduce mortality in adults with growth hormone deficiency? Data from the Dutch National Registry of Growth Hormone Treatment in adults. *J Clin Endocrinol Metab* 2011;96:3151-3159.
43. Dey S, Flather MD, Devlin G, Brieger D, Gurfinkel EP, Steg PG, Fitzgerald G, Jackson EA, Eagle KA. Sex-related differences in the presentation, treatment and outcomes among patients with acute coronary syndromes: the Global Registry of Acute Coronary Events. *Heart* 2009;95:20-26.
44. Herder M, Arntzen KA, Johnsen SH, Mathiesen EB. The metabolic syndrome and progression of carotid atherosclerosis over 13 years. The Tromso study. *Cardiovasc Diabetol* 2012;11:77. doi:1475-2840-11-77 [pii];10.1186/1475-2840-11-77 [doi].
45. Klose M, Marina D, Hartoft-Nielsen ML, Klefter O, Gavan V, Hilsted L, Rasmussen AK, Feldt-Rasmussen U. Central hypothyroidism and its replacement have significant influence on cardiovascular risk factors in adult hypopituitary patients. *J Clin Endocrinol Metab* 2013;98:9. doi:10.1210/jc.2013-1610.

XIII.

Effects of up to 15 years of recombinant human GH (rhGH) replacement on bone metabolism in adults with Growth Hormone Deficiency (GHD): The Leiden Cohort Study

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ABSTRACT

BACKGROUND: Growth Hormone Deficiency (GHD) in adulthood may be associated with a decreased bone mineral density (BMD), a decreased bone mineral content (BMC) and an increased fracture risk. Recombinant human GH (rhGH) replacement induces a progressive increase in BMD for up to 5-7 years of treatment. Data on longer follow-up are however scarce.

METHODS: Two-hundred and thirty adult GHD patients (mean age 47.1 years, 52.6% female), of whom 88% patients had adult-onset (AO) GHD, receiving rhGH replacement for ≥5 years were included in the study. Most patients had multiple pituitary hormone deficiencies. Bone turnover markers, BMC and BMD and T-scores at the lumbar spine and femoral neck were evaluated at baseline, and after 5, 10 and 15 years of rhGH replacement. In addition, clinical fracture incidence was assessed.

RESULTS: Mean lumbar spine BMD, lumbar spine BMC and T-scores gradually increased during the first 10 years of rhGH replacement and remained stable thereafter. Largest effects of rhGH supplementation were found in men. In the small subset of patients using bisphosphonates, use of bisphosphonates did not impact additional beneficial effects in the long-term. Low baseline BMD positively affected the change in BMD and BMC over time, but there was a negative effect of high GH dose at 1 year on the change in BMD and BMC over time. Clinical fracture incidence during long-term rhGH replacement was 20.1/1000 py.

CONCLUSIONS: Fifteen years of rhGH replacement in GHD adults resulted in a sustained increase in BMD values at the lumbar spine, particularly in men, and stabilization of BMD values at the femoral neck. Clinical fracture incidence was suggested not to be increased during long-term rhGH replacement.

INTRODUCTION

Growth Hormone (GH) and Insulin-like Growth Factor-1 (IGF-1) are important regulators of bone growth and metabolism during the life span (1). IGF-1 mediates most of the effects of GH on skeletal metabolism via the IGF-1 receptor (2), although GH also exerts direct effects on bone (3). GH and IGF-1 act as anabolic hormones on bone by stimulating proliferation, and to some extent, differentiation of osteoblasts. Furthermore, osteoclastic bone resorption is stimulated, resulting in an overall increase in bone remodeling (3;4).

Untreated Growth Hormone Deficiency (GHD) is characterized by low bone turnover, decreased bone mineral density (BMD) and bone mineral content (BMC)(5-7). Treatment with recombinant human GH (rhGH) replacement has shown to increase bone turnover as reflected by an increase in bone formation and bone resorption markers. Consequently, there is an initial decline in (or unchanged) BMD, followed by a small increase of about 1-2% in the first 2 years of rhGH replacement due to a net positive balance in bone formation (3;8).

Data on the skeletal effects of long-term rhGH replacement are, however, scarce. Previous studies reported that rhGH replacement induces a progressive increase in BMD and BMC up to 5-7 years of treatment, which is followed by a plateau phase (9-12). Götherström *et al.* reported an increase of total body and lumbar spine BMD and BMC even up to 10 years of rhGH replacement, whereas femoral neck BMD and BMC reached a peak value after 5-7 years (13). Only one study reported the effects of 15 years of rhGH supplementation, showing a sustained increase in total body and lumbar BMD and BMC over 15 years, whereas femoral neck BMD and BMC returned towards baseline values after 7 years (14).

The primary aim of our study was to assess the effects of 5, 10 and 15 years of rhGH replacement on bone metabolism in the Leiden Cohort Study, a well-characterized cohort of adult GHD patients. Furthermore, we studied potential influencing factors on the bone response to rhGH replacement and studied clinical fracture incidence in these patients.

PATIENTS AND METHODS

PATIENTS: Consecutive adult patients with an established diagnosis of childhood-onset (CO) or adulthood-onset (AO) GHD followed up at the Department of Endocrinology and Metabolic Diseases of the Leiden University Medical Center (LUMC) were collected in a database from 1994 onwards. In the present study, we selected all GHD patients who started rhGH replacement therapy in or before 2007, and, therefore, had the potential to receive rhGH replacement for ≥5 years. We arbitrarily defined chronic treatment as replacement for ≥5 years, since such a period will reflect a stable situation without confounding by ongoing changes induced by the start and titration of rhGH replacement (15;16). This strategy resulted in inclusion of a total of 230 eligible GHD patients, of which part of the included patients has been previously described (17).

GHD was diagnosed on the basis of a GH peak response to the Insulin Tolerance Test (ITT) <3μg/l (glucose nadir <2.2mmol/l) according to current guidelines or, in case of contraindications for ITT, to the combined Growth Hormone Releasing Hormone/Arginine-test (GHRH/Arg) with BMI-adjusted GH cut-offs (18;19). All patients receiving rhGH replacement during childhood were re-tested at the time of transition to the adult outpatient clinic, after treatment cessation for >3 months. GHD was replaced according to a standardized Leiden protocol, in which patients received rhGH replacement (Genotropin, Pharmacia/Pfizer, Capelle a/d Ijssel, The Netherlands; Zomacton, Ferring, Hoofddorp, The Netherlands; Norditropin, NovoNordisk, Alphen a/d Rijn, The Netherlands), injected subcutaneously in the evening. GH starting dose was 0.2mg/day, which was individually adjusted each month in the first half year to achieve serum IGF-1 concentrations within the age-dependent laboratory reference range, aimed at SDS between 0 and +2. When stable plasma concentrations were reached, this individualized dose was continued during the entire study period and adjusted according to the IGF-1 standard deviation score (SDS). After dose titration, patients were evaluated at least yearly at the outpatient clinic, according to a standard protocol.

Adrenocorticotropic hormone (ACTH) deficiency was defined as an insufficient increase in cortisol levels (absolute value 0.55umol/l) after an ITT or a corticotropin-releasing hormone stimulation test in case that an ITT was contra-indicated. Standard hydrocortisone replacement was 20 mg divided in 3 doses, individually adjusted when necessary. Premenopausal women were classified as gonadotropin-deficient when secondary amenorrhea persisted for >1year. In men, gonadotropin

deficiency was defined as a testosterone level <8.0nmol/l. Thyroid-stimulating hormone (TSH) deficiency was defined as total thyroxine (T_4) or free T_4 levels below the normal laboratory reference range (<10pmol/l). Hypopituitarism was supplemented by hydrocortisone, L-thyroxine, testosterone in men, and/or estrogen in combination with progestagens (in premenopausal women only) and adequacy of supplementation was regularly controlled and adjusted as required.

Patients were treated with bone-modifying medication according to the discretion of their attending physicians.

METHODS: Data were collected from the patients' electronic hospital records on the following efficacy parameters, which were assessed on a yearly basis after establishment of diagnosis and start of treatment at the outpatient clinic:

1. Anthropometric parameters: body weight was measured to the nearest 0.1kg, and body height was measured barefoot to the nearest 0.001m. BMI was calculated as weight in kilograms divided by the square of height in meters (kg/m^2).
2. Biochemical parameters: data were collected on serum concentrations of IGF-1, GH, TSH, fT4, LH, FSH, estradiol, testosterone and prolactin, which were collected after an overnight fast. Assays were presented in *Supplementary File 1*.
3. Markers of bone metabolism: Data on serum creatinine, calcium, albumin, alkaline phosphatase, vitamin D, PTH, procollagen type 1 amino-terminal propeptide (P1NP, bone formation) and β-crosslaps concentrations (bone resorption) (the latter two markers available from 2006 onwards). Assays were presented in *Supplementary File 1*.
4. BMD and BMC: Data on BMD and BMC measurements were collected. BMD was measured yearly at the lumbar spine (L1-L4) and femoral neck using dual energy X-ray absorptiometry (DXA) (Hologic QDR 1000 (until 2008) and Hologic QDR 4500 (thereafter), Hologic Inc., Waltham, MA, USA) equipped with reference values based on the National Health and Nutrition Examination Survey (NHANES III) from the time of start of rhGH replacement onwards (20). Coefficient of variation of lumbar spine and femoral neck BMD measurements was 1%. An in-house comparison using 300 measurements provided a conversion formula for all Hologic 1000 measurements, which were converted for comparison for the laterally acquired Hologic 4500 BMD measurements. BMD T-scores were used to reflect the number of standard deviations (SD) above or below the mean for a healthy age- and sex-matched adult. World Health Organization (WHO) criteria were used to define osteopenia (T-score between -1.0 and -2.5) and osteoporosis (T-score ≤-2.5). BMC was measured at the lumbar spine using the same DXA apparatus.
5. Fracture data: Specific information on clinical fractures was retrieved from the medical charts. No routine spine radiographs were performed to assess the presence of vertebral fractures; however, radiographs were made in case of height loss >3cm or specific complaints at the discretion of the treating physician (21).Vertebral fractures were defined as clinical fractures (*i.e.* pain or decrease in height) and/or radiographic fractures in case of available radiographs, defined as the presence of Genant≥2(22).
6. Data on medication use and co-morbidity was obtained from electronic files.

STATISTICAL ANALYSIS: SPSS for Windows, Version 20.0 (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. Although 19 patients did not complete 5 years of rhGH replacement for various reasons (*vide infra*), these patients were included using the last observation carried forward method according to the intention-to-treat approach used. Results are presented as mean±SD, unless stated otherwise. ANOVA repeated measurements with Bonferroni correction for multiple comparisons were used to compare anthropometric parameters, bone markers, BMD, T-scores and BMC between baseline and after rhGH treatment. Analyses were repeated after stratification for bisphosphonate use. For the subgroup of patients followed ≥10 years, we only analyzed the baseline data of this specific subgroup.We identified factors influencing the response to long-term rhGH supplementation on bone by incorporating age, sex, childhood-onset *versus* adult-onset GHD, hydrocortisone use, GH dose, hypogonadism, number of pituitary deficiencies (<2 *vs* ≥2), bisphosphonate use, baseline Vitamin D levels and baseline BMD values in a linear regression model. Finally, clinical fracture incidence rate was calculated during rhGH replacement.

RESULTS

DESCRIPTION OF PATIENTS: Baseline characteristics of the 230 adult GHD patients (202 AO/28 CO) are shown in *Table 1*. Pituitary insufficiency was mainly caused by pituitary tumors or their treatment. Most patients had multiple pituitary hormone deficiencies. Two-hundred and eleven patients completed 5 years of rhGH replacement (*Supplementary File 2*). Reasons for earlier discontinuation (19 patients, 8.3% of total) are shown in *Supplementary File 2*. There were no differences between patients completing 5 years of rhGH replacement and non-completers with respect to age, sex, pituitary hormone deficiencies or co-morbidity. Ninety-eight and 43 patients, respectively, completed10 and 15 years of rhGH replacement.

At baseline, 10 patients (4.3%) received bisphosphonates, and, respectively, 84 (36.5%) and 85 patients (37.0%) received calcium and Vitamin D supplements. After respectively 5, 10 and 15 years of rhGH replacement 28 (12.2%), 19 (19.4%) and 8 (18.6%) patients used bisphosphonates. Thirty-eight patients used bisphosphonates at any moment during the study, for a mean duration of 6.9±4.3 years. Indications for bisphosphonate use were osteoporosis (N=23), clinical fractures (N=10), osteoporosis and clinical fractures (N=8), and other (N=7). Mean age at start of bisphosphonate-therapy was 52.6±13.5 years.

GH dose, IGF-1 SDS and hydrocortisone dose during rhGH replacement: Mean GH doses after dose titration (*i.e.* after 1year) and 5 years of rhGH replacement were, respectively, 0.39±0.20mg/day and 0.43±0.27mg/day (p=0.016 compared to 1year dose). Mean doses after, respectively, 10 and 15 years of rhGH replacement were 0.44±0.26mg/day and 0.39±0.21 mg/day (both NS compared to 1 year dose).

