Long-term effects of growth hormone replacement in hypopituitary adults on body composition, bone mass and cardiovascular risk factors

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“Whosoever seeks the truth will not proceed by studying the writings of his predecessors and by simply accepting his own good opinion of them. Rather, the truth-seeker will mistrust his established opinion. He will rely solely on his understanding of the texts by following the criteria of logic rather than the statements of authors who are, after all, human, with the errors and faults which this naturally involves. Whosoever studies works of science must, if he wants to find the truth, transform himself into a critic of everything he reads. He must examine texts and explanations with the greatest precision and question them from all angles and aspects. But he must also observe himself with a critical eye in this process, so that his judgement is neither too strict nor too lax. If he follows this path, the truths will reveal themselves to him and the possible inadequacies and uncertainties in the works of his predecessors will come to the force.”

Ibn al-Haitham, Cairo 965-1040 A.D.

Contents

Abstract

Growth hormone (GH) deficient (GHD) adults have decreased bone mass and muscle strength, impaired body composition, disturbed serum lipid pattern and increased morbidity and mortality in cardiovascular and cerebrovascular diseases. GH replacement normalizes most of these aberrations within the first year of treatment.

This thesis aimed to investigate the effects of 10-15 years of GH replacement on muscle strength, bone mass and density, body composition, and cardiovascular risk factors. It also aimed to determine the effects of GH replacement in elderly patients and the importance of previous irradiation therapy for baseline characteristics and the treatment effects of GH.

All patients had adult onset GHD resulting from pituitary disease, most commonly a pituitary tumour. Upper leg muscle strength was measured using a Kin-Com dynamometer and hand grip strength was measured with Grippit®, an electronic grip force instrument. Body composition and bone data were mainly assessed using dual-energy X-ray absorptiometry (DXA). Laboratory measurements were performed using conventional methods.

After correcting for the age related decline in muscle strength, 10 years of GH replacement induced a sustained increase in knee flexor and extensor strength and hand grip strength. Fifteen years of GH replacement induced a transient decrease in body fat and sustained improvements of lean soft tissue and serum lipid profile. Fasting plasma glucose increased whereas HbA1c decreased. Sustained increases in total body and lumbar (L2-L4) spine BMC (bone mineral content) and BMD (bone mineral density) were seen. In the femur neck, BMC and BMD peaked at 7 years and then decreased toward baseline values. Men had a better treatment response in terms of bone parameters, but no major gender differences were seen in the other variables measured. Three years of GH replacement increased BMD and BMC in the lumbar (L2-L4) spine and femur neck in younger as well as elderly GHD patients, without differences in the treatment effect between the groups. Compared to non-irradiated patients, GHD patients previously treated with pituitary irradiation therapy displayed a more severely impaired cardiovascular risk profile at baseline. Both groups responded to GH replacement with improved body composition, bone mass and serum lipid pattern. However, more cardiovascular events were observed in the irradiated group.

In conclusion, 10-15 years of GH replacement in hypopituitary adults induced sustained improvements in muscle strength, body composition, bone mass and serum lipid pattern. Elderly and younger patients showed a similar treatment response in terms of bone mass and density. Previous pituitary irradiation is associated with a more severely impaired cardiovascular risk profile, which is partly reversed by GH treatment. Men had a better treatment response in bone parameters than women.

Key words: growth hormone deficiency, growth hormone replacement, bone mineral density, body composition, muscle strength, elderly, cardiovascular risk factors, pituitary irradiation.

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Summary in Swedish – Sammanfattning på svenska

Vuxna med tillväxthormonbrist har sämre benmassa och muskelstyrka, förändrad kroppssammansättning, försämrade blodfetter samt ökad risk för insjuknande och död i hjärt-kärlsjukdom och stroke. Tillväxthormonbehandling normaliserar de flesta av dessa förändringar inom första året med behandling.

Syftet med den här avhandlingen var att undersöka effekterna av 10-15 års tillväxthormonbehandling på muskelstyrka, benmassa och bentäthet, kroppssammansättning och riskfaktorer för hjärt-kärlsjukdom. Effekterna av tillväxthormonbehandling hos äldre patienter undersöktes också, liksom betydelsen av tidigare strålbehandling för patientkarakteristika före och under tillväxthormonbehandlingen.