Mean IGF-1 SDS was within the normal range during the entire study period, and increased significantly from -1.09±2.46 at baseline to 0.66±2.01 after 5 years and was 1.24±2.45 after 10 years (both p<0.01 compared to baseline). In men, mean IGF-1 SDS increased significantly from -0.60±2.21 at baseline to 0.95±1.88 after 5 years (p=0<001) and to 1.64±2.57 after 10 years (p=0.011). In women, mean IGF-1 SDS increased significantly from -1.73±2.63 at baseline to 0.35±2.11 after 5 years of rhGH replacement (p<0.001) and to 0.83±2.29 after 10 years (p=0.003) (*Figure 1*).

Mean hydrocortisone doses were 24.1±7.3mg/day, 21.4±4.10mg/day and 21.1±4.3mg/day at baseline, after 10 and after 15 years of rhGH replacement, respectively, indicating no significant change in hydrocortisone dose during rhGH replacement.

Table 1. Baseline characteristics of 230 adult GHD patients

Patient characteristics	**GHD patients (N = 230)**
Sex, female / male (N)	121 / 109
Age at start rhGH replacement, years (range)	47.1 ± 14.9 (15 – 81)
AO / CO (N)	202 / 28
BMI, kg/m^2 (range)	27.3 ± 5.1 (16.9 – 57.1)
Etiological diagnosis of GHD (N)	
NFA	76
Functioning adenoma	68
Craniopharyngeoma	21
Cerebral malignancy	12
Congenital	27
Other causes	26
Surgery, TS / TC (N)	136 / 38
Radiotherapy (N)	102
Pituitary deficiencies (N)	
TSH	190
ACTH	197
FSH / LH	173
ADH	51
Isolated GHD	10
Bisphosphonates (N)	10
Calcium supplementation (N)	84
Vitamin D supplementation (N)	85

Data are presented as mean ± SD, unless specified otherwise.

N, number of patients; GHD, Growth Hormone Deficiency; rhGH, recombinant human Growth Hormone replacement; AO, adult-onset GHD; CO, childhood-onset GHD; BMI, body mass index; NFA, non-functioning adenoma; TS, transsphenoidal; TC, transcranial; TSH, thyroid stimulating hormone; ACTH, adrenocorticotropic hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; ADH, anti-diuretic hormone; Vitamin D, vitamin D.

BIOCHEMICAL BONE MARKERS: Baseline calcium and Vitamin D levels were, respectively, 2.40±0.24mg/dl and 60.3±26.7nmol/l. After 5 years, mean calcium and Vitamin D levels were 2.40±0.11mg/dl (NS compared to baseline) and 63.3±24.5nmol/l (p=0.02 compared to baseline), respectively, and 2.39±0.26mg/dl and 61.8±26.0nmol/l (both NS compared to baseline) after 10 years of rhGH replacement. At baseline, P1NP and β-crosslaps concentrations were only available in 12 and 30 patients and were therefore not reported. After 5 years or rhGH replacement, mean P1NP and β-crosslaps concentrations were 45.3±26.6ng/mL and 0.42±0.24ng/mL, respectively, and 48.4±25.4ng/mL and 0.71±2.19ng/mL after 10 years. After 15 years of rhGH replacement, mean P1NP and β-crosslaps concentrations were 42.1±24.7ng/mL and 0.37±0.20ng/mL. These data indicate normal bone turnover throughout the entire study period with stable rhGH replacement.

BMC and BMD: Mean height, weight and BMI at the different time points during rhGH replacement are shown in *Table 2*. Mean lumbar spine BMD gradually increased during the first 10 years of rhGH replacement, from 0.98±0.15g/cm^2 at baseline to 1.03±0.16g/cm^2 after 10 years (p<0.001, 5% above the baseline value) and stabilized thereafter (*Table 2*). Mean lumbar spine T-scores increased during the first 10 years of rhGH replacement from -0.69±1.30 to -0.43±1.33 (p<0.001), and remained constant thereafter. Lumbar spine BMC increased throughout the first 10 years of rhGH replacement being 4% above the baseline level after 10 years (from 63.2±15.4kg to 65.8±18.0kg), and then reached a plateau. Similar results were obtained after stratification for the use of bisphosphonates, indicating that in the long-term there was no beneficial effect impacted from additional bisphosphonate use (*Figure 2*).

Left femoral neck BMD did not change during rhGH replacement (*Table 2)*, neither in the small subgroup of patients using bisphosphonates (*Figure 2*).

In *Supplementary File 3*, the mean BMC, BMD and T-score values at the different time points during rhGH supplementation were shown for the subgroup of GHD adults with 15 years of rhGH replacement (N=43).Within this subgroup, mean lumbar spine BMD and BMC scores increased during the first 10 years of rhGH supplementation and stabilized thereafter. With respect to left femoral neck, no changes during rhGH supplementation were found.

Table 2. Effects of 15 years of rhGH replacement in 230 adults with GHD on BMC, BMD and T-scores measured using DXA, and anthropometric parameters

	Baseline (N=230)	**5 years rhGH (N=230)**	**10 years rhGH (N=98)**	**15 years rhGH (N=43)**
DXA lumbar (L1-L4) spine				
BMC (kg)	63.2 ± 15.4	65.5 ± 15.4**	65.8 ± 18.0*	63.6 ± 13.9
BMD (g/cm^2)	0.98 ± 0.15	1.01 ± 0.14**	1.03 ± 0.16**	1.01 ± 0.14*
T-score (SD)	-0.69 ± 1.30	-0.58 ± 1.29**	-0.43 ± 1.33**	-0.54 ± 1.11
DXA left femoral neck				
BMD (g/cm^2)	0.83 ± 0.14	0.83 ± 0.15	0.84 ± 0.16	0.83 ± 0.15
T-score (SD)	-0.94 ± 1.30	-0.94 ± 1.06	-0.77 ± 1.18	-0.82 ± 1.04
Height (cm)	171.1± 13.0	171.6± 11.2	170.8± 11.1	168.4±14.7
Weight (kg)	81.7± 19.1	82.4±18.6	84.6 ±20.6	81.9±17.1
BMI (kg/m^2)	27.3±5.1	27.9 ±5.3*	28.8± 5.9*	29.6±8.4*

Statistical analyses were done by performing a Repeated Measurements ANOVA. To take into account multiple testing, p<0.01 was considered statistically significant.

N, number of patients on rhGH replacement at different time points; rhGH, recombinant human Growth Hormone; GHD, Growth Hormone Deficiency; BMC, bone mineral content; BMD, bone mineral density; DXA, dual energy X-ray absorptiometry; BMI, body mass index; SD, standard deviation.

**, p<0.01 (versus baseline)*
***, p<0.001 (versus baseline)*

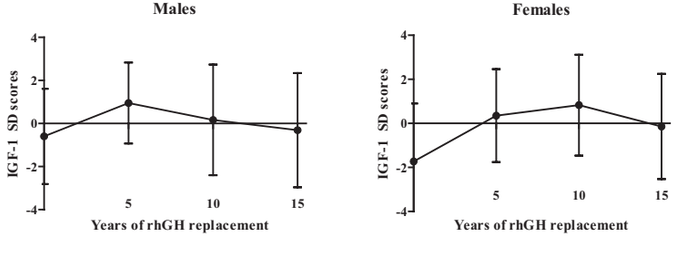


Figure 1. *Mean IGF-1 SD scores during 15 years of rhGH replacement, stratified for men (A) and women (B)*

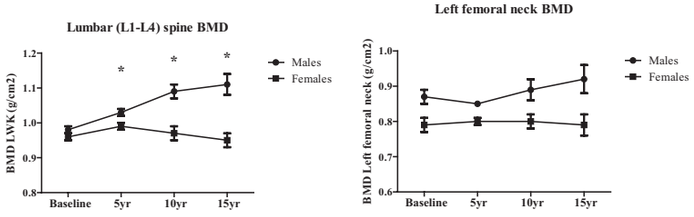


Figure 2. *BMD changes during 15 years of rhGH replacement at the lumbar spine and the left femoral neck, stratified for the use of bisphosphonates in male (A) and female GHD adults (B)*

Data are presented as mean BMD values (g/cm²), stratified by the use of bisphosphonates. Vertical bars indicate SD values.

rhGH, recombinant human Growth Hormone therapy; GHD, Growth Hormone Deficiency; BMD, bone mineral density; SD, standard deviation.
**, p<0.01 (versus baseline)*

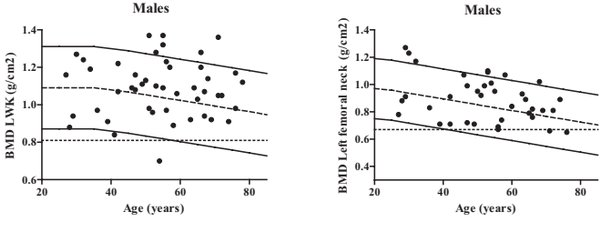


Figure 3A

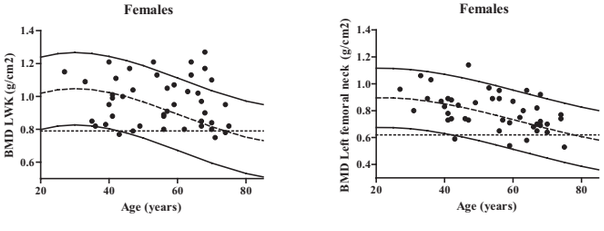


Figure 3B

Scatterplots of individual BMD values at the lumbar spine and left femoral neck in female (A) and male (B) GHD patients after 10 years of rhGH supplementation

Closed circles indicate individual scores, the interrupted line indicates mean BMD value for age and the solid lines indicate, respectively, +2 S.D. and -2 S.D. Dashed line indicates T-score -2.5 S.D.

BMD, bone mineral density; rhGH, recombinant human Growth Hormone supplementation; LWK, lumbar spine.

OSTEOPENIA AND OSTEOPOROSIS: At baseline, 29.1% and 31.7% of the patients had osteopenia (T-score<-1.0SD) in the lumbar spine and femoral neck, respectively. After 10 years of rhGH replacement, 37.8% of the patients had osteopenia at the lumbar spine and 35.7% at the femoral neck. The patients with persisting osteopenia at the femoral neck and/or lumbar spine at the study end, were older (68.5 years *vs* 59.2 years, p=0.014) and included more women (66.7% *vs* 46.7%, although NS). No differences were found with respect to 1 year GH dose or number of pituitary deficiencies between patients with and without persisting osteopenia and/or osteoporosis at the study end.

At baseline, 11 GHD patients (4.8%) had osteoporosis at the lumbar spine (4 women/7 men), with a mean age of 54.1±15.2 years. Six of these patients used bisphosphonates and respectively 7 and 5 patients had calcium and Vitamin D supplementation. Seventeen patients (7.4%) had baseline osteoporosis at the femoral neck (7 women/10 men), of whom nine patients used bisphosphonates, 11 calcium and 9 Vitamin D supplementation. In this small subgroup of patients with osteoporosis, no change in femoral neck BMD was found during rhGH supplementation. However, lumbar spine BMD increased during the first 5 years of rhGH supplementation, without a further increase afterwards. After 10 years of rhGH supplementation, osteoporosis was found in, respectively, 7.1% and 1.1% of patients at the femoral neck and the lumbar spine. BMD values of individual GHD patients after 10 years of rhGH supplementation were shown in *Figure 3A+3B*.

Influence of sex: Mean GH dose was significantly higher in women compared to men for the first 10 years of rhGH replacement, resulting in a higher GH dose/IGF-1 SDS ratio in female GHD patients. After 5 years of rhGH treatment, mean GH dose in men and women was respectively 0.37±0.26mg/day and 0.49±0.28mg/day (p=0.001) and at 10 years respectively 0.36±0.18mg/day and 0.53±0.30mg/day (p=0.001).

Men had a greater increase in lumbar spine BMD than women at all time points (all p<0.01 compared to baseline), but not in femoral neck BMD (*Figure 4A+2B*). These data were similar in patients with and without bisphosphonate use (*Figure 2A-D*). Similar sex differences, with highest response to rhGH replacement in men, were seen for lumbar spine BMC. Also in lumbar spine T-scores highest increase was seen in men after 10 and 15 years of rhGH replacement, although this was not shown for femoral neck T-scores (*data not shown*).