Alla patienter hade tillväxthormonbrist på grund av en hypofyssjukdom, oftast en hypofystumör, med debut i vuxen ålder. Muskelstyrka mättes med en Kin-Com dynamometer och handgreppsstyrka mättes med ett elektroniskt greppstyrkeinstrument, Grippit®. Kroppssammansättning och bendata mättes med dual-energy X-ray absorptiometry (DXA). Laboratorieprover analyserades med konventionella metoder.

Tio års tillväxthormonbehandling förbättrade, efter justering för den åldersrelaterade minskningen i muskelstyrka, styrkan i knäextensorer och knäflexorer samt handgreppstyrkan. Femton års tillväxthormonbehandling ledde till en övergående minskning av kroppsfett och en bestående förbättring av fettfri kroppsmassa och blodfetter. Fastesocker i plasma ökade, medan HbA1c minskade. Vidare ökade benmassa och bentäthet i helkroppsmätningar och i ländryggen (L2-L4). I lårbenshalsen ökade benmassa och bentäthet under de första sju åren, för att sedan minska mot värden som före behandlingsstart. Män hade bättre behandlingseffekt avseende benmassa och bentäthet, medan inga större könsskillnader sågs i behandlingseffekt på kroppssammansättning, muskelstyrka eller kardiovaskulära riskfaktorer. Tre års tillväxthormonbehandling ökade benmassa och bentäthet i ländrygg (L2-L4) och lårbenshals hos yngre såväl som äldre patienter, utan skillnad i behandlingseffekt mellan grupperna. Patienter som tidigare behandlats med strålning mot hypofysen hade en sämre kardiovaskulär riskprofil före behandlingsstart jämfört med patienter som inte hade strålbehandlats. Båda grupperna svarade på tillväxthormonbehandlingen med förbättrad kardiovaskulär riskprofil. Fler kardiovaskulära händelser observerades dock i den strålbehandlade gruppen.

Sammanfattningsvis hade 10-15 års tillväxthormonbehandling gynnsamma effekter på muskelstyrka, kroppssammansättning, benmassa och kardiovaskulära riskfaktorer. Äldre och yngre patienter hade lika bra behandlingseffekt på benmassa. Patienter som behandlats med strålning mot hypofysen hade en försämrad kardiovaskulär riskprofil, som endast delvis förbättrades med tillväxthormonbehandling. Män hade bättre behandlingseffekt avseende benmassa och bentäthet jämfört med kvinnor.

List of Papers

This thesis is based on the work contained in the following papers, which are referred to in the text by their roman numerals:

I. Götherström G, Elbornsson M, Stibrant-Sunnerhagen K, Bengtsson B-Å, Johannsson G, Svensson J. 2009 Ten years of growth hormone (GH) replacement normalizes muscle strength in GH-deficient adults. Journal of Clinical Endocrinology and Metabolism **94** 809-816.

II. Elbornsson M, Götherström G, Bosæus I, Bengtsson B-Å, Johannsson G, Svensson J. 2012. Fifteen years of growth hormone (GH) replacement improves body composition and cardiovascular risk factors *Submitted*

III. Elbornsson M, Götherström G, Bengtsson B-Å, Johannsson G, Svensson J. 2012 Fifteen years of growth hormone (GH) replacement increases bone mineral density in hypopituitary patients with adult onset GH deficiency. European Journal of Endocrinology **166** 787-795

IV. Elbornsson M, Götherström G, Franco C, Bengtsson B-Å, Johannsson G, Svensson J. 2012 Effects of 3-year growth hormone (GH) replacement therapy on bone mineral density in younger and elderly adults with adult onset GH deficiency. European Journal of Endocrinology **166** 181-189

V. Elbornsson M, Götherström G, Bengtsson B-Å, Johannsson G, Svensson J. 2012 Baseline characteristics and effects of ten years of growth hormone (GH) replacement therapy in adults previously treated with pituitary irradiation therapy *Submitted*

Abbreviations

ALST	Appendicular lean soft tissue
BCM	Body cell mass
BF	Body fat
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
BW	Body weight
CT	Computed tomography
CV	Coefficient of variation
DXA	Dual-energy X-ray absorptiometry
DM	Diabetes mellitus
ECW	Extracellular water
FFECS	Fat free extracellular solids
FFM	Fat-free mass
GH	Growth hormone
GHD	Growth hormone deficiency
GHRH	Growth hormone releasing hormone
HbA1c	Glycosylated haemoglobin
HDL-C	High-density lipoprotein cholesterol
ICW	Intracellular water
IGF-I	Insulin-like growth factor I
IRR	Irradiated patients
ITT	Insulin tolerance test
LDL-C	Low-density lipoprotein cholesterol
LST	Lean soft tissue
MRI	Magnetic resonance imaging
NFPA	Non-functioning pituitary adenoma
Non-IRR	Non-irradiated patients
QoL	Quality of life
SD	Standard deviation
SEM	Standard error of the mean
TBK	Total body potassium
TBW	Total body water
TC	Total cholesterol
TF	Trunk fat
TG	Triglycerides

Introduction

Historical background

The association between acromegaly and a pituitary tumour was first reported at the end of the 19th century (1). It was less obvious to link pituitary diseases to growth retardation in children, because this condition can have a number of other causes (1). Harvey Cushing was among the first to postulate the existence of a “hormone of growth” (2).

Human growth hormone (GH) was first isolated in 1956 (3). A radioimmunoassay for detection of plasma GH, which revealed significant amounts of circulating GH in adults, was developed in 1963 (4). For the diagnosis of growth hormone deficiency (GHD), the observation of hypoglycaemia as a potent stimulator of GH secretion (5) formed the basis of the insulin tolerance test (ITT), which is still the “golden standard” for evaluating the GH secretion (6, 7). Later, other tests, like the growth hormone releasing hormone (GHRH)-arginine stimulation test, have been validated (8, 9).

GH treatment in GHD children began in the late 1950s, using GH from human cadaveric pituitaries. Due to limited supply, GH treatment was restricted to patients with severe growth retardation. In 1962, Raben described the effects of GH treatment in a 35 year-old female, who had received conventional hormone replacement for 8 years prior to GH replacement (10). Raben described “increased vigour, ambition and a sense of well-being” in this patient after three months of GH replacement (10). One year later, Falkheden described the physiological consequences of hypophysectomy in adults, used as a treatment for metastatic mammary cancer and diabetic retinopathy, and concluded that these changes resulted from reduced GH secretion (11).

Concurrent with the gradual recognition of the importance of GH in adult life, recombinant GH became available in the mid-1980s. During that time, the first treatment trials with GH to adults were conducted (12, 13).

Initial GH treatment trials and dose titration

Initial GH treatment trials in adults used high GH doses that were based on body weight (BW) and adapted from the experience of paediatricians. These early studies showed increased serum insulin-like growth factor-I (IGF-I) and lean soft tissue (LST), reduced body fat (BF), and improved quality of life (QoL) (12-14). However, high GH dosage led to supraphysiological GH levels and side-effects, related mainly to fluid retention (12-14). Gradually, the weight-based dose regimen was abandoned, and replaced by individual dose titration with more physiological doses adapted to adult life (15, 16). Lower doses showed similar treatment effects with fewer side effects (15, 16).

Quality of life

GHD patients have decreased psychological well-being and QoL (17-21). They are more socially isolated, suffer from tiredness, memory and concentration problems, lack of initiative and drive and difficulties in coping with stressful situations compared to healthy controls (17, 21, 22). GH replacement therapy improves QoL with the greatest effect being shown within the first year of GH replacement, although further improvement in QoL is seen during longer treatment periods (22, 23). Biochemically, GH replacement decreases the concentration of the dopamine metabolite homovanillic acid and increases the β-endorphin immunoreactivity in cerebrospinal fluid (24, 25). Despite the improvements in QoL seen with GH replacement, hypopituitary patients receiving GH replacement worked full time to a lesser extent, and were more often on sickness leave/disability pension than the background population, in a recent Swedish multi-centre study (26). In particular, patients with childhood onset GHD, but to some extent also patients with adult onset GHD, lived less frequently with a partner, and to a higher extent with their parents (26). However, since there were no baseline data before starting GH replacement, it was not possible to evaluate the specific effect of GH replacement on psychological health in the Swedish multi-centre study (26). QoL has not been evaluated in any of the studies presented in this thesis, but a possible increase in QoL in response to GH replacement could have influenced the outcome of some of the variables measured, such as muscle strength and bone mass.