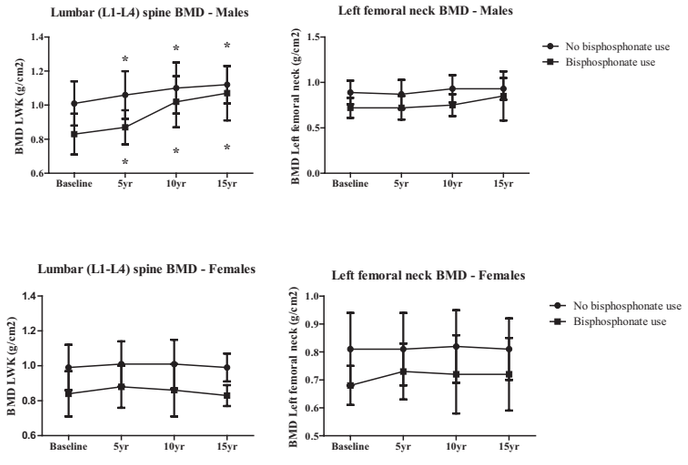


Figure 4. *Sex differences in the effects of 15 years of rhGH replacement in 230 adult GHD patients on lumbar (L1-L4) spine BMD (A) and left femoral neck BMD (B)*

Data are presented as mean BMD values (g/cm^2), stratified by sex. Vertical bars indicate SEM values. Men had a greater increase in lumbar spine BMD than women at all time points when compared to baseline (all p<0.001).

rhGH, recombinant human Growth Hormone therapy; GHD, Growth Hormone Deficiency; BMD, bone mineral density; SEM, standard error of the mean.

**, p<0.001 (versus baseline)*

OTHER POTENTIAL INFLUENCING FACTORS : Subsequently, we identified potential factors influencing the response to rhGH replacement on BMD and BMC. One hundred seventy-seven patients (77%) were <60 years and 53 patients (23%) were ≥60 years of age at baseline. The individualized rhGH dose used in older patients was significantly lower than in younger patients (0.41±0.21mg/day *vs* 0.32±0.13mg/day, p<0.001). After adjustment for the difference in rhGH dose, the rhGH response did not differ between younger and older patients with respect to IGF-1, BMD, BMC and T-scores at the lumbar spine or femoral neck. Univariate analyses including GH dose at 1year, low Vitamin D levels, hydrocortisone use, use of bisphosphonates, use of L-thyroxine, childhood- *vs* adulthood-onset, presence of ≥2 pituitary hormone deficiencies, or hypogonadism showed that these factors did not affect the effects of rhGH replacement on femoral neck BMD, lumbar spine BMD or BMC. A lower baseline lumbar BMD was found to be associated with a higher increase in lumbar BMD during 15 years of rhGH replacement, in both sexes. Bone response to rhGH replacement did not differ between patients with a history of functioning *vs* non-functioning adenoma or between patients with childhood- *vs* adulthood-onset GHD.

When incorporating all factors in a linear regression model, only higher GH dose at 1 year was associated with a decreased response to rhGH on BMC over 15 years. No other influencing factors were found.

FRACTURE DATA: Twenty-three patients (10%, 11 women/12 men) sustained fractures during rhGH supplementation, of which 12 at ≥2 sites. Fifteen patients had vertebral fractures (10 patients had ≥2 fractures), 9 patients non-vertebral fractures (hip N=6 (2 patients bilateral hip fracture), toe N=2, clavicula N=1). Consequently, the incidence rate of fractures during rhGH replacement was 46/2288.5 (mean duration of rhGH therapy 9.95 years x 230 patients) = 20.1/1000py in our GHD cohort (incidence rates for, respectively, non-vertebral and vertebral fractures in our cohort were 3.9/1000py and 16.2/1000py).

DISCUSSION

The present study describes the long-term effects of rhGH replacement on bone metabolism in a well-defined cohort of adult GHD patients. We found a sustained increase in BMD at the lumbar spine during the first 10 years of rhGH replacement, and stabilization thereafter, as was found for lumbar spine BMC and T-scores. At all time points, the largest effects of rhGH replacement on lumbar spine BMD and BMC were observed in men. In the small subset of patients using bisphosphonates, bisphosphonate use did not impact additional beneficial effects beyond an initial early effect. Lower baseline BMD and high GH dose at 1year significantly, respectively, positively and negatively influenced the bone response to long-term rhGH replacement. Clinical fracture incidence during long-term rhGH supplementation was 20.1/1000py.

Untreated GHD in adults is characterized by low bone turnover, decreased BMD and BMC (5-7), which increased or even normalized by short-term rhGH replacement (3;5;8). We have previously shown that 2 years of physiological dose of rhGH in GHD adults increased bone turnover in favor of bone formation, as reflected by a significant increase in BMD (23) that sustained after 7 years of treatment (24). In addition, during the first 4 years of rhGH supplementation, we observed a significant greater increase in BMD in GHD patients with osteoporosis with additional bisphosphonate treatment (24). These additional beneficial effects of bisphosphonates were preserved during another 3 years of rhGH replacement (25).

To date, only a single other study has reported the skeletal effects of rhGH replacement in GHD adults beyond 10 years (14). In agreement with this report, we observed a sustained increase of lumbar spine BMD and BMC during 15 years of rhGH replacement with a peak value after 5-7 years, being largest in male patients. In both studies, no changes in femoral neck BMD were found. However, since due to an ageing effect, it would be expected that femoral neck BMD decreases with increasing age over time (26). The observed stabilization of the femoral neck BMD in our study among GHD adults indicates that, in addition to an effect on lumbar spine BMD, rhGH supplementation also exerts an effect at the level of the femoral neck. The present study is the first to report that during long-term rhGH replacement, concomitant bisphosphonate use has no additional beneficial effect on BMD after an initial early effect (25;27), in the presence of stringently controlled Vitamin D and calcium status. In addition, this study also focused on clinical fracture incidence during long-term rhGH replacement.

When stratifying analyses for sex, lumbar spine BMD increases in both sexes during the first 5 years of rhGH replacement. Thereafter, we observed a further increase in BMD in men, whereas in women BMD decreased towards baseline values. Gender-specific differences in the bone response to rhGH supplementation were also demonstrated in several other studies, with largest effects in men (28;29). The lack of response in BMD in adult GHD women is suggested to due to the inhibition of the effect of GH on IGF-1 production by orally administered estrogens (30;31) or by inhibiting direct actions of GH on both osteoblasts and osteoclasts by estrogen (3). However, in this respect, it should be noted that GH doses are adjusted according to sex-adjusted IGF-1 levels. In general, given that IGF-1 levels are comparable between men and women, it is likely that other factors, such as differences in bone structure between both sexes play a role in sex-specific differences in bone response to rhGH supplementation.

Literature on fracture risk in rhGH-treated adult GHD patients is scarce and, to date, has been investigated in only three studies. Holmer *et al.* studied clinical fracture incidence in 832 rhGH-treated patients compared to 2581 matched healthy controls and reported a two-fold increase in fracture risk in CO-GHD women, although in AO-GHD men fracture risk was significantly lowered. No increased risk was found in AO-GHD women and CO-GHD men (32). Elbornsson *et al.* reported clinical fractures in a Swedish GHD cohort, and observed only 1 hip fracture and 1 symptomatic vertebral fracture (14). Mazziotti *et al.* reported a high prevalence of radiographic vertebral fractures of 53.8% in rhGH-treated GHD adults when compared to a prevalence of 78.6% in untreated GHD patients (33), suggesting that rhGH supplementation decreases fracture risk in GHD adults. In our cohort,10% of patients sustained a clinical fracture during long-term rhGH replacement, giving an incidence rate of 20.1/1000py. When compared to the Rotterdam Study in a healthy Dutch population aged ≥55 years, reporting a vertebral fracture incidence rate of 10.9/1000py and a non-vertebral fracture incidence rate of 25.0/1000py in women and 9.6/1000py in men (34;35) and in view of the younger age of our cohort, our data may suggest that fracture incidence is not increased in rhGH-treated GHD patients.

Our study has strengths as well as limitations. Its main strengths are the duration of follow-up and the size of our adult GHD cohort, who were regularly followed and stringently controlled over the years according to internationally used guidelines. Care for attention was given to Vitamin D status as reflected by normal average levels of Vitamin D and normal bone turnover rates throughout the entire study period. Limitations of the present study, and a general drawback in long-term follow-up studies, are, first, the lack of a non-rhGH treated control group. Since the beneficial effects of rhGH therapy are well-established in the short-term, rhGH replacement became a widely available therapy for adult GHD in the Netherlands, in case of no contra-indications. Consequently, it is no longer possible or ethical to perform long-term randomized placebo-controlled studies in GHD patients. A second limitation is the potential underestimation of the fracture incidence in our patients as spinal X-rays were only performed in case of a clinical fracture. Another feature that needs to be addressed is that due to the presence of multiple pituitary hormone deficiencies in almost all patients, it is difficult to examine to what extent the reported effects can be attributed to rhGH treatment. In this respect, the presence of interactions between glucocorticoids and rhGH during substitution and the negative effects of cortisol excess, either endogenous or supraphysiological supplementation, on bone has to be noted (23;36). However, in our study, there were no signs of hydrocortisone oversupplementation and when incorporating hydrocortisone use in a regression model, we found no significant association with BMD response. Finally, bisphosphonates, calcium and Vitamin D supplementation were initiated at the discretion of the treating physician and not given using a standardized protocol with only a small subset of patients using bisphosphonates. However, in other studies investigating skeletal effects of long-term rhGH supplementation, information on bisphosphonate use is often not available. In this respect, the combined use of bisphosphonates and rhGH supplementation should be a topic for future research.

In conclusion, these findings from our cohort study of GHD patients demonstrate ongoing beneficial effects on bone metabolism of 10 years of rhGH replacement therapy, particularly in men, with stabilization thereafter. Beneficial effects are most marked on BMD at the lumbar spine. In addition, clinical fracture incidence seems low in our adult GHD patients. 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.

REFERENCE LIST

1. Giustina A, Mazziotti G, Canalis E. Growth hormone, insulin-like growth factors, and the skeleton. Endocr Rev 2008; 29(5):535-559.
2. Canalis E, Giustina A, Bilezikian JP. Mechanisms of anabolic therapies for osteoporosis. N Engl J Med 2007; 357(9):905-916.
3. Ohlsson C, Bengtsson BA, Isaksson OG, Andreassen TT, Slootweg MC. Growth hormone and bone. Endocr Rev 1998; 19(1):55-79.
4. Manelli F, Carpinteri R, Bossoni S et al. Growth hormone in glucocorticoid-induced osteoporosis. Front Horm Res 2002; 30:174-183.
5. Bouillon R, Koledova E, Bezlepkina O et al. Bone status and fracture prevalence in Russian adults with childhood-onset growth hormone deficiency. J Clin Endocrinol Metab 2004; 89(10):4993-4998.
6. Rosen T, Hansson T, Granhed H, Szucs J, Bengtsson BA. Reduced bone mineral content in adult patients with growth hormone deficiency. Acta Endocrinol (Copenh) 1993; 129(3):201-206.
7. Verhelst J, Abs R. Long-term growth hormone replacement therapy in hypopituitary adults. Drugs 2002; 62(16):2399-2412.
8. Molitch ME, Clemmons DR, Malozowski S, Merriam GR, Vance ML. Evaluation and treatment of adult growth hormone deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011; 96(6):1587-1609.
9. Appelman-Dijkstra NM, Claessen KM, Roelfsema F, Pereira AM, Biermasz NR. Long-term effects of recombinant human GH replacement in adults with GH deficiency: a systematic review. Eur J Endocrinol 2013; 169(1):R1-14.
10. Clanget C, Seck T, Hinke V, Wuster C, Ziegler R, Pfeilschifter J. Effects of 6 years of growth hormone (GH) treatment on bone mineral density in GH-deficient adults. Clin Endocrinol (Oxf) 2001; 55(1):93-99.
11. Drake WM, Rodriguez-Arnao J, Weaver JU et al. The influence of gender on the short and long-term effects of growth hormone replacement on bone metabolism and bone mineral density in hypopituitary adults: a 5-year study. Clin Endocrinol (Oxf) 2001; 54(4):525-532.
12. Götherström G, Svensson J, Koranyi J et al. A prospective study of 5 years of GH replacement therapy in GH-deficient adults: sustained effects on body composition, bone mass, and metabolic indices. J Clin Endocrinol Metab 2001; 86(10):4657-4665.
13. Götherström G, Bengtsson BA, Bosaeus I, Johannsson G, Svensson J. Ten-year GH replacement increases bone mineral density in hypopituitary patients with adult onset GH deficiency. Eur J Endocrinol 2007; 156(1):55-64.
14. Elbornsson M, Götherström G, Bosaeus I, Bengtsson BA, Johannsson G, Svensson J. Fifteen years of GH replacement increases bone mineral density in hypopituitary patients with adult-onset GH deficiency. Eur J Endocrinol 2012; 166(5):787-795.
15. Janssen YJ, Frolich M, Roelfsema F. A low starting dose of genotropin in growth hormone-deficient adults. J Clin Endocrinol Metab 1997; 82(1):129-135.
16. Maison P, Griffin S, Nicoue-Beglah M, Haddad N, Balkau B, Chanson P. Impact of growth hormone (GH) treatment on cardiovascular risk factors in GH-deficient adults: a Metaanalysis of Blinded, Randomized, Placebo-Controlled Trials. J Clin Endocrinol Metab 2004; 89(5):2192-2199.
17. Claessen KM, Appelman-Dijkstra NM, Adoptie DM et al. Metabolic profile in growth hormone-deficient (GHD) adults after long-term recombinant human growth hormone (rhGH) therapy. J Clin Endocrinol Metab 2013; 98(1):352-361.
18. Ghigo E, Aimaretti G, Corneli G. Diagnosis of adult GH deficiency. Growth Horm IGF Res 2008; 18(1):1-16.
19. van der Klaauw AA, Biermasz NR, Zelissen PM et al. Administration route-dependent effects of estrogens on IGF-I levels during fixed GH replacement in women with hypopituitarism. Eur J Endocrinol 2007; 157(6):709-716.
20. Looker AC, Borrud LG, Hughes JP, Fan B, Shepherd JA, Melton LJ, III. Lumbar spine and proximal femur bone mineral density, bone mineral content, and bone area: United States, 2005-2008. Vital Health Stat 11 2012;(251):1-132.
21. Gunnes M, Lehmann EH, Mellstrom D, Johnell O. The relationship between anthropometric measurements and fractures in women. Bone 1996; 19(4):407-413.
22. Genant HK, Wu CY, van KC, Nevitt MC. Vertebral fracture assessment using a

semiquantitative technique. J Bone Miner Res 1993; 8(9):1137-1148.
23. Chiodini I, Torlontano M, Carnevale V, Trischitta V, Scillitani A. Skeletal involvement in adult patients with endogenous hypercortisolism. J Endocrinol Invest 2008; 31(3):267-276.
24. Biermasz NR, Hamdy NA, Janssen YJ, Roelfsema F. Additional beneficial effects of alendronate in growth hormone (GH)-deficient adults with osteoporosis receiving long-term recombinant human GH replacement therapy: a randomized controlled trial. J Clin Endocrinol Metab 2001; 86(7):3079-3085.
25. Biermasz NR, Hamdy NA, Pereira AM, Romijn JA, Roelfsema F. Long-term skeletal effects of recombinant human growth hormone (rhGH) alone and rhGH combined with alendronate in GH-deficient adults: a seven-year follow-up study. Clin Endocrinol (Oxf) 2004; 60(5):568-575.
26. Warming L, Hassager C, Christiansen C. Changes in bone mineral density with age in men and women: a longitudinal study. Osteoporos Int 2002; 13(2):105-112.
27. Janssen YJ, Hamdy NA, Frolich M, Roelfsema F. Skeletal effects of two years of treatment with low physiological doses of recombinant human growth hormone (GH) in patients with adult-onset GH deficiency. J Clin Endocrinol Metab 1998; 83(6):2143-2148.
28. Bex M, Abs R, Maiter D, Beckers A, Lamberigts G, Bouillon R. The effects of growth hormone replacement therapy on bone metabolism in adult-onset growth hormone deficiency: a 2-year open randomized controlled multicenter trial. J Bone Miner Res 2002; 17(6):1081-1094.
29. Snyder PJ, Biller BM, Zagar A et al. Effect of growth hormone replacement on BMD in adult-onset growth hormone deficiency. J Bone Miner Res 2007; 22(5):762-770.
30. Cook DM, Ludlam WH, Cook MB. Route of estrogen administration helps to determine growth hormone (GH) replacement dose in GH-deficient adults. J Clin Endocrinol Metab 1999; 84(11):3956-3960.
31. Janssen YJ, Helmerhorst F, Frolich M, Roelfsema F. A switch from oral (2 mg/day) to transdermal (50 microg/day) 17beta-estradiol therapy increases serum insulin-like growth factor-I levels in recombinant human growth hormone (GH)-substituted women with GH deficiency. J Clin Endocrinol Metab 2000; 85(1):464-467.
32. Holmer H, Svensson J, Rylander L et al. Fracture incidence in GH-deficient patients on complete hormone replacement including GH. J Bone Miner Res 2007; 22(12):1842-1850.
33. Mazziotti G, Bianchi A, Bonadonna S et al. Increased prevalence of radiological spinal deformities in adult patients with GH deficiency: influence of GH replacement therapy. J Bone Miner Res 2006; 21(4):520-528.
34. Schuit SC, van der Klift M, Weel AE et al. Fracture incidence and association with bone mineral density in elderly men and women: the Rotterdam Study. Bone 2004; 34(1):195-202.
35. van der Klift M, de Laet CE, McCloskey EV, Hofman A, Pols HA. The incidence of vertebral fractures in men and women: the Rotterdam Study. J Bone Miner Res 2002; 17(6):1051-1056.
36. Danilowicz K, Bruno OD, Manavela M, Gomez RM, Barkan A. Correction of cortisol overreplacement ameliorates morbidities in patients with hypopituitarism: a pilot study. Pituitary 2008; 11(3):279-285.

SUPPLEMENTARY FILE 1.

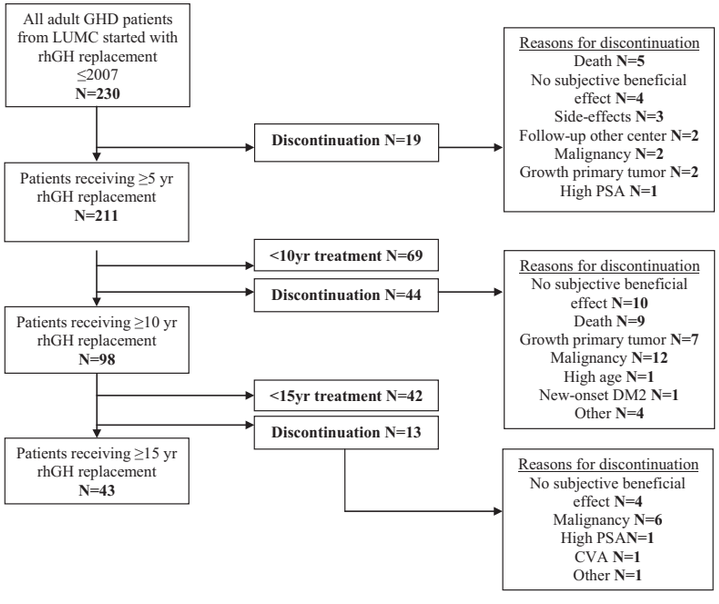
Assays

Serum GH was measured with a sensitive IFMA (Wallac, Turku, Finland), specific for the 22 kDA GH protein (detection limit: 0.01µg/l, interassay coefficient of variation (CV): 1.6-8.4% of 0.01-15.38µg/l) from 1992 onwards. For the conversion of µg/l to mU/l, multiply by 2.6. Before 1992, GH was measured by RIA (Biolab, Serona, Coissins, Switzerland), detection limit: 0.5mU/l, with an interassay CV <5%; for the conversion of µg/l to mU/l, multiply by 2. From 1986 to 2005, serum IGF-1 concentrations were determined by RIA (Incstar, Stillwater, MN) with a detection limit of 1.5nmol/l and an interassay CV less than 11%. IGF-1 is expressed as SD score for age- and gender-related normal levels determined in the same laboratory (33). Since 2005, serum IGF-1 concentrations (nmol/l) were measured using an immunometric technique on an Immulite 2500 system (Siemens Healthcare Diagnostics, Deerfield, IL, USA). The intra-assay variations at mean plasma levels of 8 and 75nmol/l were 5.0 and 7.5%, respectively. IGF-1 levels were expressed as SDS, using lambda-mu-sigma smoothed reference curves based on 906 controls(21;22).

B-crosslaps and P1NP concentrations were measured by an electrochemolumiscent immunoassay with a Modular Analytics E-170 system (Roche Diagnostics, Almere, The Netherlands). References values for β-crosslaps and P1NP concentrations were, respectively, <0.854ng/ml and <59ng/ml. Vitamin D25OH was measured by RIA (Incstar/DiaSorin, Stillwater, MN, USA) (reference range (50 – 250nmol/l).

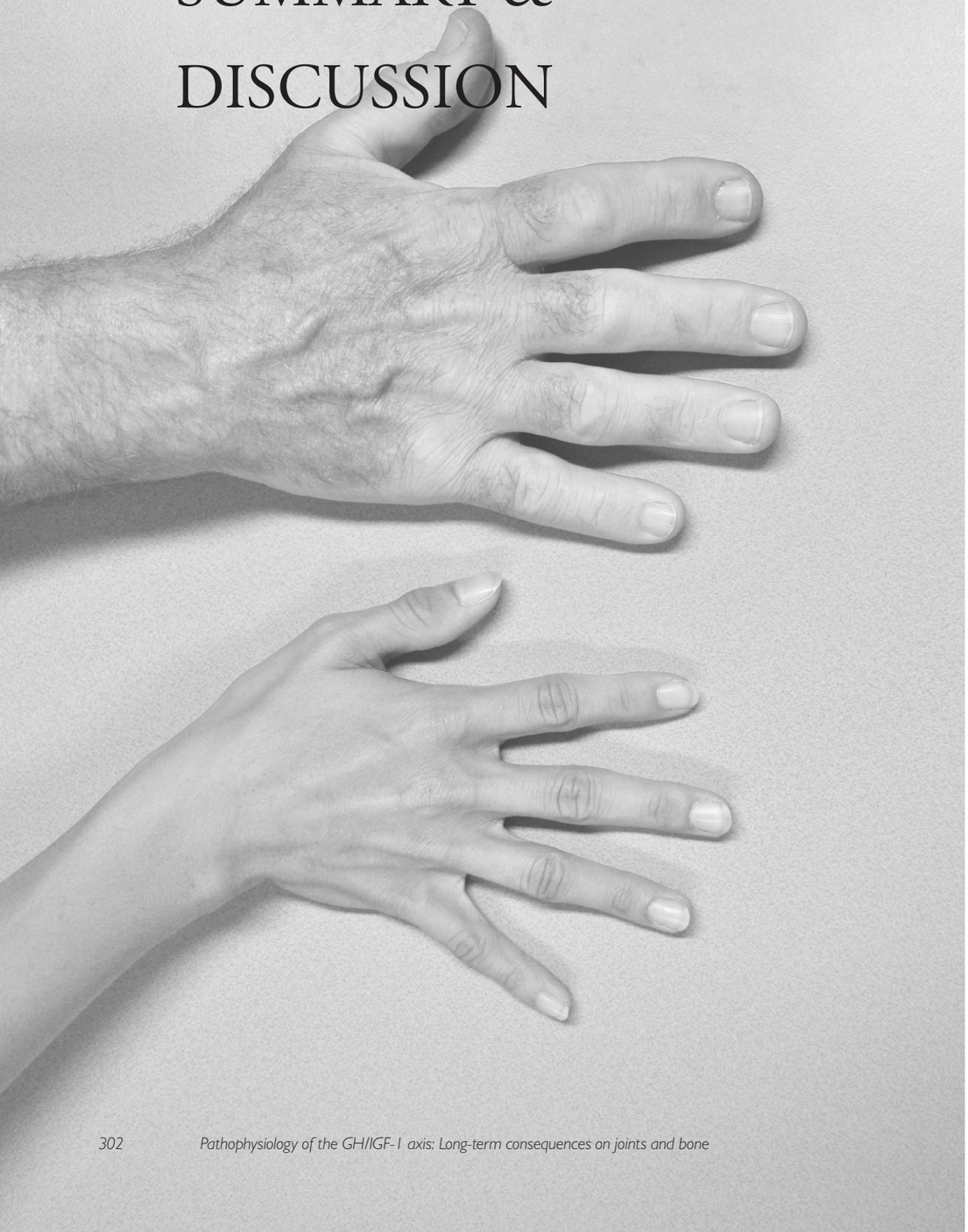
SUPPLEMENTARY FILE 2.

Flow chart of selection and follow-up of our adult GHD cohort (N=230)



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.

XV. NEDERLANDSE SAMENVATTING

Dit proefschrift beschrijft studies naar de lange termijn complicaties van acromegalie op gewrichten en botten in een cohort met gecureerde acromegalie patiënten (**Deel A**). Daarnaast is de rol van de groeihormoon (GH)/insulineachtige groeifactor-1 (IGF-1) as onderzocht in patiënten met primaire artrose (**Deel B**). Ten slotte zijn de lange termijn effecten van rhGH therapie in kaart gebracht in een groep volwassen patiënten met groeihormoon deficiëntie (GHD). Hierbij ligt de focus op de cardiovasculaire effecten van recombinant humaan GH (rhGH) therapie en de effecten op het botmetabolisme (**Deel C**).

Deel A. Lange termijn effecten van acromegalie op gewrichten en bot

Acromegalie is een endocriene ziekte die in de meeste gevallen wordt veroorzaakt door een hypofyseadenoom. Dit adenoom produceert overmatige hoeveelheden GH, wat resulteert in sterk verhoogde GH en IGF-1 spiegels in het bloed. De klinische, vaak aspecifieke symptomen ontstaan langzaam, waardoor de diagnose acromegalie vaak pas na 6 tot 9 jaar na het ontstaan van de eerste klachten wordt gesteld. Adequate behandeling middels transsfenoïdale operatie, radiotherapie, medicatie of een combinatie van deze behandelopties geeft een sterke verbetering van een aantal systemische effecten van acromegalie. Echter zelfs na het bereiken van langdurige biochemische remissie blijven veel manifestaties van de ziekte bestaan en ervaart een groot deel van de patiënten een sterk verminderde kwaliteit van leven. In dit opzicht zijn de skeletale manifestaties van acromegalie één van de meest voorkomende en invaliderende complicaties.