Effects of GH replacement on bone mass and density

Young GHD adults have reduced bone mineral content (BMC) and bone mineral density (BMD) (7, 27-31), and this reduction is more prominent in patients with childhood onset (CO) than with adult onset (AO) GHD (32). Compared to age-matched healthy controls, elderly GHD adults do not have a reduced bone mass and density (31, 33, 34). However, adult GHD patients without GH replacement are at a higher risk of fractures than healthy controls of the same age (35-37). Therefore, factors other than reduced BMD (e.g. an increased number of falls due to visual deficits, caused by pituitary tumours or their treatment) might contribute to the increased fracture risk in GHD adults.

GH replacement in adults with GHD induces an initial increase in bone resorption, which may result in unchanged or even reduced bone mass (7, 28). This is followed by increased bone formation and a net increase in bone mass after 12-18 months of GH replacement (7, 28). Although GH exerts direct effects on bone (28, 38), it has been questioned whether the direct effects can fully explain the effects of GH on bone. Indirect effects, such as increased muscle performance by GH, could also be of importance (39). Responsiveness to GH replacement depends on the group of patients studied. The increase in BMC and BMD is larger in patients with CO GHD compared to patients with AO GHD (32), and more prominent in men compared to women (40-45). Finally, the response to GH is most conspicuous at weight-bearing locations and the increase in BMC exceeds that in BMD (42, 44).

GH replacement induces a progressive increase in bone mass and density up to 5-6 years of treatment (42, 46-48). A 7-year study of GH replacement showed that BMC and BMD increased up to 4 years and then plateaued (40). In another study, total body and lumbar (L2-L4) spine BMD and BMC increased progressively up to 10 years of GH replacement, but femur neck BMC and BMD reached a peak value after 5-7 years of treatment (49).

Effects of GH replacement on muscle strength

Compared with healthy controls, adults with GHD show reduced isometric muscle strength and reduced or low-normal isokinetic muscle strength and local muscle endurance (12, 50, 51). GH replacement increases muscle mass and maximum voluntary isometric and isokinetic strength (12, 32, 50-53). No major effects on muscle morphology or on intrinsic factors in the muscles have been observed (54-56). Therefore the increase in muscle strength previously observed in GH treatment trials is probably a consequence of the increased muscle mass. The increase in muscle strength, observed mainly in open label GH treatment studies, is not seen until after approximately one year of therapy (12, 32, 50-53).

Increased muscle strength in response to GH is more predominant in adults with childhood onset GHD compared to patients with adult onset disease (32). In elderly GHD adults, older than 60 years, GH replacement mainly prevents age-related reduction of muscle strength, although the increase is relatively small in terms of absolute values (52). In normal elderly subjects, decreased neuromuscular function with reduced motor unit activation could be one mechanism underlying the age-related decrease in muscle strength (54). In a previous 2-year study, GH replacement did not affect the estimated torque at maximal motor unit activation (51). However, the effect of long-term GH replacement on neuromuscular function remains unknown.

Effects of GH replacement on body composition

The body composition of GHD adults is abnormal, characterized by increased body fat (BF) – especially visceral fat – and decreased lean mass (12, 13, 19, 50, 57). In comparison, patients with acromegaly have increased lean mass, decreased relative amount of body fat and fluid retention (58, 59). Within the first year of treatment, GH replacement therapy in GHD adults normalizes most of the alterations in body composition by reducing body fat and increasing lean mass (60-63). Although GH replacement sustains lean mass up to 10 years (64, 65), body fat gradually returns toward baseline values during prolonged GH replacement (64, 65), which might result from normal aging of the patients (65).

GH affects protein and fat metabolism in several ways (66, 67). In the basal state, i.e. after an overnight fast, GH mainly stimulates lipolysis and lipid oxidation, resulting in increased levels of free fatty acids (FFA) (66-70). The lipolytic effects of GH are at least partly mediated via stimulation of the hormone-sensitive lipase in fat cells, leading to increased degradation of triglycerides to FFAs (66, 67). In accordance with this, administration of acipimox, which blocks the action of hormone-sensitive lipase, suppresses the lipolytic effects of GH in humans (71-73). Further, some results suggest that GH also suppresses lipoprotein lipase in human adipose tissue, thereby decreasing the uptake of FFA from plasma by the adipocytes (66, 67, 74). Finally, GH, probably via IGF-I, inhibits the conversion of cortisone to cortisol in human adipose tissue by inhibiting the expression and activity of 11β-hydroxysteroid dehydrogenase 1 (75, 76). However, it is not clear to what extent this effect contributes to the lipolytic and insulin-antagonist effect of GH (66). Especially in the fasting state, GH is important for the conservation of muscle protein (77, 78). Experimental evidence suggests that lipolysis may act as a mechanism to preserve muscle protein (79). Some studies have shown increased protein synthesis and decreased breakdown after prolonged exposure to GH (66, 67, 80, 81). However, there are some conflicting results and the mechanisms are not fully understood (66, 67). Taken together, GH induces lipid oxidation and preserves muscle protein. GH has been described as a metabolic switch, altering fuel consumption from the use of carbohydrates and protein to the use of fat (66, 67). Indeed it has been known for decades that GH regulates fuel distribution and metabolism during fasting in adult life (66). These metabolic effects are consistent with the changes in body composition seen during the first year of GH replacement.