In **Deel A** worden de lange termijn effecten van acromegalie op gewrichten en botten beschreven in een goed gekarakteriseerd cohort van patiënten met acromegalie in langdurige remissie. In het bijzonder is er gekeken naar het beloop van gewrichtsafwijkingen en wervelinzakkingen en naar risicofactoren voor een slecht beloop van deze afwijkingen. Om deze vragen te beantwoorden werd een prospectieve follow-up studie uitgevoerd met een gemiddelde vervolgtijd van 2.5 jaar.

Artropathie is één van de meest voorkomende complicaties van acromegalie en heeft een hoge prevalentie onder zowel patiënten met actieve ziekte als patiënten die gecureerd zijn. In eerdere studies is aangetoond dat een verhoogde GH en IGF-1 activiteit geassocieerd is met het ontstaan van gewrichtsklachten bij acromegalie. Voornamelijk patiënten met zeer hoge IGF-1 spiegels ten tijde van het stellen van de diagnose en met het veelvoorkomende exon 3 deletie GHR (d3-GHR) polymorfisme, dat is geassocieerd met een versterkte sensitiviteit voor GH, hadden een verhoogde kans op het ontwikkelen van artropathie. Hoewel artropathie bij acromegalie gelijkenissen vertoont met de gewrichtsafwijkingen zoals deze gezien worden bij patiënten met primaire artrose, verschillen de radiologische karakteristieken duidelijk. Artrose in acromegalie patiënten wordt gekenmerkt door een ernstige osteofytose in combinatie met verwijde gewrichtsspleten. Dit in tegenstelling tot de vernauwde gewrichtsspleten die kenmerkend zijn voor het kraakbeenverlies bij patiënten met primaire artrose. Recent werd middels een nieuwe kwantitatieve semiautomatische methode de karakteristieke gewrichtsspleetverwijding in acromegalen gemeten en deze methode bleek sensitief genoeg om de gewrichtsspleetdikte te relateren aan markers van ziekteactiviteit in acromegalie patienten.

Het beloop van artropathie na behandeling voor acromegalie is onbekend. Daarnaast bestaat er geen literatuur over risicofactoren voor een ongunstig beloop van gewrichtsafwijkingen. In **Hoofdstuk 2** en **Hoofdstuk 3** is het beloop van artropathie bij acromegalie patiënten en risicofactoren voor een slechte uitkomst bestudeerd in een prospectieve follow-up studie met een gemiddelde follow-up duur van 2.5 jaar. In deze studie werden 58 acromegalie patiënten geïncludeerd, welke allen gemiddeld 17.6 jaar in biochemische remissie waren na een transsfenoïdale operatie, radiotherapie en/of medicamenteuze behandeling.

Hoofdstuk 2 beschrijft het radiologische beloop van artropathie bij behandelde acromegalen. Er werd gevonden dat ondanks biochemische ziektecontrole meer dan 70% van de acromegalie patiënten progressieve osteofytose en gewrichtsspleetvernauwing heeft. Een hogere leeftijd en een toegenomen GH/IGF-1 activiteit gedurende de studie waren geassocieerd met progressieve gewrichtsziekte. Opvallend was een sterk verhoogd percentage radiologische progressie in patiënten door middel van behandeling met somatostatine (SMS) analogen in remissie waren in vergelijking met operatief gecureerde patiënten, onafhankelijk van leeftijd, geslacht, BMI en IGF-1 waarden. Deze bevindingen zijn in overeenstemming met eerdere studies die aantoonden dat, in vergelijking met operatief gecureerde patiënten, patiënten behandeld met SMS analogen ongunstigere uitkomsten hadden op het gebied van kwaliteit van leven en diastolische hartfunctie. Daarnaast is eerder aangetoond dat gedurende behandeling met SMS analogen de GH secretie niet volledig normaliseert. De bevindingen van de hier beschreven studie impliceren

dat er een indicatie bestaat voor agressievere therapie, wellicht door toevoeging van Pegvisomant. Toekomstige studies zullen moeten uitwijzen of een combinatiebehandeling met Pegvisomant de ziektecontrole in acromegalie patiënten kan optimaliseren. Een alternatieve verklaring voor een toegenomen progressie van artrose in patiënten behandeld met SMS analogen is de mogelijkheid van een IGF-1 onafhankelijk effect van SMS analogen op gewrichtsstructuren. Daarnaast hebben met SMS behandelde patiënten over het algemeen een ongunstiger ziektebeloop. Gezien het feit dat behandeling met SMS analogen de voorkeur geniet als additionele therapie na een incomplete operatie en als primaire behandeling wordt beschouwd als een goed alternatief voor operatie, hebben de bevindingen van deze studie implicaties voor de huidige behandelstrategieën.

Hoofdstuk 3 beschrijft het klinische beloop van artropathie bij acromegalie patiënten in langdurige remissie en de relatie tussen het klinische en radiologische beloop van artrose. Klinische artrose werd vastgesteld middels gevalideerde vragenlijsten naar gewrichtsklachten (pijn, stijfheid en functiebeperking) van de hand, knie en heup. Daarnaast werd gestructureerd lichamelijk gewrichtsonderzoek verricht en werden functietesten van de gewrichten uitgevoerd. Deze studie toonde een grote variatie in klinisch beloop van artrose tussen patiënten onderling. Echter, gemiddeld genomen werd een verslechtering waargenomen van de gewrichtsfuncties van de hand, knie en heup ondanks langdurige biochemische remissie. Gewrichtspijn was daarentegen stabiel. Veel pijn en functiebeperkingen bij aanvang van de studie waren voorspellend voor een toename in pijnklachten en functiebeperking in de hand gedurende de onderzoeksperiode. Daarnaast was een hoger BMI een risicofactor voor een toename van functiebeperkingen in de knie en heup. Klinische en radiologische progressie van artrose waren niet aan elkaar gerelateerd, zoals ook in de primaire artrose beschreven is.

Uit **Hoofdstuk 2** en **Hoofdstuk 3** volgt dat artropathie bij acromegalie patiënten een progressieve ziekte is welke niet gestopt wordt door biochemische ziektecontrole. Dit onderstreept de irreversibiliteit van een eerder GH overschot. Gezien het ontbreken van een relatie tussen klinische en radiologische progressie van artrose, is het in de klinische praktijk belangrijk een combinatie van klinische en radiologische gewrichtsbeoordeling te hanteren. Toekomstig onderzoek zal moeten uitwijzen wat de optimale behandelstrategie is voor artropathie in deze specifieke patiëntengroep en of specifieke interventiebehandelingen, zoals fysiotherapie, in dit opzicht zinvol zijn.

In **Hoofdstuk 4** werden kniegewrichten van 26 acromegalie patiënten nader gekarakteriseerd middels 3.0 Tesla MRI scans. Tot op heden zijn gewrichtsafwijkingen bij acromegalen alleen gekarakteriseerd middels röntgenfoto's en in het verleden is door één onderzoeksgroep met behulp van echografie gekeken naar gewrichtsafwijkingen. MRI verschaft additionele informatie ten opzichte van conventionele röntgenfoto's, voornamelijk over kraakbeendefecten, maar ook over andere structurele afwijkingen zoals cysten, beenmerglaesies en meniscusschade. In deze studie werden structurele gewrichtsafwijkingen onderzocht middels de gevalideerde KOSS score voor knie MRI's. Daarnaast werd de kraakbeendikte gemeten en werden T2 relaxatietijden in het kraakbeen gemeten, welke informatie verschaffen over de biochemische compositie van kraakbeen. Allereerst werden patiënten met actieve en gecureerde acromegalie vergeleken en daarnaast werden als controlegroep patiënten met primaire knieartrose geïncludeerd van de geMstoan studie. Er werd reeds in de actieve fase van acromegalie een hoge prevalentie van structurele gewrichtsafwijkingen gezien. Tevens werd gevonden dat patienten met actieve acromegalie dikker gewrichtskraakbeen hadden dan gecureerde acromegalie patienten en tevens verhoogde T2 relaxatietijden. Dit laatste wijst op een veranderde biochemische samenstelling van kraakbeen met een toegenomen hoeveelheid water. In vergelijking met primaire artrose patiënten hadden acromegalie patiënten minder cystes, maar een gelijke prevalentie van kraakbeendefecten, osteofyten en beenmerglaesies. De acromegalie-groep had gemiddeld een verdikt gewrichtskraakbeen en verhoogde T2 relaxatietijden in de knie. Op basis van huidige bevindingen is de hypothese dat in actieve acromegalen het verdikte gewrichtskraakbeen bestaat uit twee componenten: een structurele component van kraakbeenhypertrofie, welke (partieel) irreversibel is, en een oedemateuze component, welke afneemt na succesvolle behandeling. Een afname van deze laatste component verklaart mogelijk waarom gecureerde acromegalen in vergelijking met controles een verdikt gewrichtskraakbeen hebben, maar minder dik dan in de actieve fase door een afname van de oedemateuze component. Geconcludeerd werd dat de artrosekenmerken verschillen tussen acromegalie patiënten en patiënten met primaire artrose. Bovendien suggereren de resultaten van deze studie dat naast een dikker gewrichtskraakbeen het kraakbeen van acromegalie patiënten een andere biochemische samenstelling heeft dan het kraakbeen van primaire artrose patiënten. Geconcludeerd werd dat de artrose kenmerken verschillen tussen acromegalie patienten en patienten met primaire artrose, voornamelijk op het gebied van gewrichtskraakbeen. De relevantie van de verschillen op kraakbeenniveau dient in toekomstige studies verder uitgezocht te worden.

In een klein percentage behandelde acromegalie patiënten wordt

op röntgenfoto's in plaats van de voor acromegalie karakteristieke gewrichtsspleetverwijding juist een versmalde gewrichtsspleet gezien. Het is onbekend of dit een eindstadium is van artropathie bij acromegalie of dat dit een proces is dat zich onafhankelijk van de ziekte acromegalie ontwikkelt. In **Hoofdstuk 5** werden knieën en heupen van 89 behandelde acromegalie patiënten radiologisch onderzocht op de aanwezigheid van gewrichtsspleetversmalling. Daarnaast werden risicofactoren voor gewrichtsspleetversmalling onderzocht en de relatie tussen gewrichtsspleetvernauwing en klinische symptomen. Gewrichtsspleetversmalling werd gezien in 10.3% van de heupen en 15.4% van de knieën. Bekende risicofactoren zoals leeftijd en het vrouwelijk geslacht waren geassocieerd met gewrichtsspleetversmalling. Daarnaast waren er acromegalie-specifieke risicofactoren voor gewrichtsspleetversmalling, welke verschilden voor de knie en heup. In de heup werd een relatie gevonden met meer actieve ziekte, gereflecteerd door hogere GH en IGF-1 spiegels voor de start van behandeling, langere GH expositie en minder curatie na operatie en/of radiotherapie. In de knie waren voornamelijk eerdere knieoperaties en niet zozeer acromegalie-specifieke kenmerken, voorspellend voor het krijgen van gewrichtsspleetversmalling. Patiënten met gewrichtsspleetversmalling rapporteerden meer gewrichtsklachten. Deze studie laat zien dat gewrichtsspleetversmalling weinig voorkomt bij acromegalie patiënten, maar desondanks geassocieerd is met een toename van klachten. Daarnaast toont deze studie aan dat excessieve GH/IGF-1 activiteit niet alleen een rol speelt in de vroege fase, maar dat ook in de late fase van arthropathie, gereflecteerd door gewrichtsspleetversmalling, een associatie bestaat met ziekteactiviteit.

Een andere veel voorkomende complicatie van acromegalie is het optreden van wervelfracturen. In eerdere studies werd in 60% van de acromegalie patiënten, ondanks langdurige biochemische remissie, wervelfracturen gevonden, vaak in multipele wervels. In de meeste patiënten is de botmassa (BMD) normaal en dit is hiermee een slechte predictor voor fracturen in deze specifieke patiëntengroep. Het lijkt erop dat in acromegalie het verhoogde fractuurrisico berust op afwijkingen in de botkwaliteit en niet zozeer in de botkwantiteit, gezien de normale BMD. Op dit moment is onbekend of het bereiken van biochemische remissie het risico op (wervel)fracturen normaliseert of dat patiënten in langdurige remissie een persisterend verhoogd risico op fracturen hebben.