Effects of GH replacement on glucose metabolism

Patients with acromegaly are insulin resistant due to the insulin antagonist effects of GH. Children with isolated GHD frequently display fasting hypoglycaemia and are hyperresponsive to insulin (82). However, GHD adults have decreased insulin sensitivity, as measured using the hyperinsulinaemic, euglycaemic clamp technique (83, 84), possibly due to altered body composition with increased visceral fat.

The effect of GH replacement on glucose homeostasis remains controversial. In the early studies using a fixed GH dose based on body weight, short-term GH replacement further decreased insulin sensitivity (63, 85-88), despite favourable changes in body composition (63, 87, 88). In some studies, insulin sensitivity returned to the baseline level after 3-6 months of GH replacement (85, 86). During treatment periods of more than 6 months some studies showed an insulin sensitivity that was still decreased (87-89), whereas other studies reported unchanged insulin sensitivity as compared to baseline during long-term GH replacement (90-92). In a study by Hwu *et al*. insulin sensitivity was normalized after one year of GH replacement (93). In a study from our centre, 7 years of GH replacement protected against the normal age-related decline in insulin sensitivity (94), possibly resulting from improved body composition (94). In addition, an increase in circulating IGF-I by GH replacement could be beneficial in terms of insulin sensitivity (95). Yuen *et al*. randomized patients to receive either a fixed low GH dose of 0.1 mg/day or a standard dose aiming to normalize serum IGF-I levels (96). Patients in the low-dose group had improved insulin sensitivity compared to unchanged insulin sensitivity with the standard dose, although improvements in body composition were only seen with the standard dose (96). In another study, a mean GH dose of 0.3 mg improved insulin sensitivity (97). A recent study in GHD adults who had received continuous GH replacement for around 5 years prior to the test, used the euglycaemic-hyperinsulinaemic glucose clamp technique (98). Insulin sensitivity was similar to that of healthy controls when GH infusion was terminated 5 h before starting the clamp, and continuing GH infusion into the first part of the clamp caused decreased insulin sensitivity (98). The authors conclude that GH-induced insulin resistance is of rapid onset and transient in nature, since insulin sensitivity was normalized 5 h after the termination of GH exposure (98). It is likely that the 5 years of GH replacement prior to the test had induced positive effects on body composition, and this might be the reason why insulin sensitivity was comparable to that of healthy controls when GH exposure was terminated 5 h before the clamp (98). GH replacement therapy increases lipolysis, thereby increasing circulating levels of FFA (85, 86). According to the glucose-FFA cycle postulated by Randle *et al*. (99), these increased FFA concentrations may decrease the uptake of glucose in skeletal muscle. In later studies, inhibition of lipolysis with acipimox increased insulin sensitivity, confirming the inverse relationship between FFA levels and insulin sensitivity in GHD adults (72, 73). As a further support of this relationship, insulin sensitivity decreases with increasing levels of FFA above physiological FFA levels (100). Taken together, GH replacement induces lipolysis, with an increase in FFA, which is an important mechanism behind the acute insulin-antagonist effect of GH. Long-term GH replacement improves body composition, which, on the contrary, has favourable effects on insulin sensitivity.

GHD patients with and without GH replacement have a higher prevalence of the metabolic syndrome than the general population (101, 102), and the incidence and prevalence of diabetes mellitus (DM) type 2 may either be increased (103) or similar compared to that in the background population (104). One study showed increased risk of diabetes in women, but not in men, at least partly explained by the higher body mass index (BMI) and lower physical activity in women (105). Obesity and impaired metabolic profile prior to GH replacement are associated with an increased risk of developing diabetes during GH therapy (104, 106).