In **Hoofdstuk 6** werd het natuurlijk beloop van wervelfracturen en potentiële determinanten van progressie onderzocht in behandelde acromegalie patiënten. Patiënten met bisfosfonaten werden uitgesloten van deelname, waarna 49 patiënten geschikt bevonden werden voor inclusie. De prevalentie van wervelfracturen was 63% bij de start van de studie en was het hoogst in mannelijke acromegalen. Er bestond geen relatie met BMD. Gedurende een periode van 2.5 jaar vervolgtijd in biochemische remissie progressieve wervelfracturen gezien in 20% van de patiënten. Voornamelijk mannen en patiënten met 2 of meer fracturen bij de start van de studie toonden progressieve wervelinzakkingen gedurende de vervolgperiode. Progressie was niet gerelateerd aan BMD waarden bij aanvang van de studie of BMD veranderingen in de tijd. Geconcludeerd werd dat bij patiënten met acromegalie wervelfracturen niet alleen tijdens de actieve fase van de ziekte ontstaan, maar dat deze patiënten nieuwe fracturen ontwikkelen en bestaande fracturen ernstiger worden gedurende de periode van remissie. De bevinding dat deze wervelfracturen ontstaan ondanks een normale BMD wijst op een probleem in de botkwaliteit. Toekomstige studies moeten zich richten op (persisterende) veranderingen in de botkwaliteit in deze patiëntengroep met het oog op het identificeren van acromegalie patiënten met een verhoogde kans op toekomstige fracturen. Tevens dient onderzoek gedaan te worden naar de juiste behandelstrategie van fracturen bij deze specifieke patiëntenpopulatie.

Deel B. De rol van de GH/IGF-1 as in primaire artrose

Deel B richt zich op de betrokkenheid van de GH/IGF-1 as bij het ontstaan en de progressie van primaire artrose. Artrose is een veelvoorkomende verouderingsgerelateerde ziekte, welke wordt gekenmerkt door progressieve slijtage van gewrichtskraakbeen en bot remodelling. Artrose leidt tot gewrichtspijn, stijfheid en functiebeperking van de gewrichten. Ondanks dat de laatste jaren de kennis betreffende het ontstaan van artrose is toegenomen, is de exacte pathogenese onbekend. Artrose wordt gezien als een ziekte met een multifactoriële etiologie, waarin naast genetische predispositie, leeftijd, body mass index (BMI), hormonale factoren en lokale biomechanische factoren een rol spelen.

Verschillende onderzoekslijnen suggereren de betrokkenheid van de GH/IGF-1 as in de pathogenese van artrose. Ten eerste is aangetoond dat IGF-1 in normaal kraakbeen zorgt voor stimulatie van de chondrocytproliferatie en proteoglycaan- en collageensynthese van chondrocyten, ook tijdens expositie aan cytokinen. Deze anabole en beschermende kenmerken van IGF-1 wijzen op een mogelijke betrokkenheid bij kraakbeenherstel. Ten tweede is bij acromegalie patiënten een duidelijke relatie gevonden tussen de GH/IGF-1 activiteit

en het risico op het ontwikkelen van arthropathie. De prevalentie en de ernst van de radiologische artrose nemen toe bij een langere duur van onbehandelde acromegalie en hogere IGF-1 spiegels bij de diagnose. Ten slotte werd eerder aangetoond dat genen die de aanmaak, afbraak en herstel van gewrichtskraakbeen en de remodelling van het subchondrale bot reguleren betrokken zijn in de pathogenese van artrose. Hierbij wordt in het bijzonder het proces van endochondrale ossificatie genoemd (*zie onder*).

In **Hoofdstuk 7** zijn de resultaten van studies naar de associatie tussen serum IGF-1 spiegels en polymorfismen in het IGF-1 gen en primaire artrose samengevat in een systematisch literatuuroverzicht. In totaal werden elf studies geschikt bevonden voor inclusie. Aan de hand van een gestandaardiseerde set met kwaliteitscriteria werd de methodologische kwaliteit van elke individuele studie beoordeeld. Samenvattend impliceren *in vitro* studies, genetische associatiestudies en het hoge risico op secundaire artrose in acromegalie allen een relatie tussen het GH/IGF-1 systeem en het ontstaan van artrose. Hoewel in de grootste cross-sectionele studie en de enige prospectieve studie een associatie werd gezien tussen hoge IGF-1 spiegels en artrose, werd in het systematische literatuuroverzicht geen overtuigend bewijs gevonden voor een associatie tussen IGF-1en het ontwikkelen van radiologische artrose. Echter, omdat een invloed van kleine patiëntenaantallen, methodologische beperkingen en publicatie bias op de resultaten niet uitgesloten kan worden en slechts in enkele studies is gecorrigeerd voor leeftijd, geslacht en BMI in de interpretatie van de IGF-1 spiegels, is verder onderzoek in grote prospectieve studies noodzakelijk is om hier een uitspraak over te doen.

Gezien de inconsistente resultaten van het kleine aantal beschikbare epidemiologische studies naar de relatie tussen serum IGF-1 en primaire artrose, zoals beschreven in **Hoofdstuk 7**, werd in **Hoofdstuk 8** de relatie tussen serum IGF-1 en het ontstaan en progressie van primaire artrose onderzocht in een groot cohort van patiënten met een ernstige familiaire vorm van artrose. Deze patiënten zijn afkomstig van de GARP (Genetics osteoARthritis and Progression) Studie, een prospectieve follow-up studie onder 384 patiënten uitgevoerd in het LUMC. Gemiddeld genomen werd een significant verhoogde IGF-1 SD score gezien in GARP patiënten ten opzichte van beschikbare referentiewaarden. Er werd echter geen dosis-respons relatie gevonden met de kans op het ontstaan of de progressie van radiologische artrose. In dit opzicht is het van belang om op te merken dat alle GARP patiënten een ernstig fenotype van symptomatische artrose hebben in meerdere gewrichten met een familiaire component, wat het lastig maakt een dosis-respons relatie te detecteren met ernst van artrose. Daarnaast is de meting van serum IGF-1 erg complex gezien de gevoeligheid van IGF-1 voor temporaire variaties en de substantiële invloed van leeftijd, oestrogenen en BMI. Geconcludeerd werd dat een hoog serum IGF-1 geassocieerd is met het ontstaan van artrose, maar niet met de radiologische progressie van artrose.

Genetische factoren spelen een grote rol in de pathogenese van primaire artrose. Inmiddels zijn in genetische studies associaties gevonden met verschillende polymorfismen in genen die allen betrokken lijken te zijn bij de endochondrale ossificatie, dat het belangrijkste proces is in de lengtegroei van het skelet. Chrondrocyten in de groeischijf spelen de hoofdrol in dit proces, die door een combinatie van proliferatie, secretie van extracellulaire matrix componenten en hypertrofie resulteren in lengtegroei van het bot. Het kraakbeenmodel wordt uiteindelijk vervangen door bot. Het gedrag van de chondrocyten wordt in elk van de genoemde fases gereguleerd door een complex netwerk van circulerende hormonen, lokale groeifactoren en extracellulaire matrixcomponenten. Eén van de sterkste stimulatoren van de chondrocytproliferatie is GH, voornamelijk via secretie van IGF-1. Dit maakt de betrokkenheid van de GH/IGF-1 as in het ontstaan van artrose aannemelijk en kwalificeert polymorfismen in GH/IGF-1 genen als potentiële kandidaten voor genetische associatiestudies.

De effecten van GH op doelweefsels worden gemedieerd door de GHR. In 2004 werd voor het eerst door Dos Santos *e.a.* een veelvoorkomend polymorfisme van de GHR (30-35% van de populatie) beschreven. De deletie van exon 3 (d3-GHR) resulteert in een verkorte GHR en is geassocieerd met een verhoogde responsiviteit van de GHR voor GH vanwege een toegenomen signaaltransductie. Binnen patiëntengroepen met verschillende klinische aandoeningen, zoals vertraagde lengtegroei bij kinderen en in acromegalen, zijn de functionele effecten van het d3-GHR polymorfisme reeds aangetoond. Zo werd in acromegalie patiënten een relatie gevonden tussen het d3-GHR polymorfisme en het vaker en ernstiger voorkomen van radiologische artrose. De associatie tussen het d3-GHR polymorfisme en primaire artrose is nooit eerder onderzocht.

In **Hoofdstuk 9** werd de associatie van het d3-GHR polymorfisme bekeken in patiënten met (symptomatische) primaire artrose in vergelijking met controlepersonen zonder artrose. De GARP studie, waarin patiënten met familiaire primaire artrose in meerdere gewrichten zijn geïncludeerd, was gekozen als uitgangsstudie, omdat deze studie informatie verschaft over meerdere gewrichtslokalisaties in genetisch verrijkte artrose patiënten. Alle GARP patiënten werden gegenotypeerd voor 7 Single Nucleotide Polymorphisms (SNPs) rondom

het d3-GHR polymorfisme (rs4590183, rs13354167, rs7721081, rs7701605, rs4242116, rs6878512, rs10941583), die vanwege een hoog linkage disequilibrium als marker konden dienen voor het d3-GHR polymorfisme. Deze methode is voor grote cohorten efficiënter dan de arbeidsintensieve conventionele polymerase chain reaction (PCR) techniek. De SNP rs4590183 werd geselecteerd als marker voor het d3-GHR polymorfisme. In de GARP studie werd een associatie gevonden tussen het d3-GHR polymorfisme en artrose in vrouwen, echter niet in mannen. Ter replicatie werd de bovengenoemde SNP in 3 additionele patiëntencohorten bepaald, te weten de PAPRIKA/RAAK studie, Rotterdam studie en de ACRO studie, waarin allen patiënten zijn geïncludeerd met symptomatische artrose. De hierboven genoemde studies werden samen met de GARP studie in een meta-analyse geanalyseerd. Alleen vrouwen werden in de analyse meegenomen, gezien het effect van het d3-GHR polymorfisme in de GARP studie alleen in vrouwen werd gevonden. In totaal werden 2175 OA patiënten en 2623 controles geanalyseerd. In de meta-analyse werd de associatie tussen het d3-GHR polymorfisme en artrose bevestigd (OR=1.17 (1.04-1.32), ook na exclusie van de GARP studie. De sterkste relatie werd gevonden met heupartrose (OR=1.34 (1.11-1.62). De resultaten van deze studie verschaffen additioneel bewijs voor de betrokkenheid van de GH/IGF-1 as bij het ontstaan van artrose. Er werd geconcludeerd dat overactiviteit van de GH/IGF-1 as het artroseproces versnelt in patiënten die hiervoor gevoelig zijn en daarmee het risico op artrose verhoogt. Toekomstige studies moeten uitwijzen of een toegenomen activiteit van de GH/IGF-1 as therapeutische consequenties heeft.

Deel C. Lange termijn effecten van recombinant humaan GH (rhGH) therapie bij volwassenen met GH deficiëntie (GHD)

Deel C beschrijft de lange termijn effecten van rhGH therapie bij volwassen patiënten met GHD. GHD bij volwassen patiënten is geassocieerd met een ongunstig metabool profiel en wordt gekenmerkt door abdominale obesitas, dislipidemie en een verhoogde mortaliteit. Daarnaast is GHD geassocieerd met een afgenomen botmassa en een verhoogd fractuurrisico. In eerdere studies is aangetoond dat rhGH therapie op korte termijn een verbetering van de lichaamssamenstelling en het vetmetabolisme geeft, met een afname van lichaamsvet, een toename van de vetvrije lichaamsmassa en een verlaging van zowel het totaal cholesterol als het LDL-cholesterol. Daarnaast leidt rhGH therapie tot een toename van de botdichtheid. Tevens werd in enkele studies daarnaast een gunstig effect beschreven op de kwaliteit van leven. Gebaseerd op deze positieve korte termijn resultaten, is rhGH suppletie sinds 1994 in Nederland geregistreerd als behandeling voor GHD bij volwassenen. Eerdere studies toonden aan dat de positieve effecten van rhGH therapie op metabool gebied, maar ook op de botmassa, blijven bestaan gedurende de eerste 5 jaren van rhGH suppletie. Gegevens over de effecten van rhGH therapie op langere termijn zijn echter schaars.

In **Hoofdstuk 10** zijn in een systematisch literatuuroverzicht de effecten van chronische rhGH suppletie, gedefinieerd als ten minste 5 jaar, bij volwassen patiënten met GHD op een rij gezet. In dit overzicht werden 21 studies opgenomen die de effecten van rhGH suppletie op biochemische en antropometrische parameters, kwaliteit van leven, botmetabolisme, spierkracht, serious adverse events (SAEs) en mortaliteit hebben onderzocht. De studies opgenomen in het literatuuroverzicht suggereerden dat rhGH behandeling ook op de lange termijn leidt gunstige effecten heeft op de lichaamssamenstelling, het lipidenprofiel en de intima-mediadikte van de carotiden. Echter, de prevalentie van het metabool syndroom, glucose spiegels en BMI werden niet of zelfs negatief beïnvloed door langdurig rhGH gebruik. Hierbij moet worden opgemerkt dat in de meeste studies nauwkeurige data over het gebruik van vetverlagende middelen en antidiabetica ontbraken. Wat betreft spiermassa werden slechts in de eerste 5 jaar van rhGH therapie gunstige effecten waargenomen. Daarnaast werd in een selecte groep patiënten een verbetering van de kwaliteit van leven gevonden. Mortaliteit was in verschillende studies licht verhoogd gedurende rhGH suppletie, echter dit kon, mogelijk gezien de heterogeniteit van studies, in andere studies niet bevestigd worden. Geconcludeerd werd dat de beschikbare literatuur over de lange termijn effecten van rhGH therapie bij volwassenen met GHD inconsistente resultaten laat zien met betrekking tot de verwachte gunstige effecten, in de aanwezigheid van verschillende beperkingen, welke hierna worden genoemd. Allereerst waren de meeste lange termijn studies ongecontroleerd voor het effect van veroudering. Ten tweede hadden slechts een beperkt aantal centra hun resultaten gepubliceerd, waardoor de helft van de geïncludeerde studies (deels) hetzelfde patiëntencohort beschreef. Op basis van de resultaten van dit literatuuroverzicht kan geconcludeerd worden dat nader onderzoek naar het effect en de veiligheid van langdurige rhGH therapie bij volwassenen met GHD geïndiceerd is. Daarnaast is het in de klinische praktijk van belang de indicatie voor langdurige rhGH suppletie bij elke patiënt zorgvuldig afgewogen dient te worden.

Gezien het ontbreken van voldoende studies naar de effecten van 10 jaar rhGH suppletie of langer bij volwassenen met GHD, waarbij de beschikbare studies veelal kleine patiëntengroepen beschreven, zijn in **Hoofdstuk 12** de metabole effecten van 10 jaar rhGH behandeling onderzocht in ons eigen GHD cohort. In 98 volwassen GHD patiënten werd gekeken naar het effect van rhGH therapie op biochemische en antropometrische parameters, de prevalentie van metabool syndroom en de incidentie van ernstige cardiovasculaire gebeurtenissen. In een subgroep van 49 patiënten werden de 15-jaars effecten van rhGH therapie beschreven. IGF-1 spiegels werden in alle GHD patiënten strikt gecontroleerd om fysiologische rhGH suppletie te bewaken. De gunstige effecten op het lipidenprofiel werden na 10 jaar rhGH therapie nog steeds gezien, met een significante verlaging van het totale cholesterol en LDL-cholesterol en een toename van het HDL-cholesterol in vergelijking met de onbehandelde situatie. De gemiddelde middelomtrek, BMI en nuchtere glucose spiegels, daarentegen, namen significant toe gedurende rhGH therapie. In de groep patiënten met ten minste 15 jaar rhGH therapie werden dezelfde effecten gevonden. Ondanks verbetering van enkele cardiovasculaire parameters, nam de prevalentie van het metabool syndroom, als maat voor het algehele cardiovasculaire risico, significant toe gedurende rhGH therapie, van 32.7% bij de start van rhGH therapie tot 57.1% na 10 jaar behandeling. Dit was het meest evident in mannen. De incidentie van ernstige cardiovasculaire gebeurtenissen was laag (1.5/1000 patiënten jaren). Geconcludeerd werd dat gezien het feit dat op dit moment verbetering van het cardiovasculaire risico en verbetering van de kwaliteit van leven de belangrijkste doelen zijn van rhGH therapie bij volwassenen, grotere gecontroleerde studies essentieel zijn om de netto cardiovasculaire winst van langdurige rhGH suppletie vast te stellen. Daarnaast dienen ook de kosten van langdurige rhGH therapie meegenomen te worden. In dit opzicht is een vergelijking met statines, die bewezen effectief zijn gebleken in secundaire cardiovasculaire risicopreventie, zinvol.

Een beperking van de studie beschreven in **Hoofdstuk 11** is het ontbreken van een niet-behandelde controlegroep. Gezien de vastgestelde gunstige effecten van rhGH therapie op korte termijn, worden alle patiënten met vastgestelde GHD zonder contra-indicaties met rhGH gesuppleerd. Dit maakt het onmogelijk een langdurige vervolgstudie op te zetten met een gerandomiseerde gecontroleerde opzet, waarin patiënten met GHD zonder rhGH therapie geïncludeerd zijn. Recent is in het LUMC een grote populatiestudie uitgevoerd met de focus op de pathofysiologie van obesitas (NEO, Nederlandse Epidemiologie van Obesitas Studie). Hiermee zijn grootschalige recente controledata beschikbaar gekomen van zowel controlepersonen met een normaal gewicht als controlepersonen met obesitas, welke als alternatief, bij gebrek aan een niet-geselecteerde controlegroep van GHD patiënten zonder rhGH therapie, gebruikt kan worden.

In **Hoofdstuk 12** werden de metabole effecten van chronische rhGH therapie (gedefinieerd als 5 jaar), beschreven in 161 GHD patiënten van middelbare leeftijd. Deze data werden vergeleken met gezonde controlepersonen met een normaal gewicht van het Leiderdorp cohort van de NEO studie. GHD patiënten hadden, ondanks chronische rhGH therapie, een 1.3 keer verhoogde prevalentie van metabool syndroom dan controles, onafhankelijk van leeftijd, geslacht en BMI (53.4% *vs* 30.3%). Daarnaast werd in behandelde GHD patiënten een ander metabool profiel gezien dan bij controlepersonen met een gelijk BMI, gekenmerkt door een verhoogd prevalentie van hypertriglyceridaemie en laag HDL-cholesterol, maar minder vaak hyperglycaemie. Het is opvallend dat ondanks dat het beschreven GHD cohort verondersteld wordt adequaat gesuppleerd te zijn, het metabool profiel na chronische rhGH therapie lijkt op het profiel van een patiënt met onbehandelde GHD, met in het bijzonder afwijkingen in het lipidenprofiel. Deze bevindingen plaatsen vraagtekens bij de eerder gepropageerde gunstige effecten van rhGH therapie op metabool gebied. Het is van belang dat in de context van, aan de ene kant, het toenemende bewijs voor een (negatieve) rol van GH en IGF-1 in kanker, veroudering en cardiovasculaire ziekten, en, aan de andere kant, het beperkte bewijs voor een gunstig effect van rhGH substitutie in de oudere GHD populatie, een kritische re-evaluatie van de indicatie voor langdurige rhGH suppletie bij GHD volwassenen noodzakelijk is.

In **Hoofdstuk 13** werden de effecten van chronische rhGH therapie (≥5 jaar) op de botmassa en botmetabolisme onderzocht in 230 volwassen patiënten met GHD. Patiënten werden nauwkeurig gemonitord gedurende de rhGH therapie en overige hormonale deficiënties werden adequaat gesuppleerd. Er werd een persisterende toename van de BMD in de lumbale wervelkolom gezien, echter niet op het niveau van femurnek, gedurende 15 jaar rhGH suppletie. Lumbale wervelkolom BMC en T-scores namen gedurende de eerste 10 jaar rhGH behandeling toe en stabiliseerden hierna. De grootste stijging in BMD en BMC in de lumbale wervelkolom werd gezien in mannen, zoals reeds in eerdere studies is beschreven. In patiënten die naast rhGH suppletie bisfosfonaten gebruikten werd geen additioneel effect van bisfosfonaten op de BMD gevonden. Een lagere BMD op baseline, bisfosfonaatgebruik en een hogere GH dosis op 1 jaar waren van invloed op de botrespons tijdens langdurige

rhGH suppletie. De incidentie van klinische fracturen van gedurende langdurige rhGH suppletie was 20.1 / 1000 persoonsjaren en leek laag in dit cohort GHD patiënten. Geconcludeerd werd dat in volwassen GHD patiënten rhGH suppletie een persisterend gunstig effect heeft op de botmassa, voornamelijk in mannen, en dat de incidentie van klinische fracturen laag is. Het verschil in de botrespons op rhGH suppletie tussen mannen en vrouwen is een onderwerp voor nader onderzoek. Tevens dient er in toekomstige studies aandacht te zijn voor het fractuurrisico gedurende langdurige rhGH suppletie in een gecontroleerde setting en de rol van additionele behandeling met bisfosfonaten hierin.

CURRICULUM VITAE

Kim Claessen werd geboren als jongste van een tweeling op 24 maart 1989 te Roosendaal. Zij groeide op in Roosendaal in een gezin van vier kinderen, en bracht vele uren door op de tennisbaan verbonden aan de tennisschool Junior Top Tennis (JTT) van trainer Gillis van der Gruiter. Al sinds haar jeugd wist Kim dat zij dokter wilde worden. In 2007 behaalde zij *cum laude* haar Gymnasium diploma aan het Norbertus Lyceum, en begon zij aan de studie Geneeskunde aan de Universiteit Leiden. Zowel haar Propedeuse (2008) als Bachelor diploma (2010) werden *cum laude* behaald. Naast de tennistrainingen en –toernooien was Kim tijdens haar studententijd op vele andere fronten actief, en nam zij onder andere deel aan enkele Honours Classes, was zij snijzaal-assistent Anatomie en werkte zij als medisch triagist op de Huisartsenpost.

Reeds vroeg in haar studie Geneeskunde ontstond bij Kim de interesse voor wetenschappelijk onderzoek. In september 2009 werd zij geselecteerd voor het M.D./Ph.D.-traject voor Excellente Studenten, en startte zij vol enthousiasme met onderzoek aan de afdeling Endocrinologie en Metabolisme van het Leids Universitair Medisch Centrum (LUMC), onder leiding van Dr. N.R. Biermasz, Prof. Dr. M. Kloppenburg en Prof. dr. A.M. Pereira. Hiermee legde zij de fundering voor dit proefschrift. In april 2011, na de afronding van haar eerste 4 studiejaren van Geneeskunde en inmiddels verhuisd naar Amsterdam, ontving Kim van de Raad van Bestuur van het LUMC een 2-jarige beurs voor fulltime promotieonderzoek. In deze periode werden klinische studies bij patiënten met acromegalie en groeihormoondeficiëntie verricht. Daarnaast werden (genetische) studies uitgevoerd naar de rol van groeihormoon en IGF-1 bij primaire artrose, in samenwerking met de afdelingen Reumatologie en Moleculaire Epidemiologie van het LUMC. Tijdens haar onderzoeksperiode kreeg Kim de mogelijkheid een aantal cursussen op het gebied van Epidemiologie, Statistiek en Genetica te volgen, heeft zij meerdere posterpresentaties en mondelinge presentaties op nationale en internationale congressen gegeven, waarvoor haar enkele reisbeurzen werden toegekend.

In april 2013 is Kim gestart met haar coschappen aan het LUMC en zij zal in januari 2015 haar artsenbul in ontvangst nemen.

LIST OF PUBLISHED ABSTRACTS

1. Claessen Kim, Ramautar Sharita, Pereira Alberto, Smit Jan, Roelfsema Ferdinand, Romijn Hans, Kroon Herman, Kloppenburg Margreet, Biermasz Nienke. Progression of acromegalic arthropathy despite long-term biochemical control: a prospective follow-up study. *Endocrine Reviews 2012*; 33: OR 41-3.

2. Claessen Kim, Kloppenburg Margreet, Kroon Herman, Bijsterbosch Jessica, Pereira Alberto, Romijn Hans, Van der Straaten Tahar, Beekman Marian, Slagboom Eline, Biermasz Nienke, Meulenbelt Ingrid. A functional growth hormone receptor polymorphism, exon 3 deleted GHR, is associated with radiographic knee osteoarthritis in females with familial osteoarthritis at multiple sites: the GARP Study. *Endocrine Reviews 2012*; 33: SUN-LB7.

3. Claessen KMJA, Kloppenburg M, Kroon HM, Bijsterbosch J, Pereira AM, Romijn JA, Van der Straaten T, Beekman M, Slagboom PE, Biermasz NR, Meulenbelt I. A functional growth hormone receptor polymorphism, exon 3 deleted GHR, is associated with radiographic knee osteoarthritis in females with familial osteoarthritis at multiple sites: the GARP Study. *Arthritis & Rheumatism 2012*; 64(10): S472-S473.

4. Claessen Kim, Appelman-Dijkstra Natasha, Pereira Alberto, Hamdy Neveen, Kroon Herman, Kloppenburg Margreet, Biermasz Nienke. Progression of vertebral fractures despite long-term biochemical control of acromegaly: a prospective follow-up study. *Endocrine Reviews 2013*; 34; FP27-6.

5. Claessen Kim, Kloppenburg Margreet, Kroon Herman, Bijsterbosch Jessica, Pereira Alberto, Romijn Johannes, van der Straaten Tahar, Nelissen Rob, Hofman Albert, Uitterlinden André, Duijnisveld Bouke, Lakenberg Nico, Beekman Marian, van Meurs Joyce, Slagboom Eline, Biermasz Nienke, Meulenbelt Ingrid. A functional growth hormone receptor polymorphism, exon 3 deleted GHR, is associated with osteoarthritis in females: the results of a meta-analysis. *Endocrine Reviews 2013*; 34: SAT-123.

6. Joustra Sjoerd, Claessen Kim, Appelman-Dijkstra Natasha, Dekkers Olaf, van Beek André, Wolffenbuttel Bruce, Pereira Alberto, Biermasz Nienke. High prevalence of metabolic syndrome features in patients previously treated for non-functioning pituitary macroadenoma. *Endocrine Reviews 2013*; 34: MON-104.