Effects of GH replacement on lipid metabolism

GH deficient adults have an impaired lipid profile (107-109). GH replacement improves the serum lipid profile, decreasing serum low density lipoprotein (LDL)-cholesterol (LDL-C) and, in most studies, increasing serum high density lipoprotein (HDL)-cholesterol (HDL-C) (62, 64, 65, 110, 111). Depending on the duration and dose of GH replacement, serum triglyceride (TG) level may increase, decrease or remain unchanged (64, 65, 110, 111). Although improved body composition might explain the improved lipid profile, some studies suggest that GH directly affects lipid metabolism, by increasing the expression of LDL receptors in the liver (112) and enhancing LDL catabolism (113). Further, GH administration may increase the turnover of LDL to a higher degree than indicated by the changes in serum LDL-C concentrations (114) and also increases the turnover of very low density lipoprotein (VLDL)-apolipoprotein B (apoB) (115).

Elderly with GHD

GH secretion declines with increasing age (116, 117), but distinct differences exist between normal elderly subjects and elderly adults with structural hypothalamic-pituitary disease. Elderly GHD adults have lower GH secretion (118) and increased total body fat (119) compared to age-matched healthy subjects, but show little difference in lean mass (119). The results of several studies suggest that GH replacement in elderly GHD patients have approximately similar efficacy as that in younger GHD adults in terms of quality of life, body composition and serum lipid pattern (52, 120-122).

In elderly GHD adults not receiving GH replacement, bone mass and density are approximately similar to that of healthy age-matched controls (31, 33, 34, 121). Little is known about whether GH replacement affects BMC and BMD in elderly GHD adults. A recent review of studies in elderly GHD adults (123) identified no significant effect of GH replacement on BMD, but previous studies have been few and of short duration and/or included relatively few patients (123).

Importance of previous pituitary irradiation therapy

Pituitary irradiation therapy is used as an adjuvant treatment of pituitary tumours, predominantly to prevent regrowth of incompletely resected or relapsing tumours (124-126). Until the 1980s pituitary irradiation was used as a standard treatment after pituitary surgery in our centre. A total dose of 40 Gy was delivered in 20 fractions of 2 Gy/fraction (4 days per week during 5 weeks). In most cases a 2-field technique with two lateral opposed fields was used, and in some cases a 3-field technique was used. After pituitary or cranial irradiation therapy, hypopituitarism develops gradually over time (127-129). Although this development depends on radiation dose, patient age, and the nature of the underlying deficit, most patients will have GHD and a relatively large proportion will develop panhypopituitarism within 5 years after radiotherapy (128, 130). Other late consequences of pituitary irradiation therapy may include decreased QoL (131-133) and neuropsychological changes (133, 134). Some studies have suggested that radiation-induced angiopathy is risk factor for cerebrovascular events (135, 136), and previous radiotherapy could therefore be of importance for the increased cerebrovascular morbidity and mortality in hypopituitary patients not receiving GH replacement (137-140).

In childhood cancer patients, cranial irradiation therapy is associated with weight gain, risk of obesity and signs of the metabolic syndrome (128, 134, 141). Adults might be less sensitive than children to the effects of radiotherapy (128, 130). Little is known about whether previous pituitary irradiation therapy affects baseline characteristics and the response to GH replacement in adult GHD patients. A study based on the Pfizer Metabolic Database (KIMS), which is a large post marketing surveillance program, demonstrated that previously irradiated GHD patients at baseline had lower QoL, similar BMI but higher fat mass, lower HDL-C levels, and lower BMC compared to non-irradiated GHD patients (131). One year of GH treatment induced approximately similar changes in both groups, although irradiated patients had a better response in terms of serum lipid profile (131).

Gender differences in responsiveness to GH replacement

GH secretion is markedly higher in premenopausal women compared to men of the same age (142). Oral, but not transdermal, oestrogen inhibits IGF-I formation in the liver, thus decreasing the serum IGF-I level (143). The reason for this may be the so called first-pass effect when orally administered oestrogen has to pass through the liver before entering the systemic circulation (143). Decreased serum IGF-I in women receiving oral oestrogens leads to increased GH level, most likely through feed-back mechanisms on the pituitary gland (143). Testosterone replacement in men could also influence gender differences in response to GH replacement. Testosterone can act on the liver together with GH to increase the IGF-I production (144). Also, the anabolic effects of testosterone increase lean and bone mass and decrease body fat (51).