7. Claessen Kim, Appelman-Dijkstra Natasha, Hamdy Neveen, Pereira Alberto, Biermasz Nienke. Effects of up to 15 years of recombinant human GH (rhGH) replacement on bone metabolism in adults with Growth Hormone Deficiency (GHD): The Leiden cohort study. *Endocrine Reviews 2014*; 35: SAT-680.

8. Claessen Kim, de Bruin Paul, Visser Willemien, de Lange-Brokaar Badelog, Pereira Alberto, Kroon Herman, Kloppenburg Margreet, Biermasz Nienke. Acromegalic arthropathy assessed by 3.0 Tesla Magnetic Resonance Imaging (MRI): a case-control study. *Endocrine Reviews 2014*; 35: MON-735.

LIST OF SCIENTIFIC PUBLICATIONS

1. Claessen KMJA, van Lieshout L, Visser LG. HIV and malaria co-infection: a Systematic Review. *JEMSA 2010*; 5: 1-11

2. Biermasz NR, van 't Klooster R, Wassenaar MJE, Malm SH, Claessen KMJA, Nelissen RGHH, Roelfsema F, Pereira AM, Kroon HM, Stoel BC, Romijn JA, Kloppenburg M. Automated image analysis of hand radiographs reveals widened joint spaces in patients with long-term control of acromegaly: relation to disease activity and symptoms. *European Journal of Endocrinology 2012*; 166 (3): 407-413

3. Claessen KMJA, Ramautar SR, Pereira AM, Smit JWA, Biermasz NR, Kloppenburg M. Relationship between insulin-like growth factor-1 and radiographic disease in patients with primary osteoarthritis: a Systematic Review. *Osteoarthritis & Cartilage 2012*; 20 (2): 79-86

4. Claessen KMJA, Ramautar SR, Pereira AM, Smit JWA, Roelfsema F, Romijn JA, Kroon HM, Kloppenburg M, Biermasz NR. Progression of acromegalic arthropathy despite long-term biochemical control: a prospective, radiological study. *European Journal of Endocrinology 2012*; 167 (2): 235-244. Comment in: *Nature Reviews Endocrinology 2012*; 8: 447 (Research Highlight)

5. Claessen KMJA, Appelman-Dijkstra NM, Adoptie DMMM, Roelfsema F, Smit JWA, Biermasz NR, Pereira AM. Metabolic profile in growth hormone deficient (GHD) adults after long-term recombinant human growth hormone (rhGH) therapy. *Journal of Clinical Endocrinology & Metabolism 2013;* 98 (1): 352-361
Comment in: *Reuters Health News 2012, 30 November*

6. Claessen KMJA, Ramautar SR, Pereira AM, Romijn JA, Kroon HM, Kloppenburg M, Biermasz NR. Increased clinical symptoms of acromegalic arthropathy in patients with long-term disease control: a prospective follow-up study. *Pituitary 2013;* 17(1): 44-52

7. Claessen KMJA, Appelman-Dijkstra NM, Roelfsema F, Pereira AM, Biermasz NR. Therapy of Endocrine Disease: Long-term effects of recombinant human GH replacement in adults with GH deficiency: a systematic review. *European Journal of Endocrinology 2013;* 169 (1): R1-R14

8. Claessen KMJA, Kloppenburg M, Kroon HM, Bijsterbosch J, Pereira AM, Romijn JA, van der Straaten T, Nelissen RGHH, Hofman A, Uitterlinden AG, Duijnisveld BJ, Lakenberg N, Beekman M, van Meurs JB, Slagboom PE, Biermasz NR, Meulenbelt I. Relationship between the functional exon 3 deleted growth hormone receptor polymorphism and symptomatic osteoarthritis in women. *Annals of Rheumatic Diseases 2014;* 73(2): 433-436

9. Claessen KMJA, Kloppenburg M, Kroon HM, Romijn JA, Pereira AM, Biermasz NR. Two phenotypes of arthropathy in long-term controlled acromegaly? A comparison between patients with and without joint space narrowing (JSN). *Growth Hormone & IGF Research 2013;* 23(5): 159-164

10. Claessen KMJA, Kroon HM, Pereira AM, Appelman-Dijkstra NM, Verstegen MJ, Kloppenburg M, Hamdy NAT, Biermasz NR. Progression of vertebral fractures despite long-term biochemical control of acromegaly: a prospective follow-up study. *Journal of Clinical Endocrinology and Metabolism 2013;* 98(12): 4808-4815
Comment in: *Lancet Diabetes & Endocrinology 2013*; 1 (3): 178 (Research Highlight)

11. Claessen KMJA, Appelman-Dijkstra NM, Pereira AM, Joustra SD, de Mutsert R, Gast KB, den Heijer M, Smit JWA, Dekkers OM, Biermasz NR. Abnormal metabolic profile in middle-aged GH-deficient adults despite long-term recombinant human GH replacement. *European Journal of Endocrinology 2013;* 170(2): 265-274

12. Joustra SD, Claessen KMJA, Dekkers OM, van Beek AP, Wolffenbuttel BHR, Pereira AM, Biermasz NR. High prevalence of metabolic syndrome features in patients previously treated for Nonfunctioning Pituitary Macroadenoma. *PLOS One 2014;* 9(3): 1-7

13. Claessen KMJA, Appelman-Dijkstra NM, Hamdy NAT, Pereira AM, Biermasz NR. Effects of up to 15 years of recombinant human GH (rhGH) replacement on bone metabolism in adults with Growth Hormone Deficiency (GHD): The Leiden Cohort Study. *Clinical Endocrinology 2014, in press*

14. Claessen KMJA, Meulenbelt I, Pereira AM, Kroon HM, Biermasz NR, Kloppenburg M. High serum insulin-like growth factor-1 (IGF-1) levels are associated with the presence of primary osteoarthritis, but not with radiographic progression: the GARP Study. *Submitted*

15. Claessen KMJA, de Bruin PW, Visser AW, de Lange-Brokaar BJE, Yusuf E, Pereira AM, Kloppenburg M, Kroon HM, Biermasz NR. Acromegalic arthropathy in various stages of the disease: a Magnetic Resonance Imaging (MRI) study. *Submitted*

16. Appelman-Dijkstra NM, Claessen KMJA, Hamdy NAT, Van de Bent C, Kroon HM, Pereira AM, Biermasz NR. Sclerostin levels are low after long-term remission of acromegaly. *In progress*

17. Claessen KMJA, van Vliet NA, Verstegen MJ, Roelfsema F, Pereira AM, Biermasz NR. Long-term outcome of transsphenoidal surgery in acromegaly: The Leiden Cohort Study. *In progress*

18. Claessen KMJA, Biermasz NR, Mazziotti G, Giustina A. Bone and joint disorders in acromegaly. ***In progress***

APPENDICES

APPENDIX 1: Comment in Nature Reviews Endocrinology 2012; 8: 447 (Research Highlight) on 'Progression of acromegalic arthropathy despite long-term biochemical control: a prospective, radiological study (European Journal of Endocrinology 2012; 167(2): 235-244)'

pituitary function

Arthropathy in acromegaly

Long-term biochemical control of acromegaly does not prevent progression of acromegalic arthropathy in many patients, report researchers from The Netherlands.

Arthropathy is a complication of acromegaly that impairs quality of life both physically and psychologically. Cross-sectional studies had previously shown a high prevalence of arthropathy in patients with long-term biochemical control of acromegaly.

"However, the disease course of acromegalic arthropathy during prolonged follow-up is unknown in treated patients," explains lead author Kim Claessen of Leiden University Medical Center. "Therefore, the aim of the present study was to assess the course of acromegalic arthropathy in a cohort of long-term control patients and to identify potential risk factors for progression over 2.6 years of prospective follow-up."

The researchers studied 58 patients with acromegaly who had been in biochemical remission of the disease for a mean duration of 15 years. The investigators obtained radiographs of the knees, hips and hands of the patients at two study visits a mean interval of 2.6 years apart. Radiographic progression of arthropathy from baseline to follow-up was defined as a ≥1 point increase in the score for osteophytes or joint space narrowing according to the Osteoarthritis Research Society International atlas.

Radiographic progression of osteophytes and joint space narrowing at any joint site was observed in 42 (72%) and 43 (74%) patients, respectively. Surprisingly, patients whose disease was biochemically controlled by somatostatin analogues had a higher risk of osteophyte progression than patients cured by surgery or additional radiotherapy.

The findings might indicate insufficient control of growth hormone in patients treated with somatostatin analogues, the researchers suggest.

"Further studies, preferably randomized controlled trials, with longer follow-up duration are required to explore whether more aggressive treatment is beneficial for the outcome of acromegalic arthropathy," concludes Claessen.

Carol Wilson

Original article Claessen, K. M. *et al.* Progression of acromegalic arthropathy despite long-term biochemical control: a prospective, radiological study. *Eur. J. Endocrinol.* doi:10.1530/EJE-12-0147

APPENDIX 2: Comment in Reuters Health (2013, Nov 30th) on 'Metabolic profile in growth hormone deficient (GHD) adults after long-term recombinant human growth hormone (rhGH) therapy (Journal of Clinical Endocrinology and Metabolism 2013;98(1): 352-361)'

Long-term growth hormone treatment ups risk of metabolic syndrome

Nov 30, 2012 | Reuters Health News
By Anne Harding

NEW YORK (Reuters Health) – Adults with growth hormone deficiency (GHD) who take recombinant human growth hormone (rhGH) for 10 years or more are at increased risk of metabolic syndrome, a new study in 98 patients shows.

Given that improving cardiovascular risk is one of the major targets of rhGH therapy for adults with GHD, "the effects of long-term rhGH therapy on overall cardiovascular profile needs to be established in a larger GHD cohort and should also be compared to healthy controls to control for the effect of aging," Dr. Kim M. J. A. Claessen of Leiden University Medical Center in The Netherlands, one of the study's authors, told Reuters Health.

In the short term, the researchers note, rhGH therapy improves several cardiovascular risk factors, such as low-density lipoprotein (LDL) levels and body fat. However, they add, there is some evidence that rhGH therapy could actually increase cardiovascular risk.

To better understand the cardiovascular effects of long-term rhGH therapy in adults with GHD, the researchers looked at several efficacy parameters in patients who had been treated at their center for 10 years or longer. They recorded patients' data at baseline and after five, 10 and 15 years of therapy with rhGH, and reported the findings online November 15 in the Journal of Clinical Endocrinology and Metabolism.

The mean age of patients in the study was nearly 60 years. Total cholesterol and LDL cholesterol were significantly lower than at baseline, while high-density lipoprotein levels were higher, the researchers found. However, at 10 years, waist circumference, body mass index, and fasting plasma glucose levels were all higher than at baseline.

And the prevalence of metabolic syndrome nearly doubled, showing a greater increase than would have been expected based on aging alone. "This was mainly due to a gradual increase in abdominal obesity, hypertriglyceridemia, and hyperglycemia," the researchers wrote.

At baseline, 32.7% of patients had metabolic syndrome, while after 10 years on rhGH, 57.1% did. While men and women were equally likely to have the metabolic syndrome at baseline, the prevalence at 10 years was 44.9% in women and 69.4% in men.

"Since the profile of GHD patients changes over time, critical re-evaluation of the net benefit of long-term rhGH therapy is of paramount importance," Dr. Claessen said via email. "Therefore, at present, the indication for long-term rhGH therapy should be established in every individual patient, and critically be re-evaluated during long term treatment."

It is also important to look at the cost-benefit ratio of rhGH therapy compared to other widely available medications, such as statins, that have proven effectiveness in secondary cardiovascular risk prevention, the researcher added.

SOURCE: Journal of Clinical Endocrinology and Metabolism, online November 15, 2012.

APPENDIX 3: Comment in: Lancet Diabetes & Endocrinology 2013 (Research in Brief) on 'Progression of vertebral fractures despite long-term biochemical control of acromegaly: a prospective follow-up study (Journal of Clinical Endocrinology and Metabolism 2013, in press)'

Progressive vertebral fractues in acromegaly

Written by Iley Ozerlat-Gunduz

In a prospective study including 49 patients with mean age of 61.3±11.1 years having controlled acromegaly for a mean of 17 years the natural course of vertebral fractures was assessed over a follow-up of 2.5 years. Remission of acromegaly was achieved by surgery, radiotherapy and/or medical therapy and patients did not use bisphosphonates. In these patients, baseline prevalence of vertebral fractures was 63%, and at the end of the follow up period, progressive fractures were found in 20% of patients despite adequate biochemical remission of acromegaly. Progression rate was highest in patients with 2 or more fractures at baseline and in men, but was independent of bone mineral density.

The data of the present study suggest that despite long-term biochemical remission acromegaly patients are at risk for progressive vertebral fractures, indicating persisting abnormalities in bone quality in these patients, possibly related to pretreatment long-term exposure to high circulating levels of GH. Further research has to elucidate the pathophysiological basis of the changes in bone quality leading to the high vertebral fracture risk and whether acromegaly patients require treatment for these fractures.

PA/PA Archive/Press Association Images

Her Majesty the Queen views the skeleton of Charles Byrne (1761–83), an Irish-born man who had acromegaly