Because early GH replacement studies based GH dose on body weight, men received higher doses of GH than women (14, 43, 145). However, when an individualized GH dose was used, women received a similar (146) or higher (43, 48, 65, 110) GH dose compared to men, which may be more physiological considering the interaction between sex steroids and the GH/IGF-I axis.

Most studies have shown a better treatment response in bone mass and density in men than in women (40, 42-46, 147, 148), but other studies have shown no gender difference (49, 149). Adjusted for age and gender, hypopituitary women have lower muscle strength than men before starting GH replacement (53), but treatment response in muscle strength during GH replacement is similar in both genders (51, 53). In terms of body composition some studies have shown similar treatment response in men and women (146, 150). A five-year study, conducted by our centre, showed greater reduction in body fat in men compared to women when using a four-compartment and a five-compartment model, whereas dual-energy X-ray absorptiometry (DXA) showed no gender differences (42). During 10 years of GH replacement, men had a more pronounced decrease in body fat and a greater increase in lean mass compared to women (65). Except for a more marked increase in HDL-C in men than in women in one study (65), no gender differences have been noticed in the treatment response in lipid profile or glucose metabolism (42, 65, 110).

Safety of GH replacement

In a meta-analysis of population-based studies, a U-shaped relation was observed between circulating IGF-I concentration and all-cause mortality (151). This suggests that both low and high serum IGF-I levels are associated with increased all-cause mortality in the normal population. Adult GHD patients receiving conventional hormonal therapy but not GH replacement, show increased cerebrovascular and cardiovascular mortality (137, 140, 152, 153). The greatest increase was seen in cerebrovascular disease (137, 140), with a more pronounced risk of cerebrovascular, but not cardiovascular, risk in women (137). GHD patients further display an increased incidence of non-fatal cardiovascular and cerebrovascular disease (139), and GHD women have an increased prevalence of cardiovascular risk factors (154).

There are still few data on mortality during GH replacement, because GH replacement in adults has not been in use for more than approximately 25 years. In one study from our centre, overall mortality was lower in GHD patients receiving GH replacement compared to that reported in untreated GHD adults (139). Mortality among GH treated patients was approximately similar to that of the background population (139). In a Dutch national study based on 2,229 GH treated patients, overall mortality was 27% higher than in the background population (155). Moreover, in a study based on 13,983 patients from the KIMS database, overall mortality was 13% higher than in the background population (156). Both studies observed increased mortality in women, younger patients, patients with craniopharyngeoma or aggressive underlying pituitary tumour (155, 156). The higher mortality was due mainly to cardiovascular and cerebrovascular disease (155, 156). In the KIMS study, patients with better response to GH in terms of increased IGF-I had a lower mortality rate (156). No increased mortality from malignancies was seen (155, 156).

In some studies, the incidence of colorectal cancer is increased among patients with acromegaly (157, 158). Furthermore, some population-based studies suggest that high serum IGF-I levels are associated with increased risk of colonic, prostate and breast cancer in the normal population (159-161). However, a recent population-based study showed a U-shaped relation between serum IGF-I concentration and cancer mortality in older men (162), suggesting that both high and low serum IGF-I concentration may be associated with increased cancer mortality. The results of some studies have also suggested that hypopituitarism and GHD may be associated with increased cancer incidence or mortality (139, 153, 163, 164). Safety concerns have been raised of a potentially increased risk of malignancy during GH replacement (138), especially if serum IGF-I concentration is increased to supraphysiological levels. However, available safety data indicate a cancer risk during GH replacement in adults of about the same magnitude as that in the general population (139, 165).

Aims of the thesis

This thesis aimed mainly to study the effects of long-term GH replacement in hypopituitary patients with adult onset GHD and to determine whether responsiveness to such treatment differed between different subgroups of patients. Specific aims were:

Paper I

To study the effects of 10 years of GH replacement on muscle strength in hypopituitary adults with GHD.

Paper II

To determine the effects of 15 years of GH replacement in GHD adults on body composition and cardiovascular risk factors, and to compare the treatment response in men and women.

Paper III

To evaluate the effects of 15 years of GH replacement on bone mineral content and bone mineral density in hypopituitary adults with GHD and to investigate whether the treatment response differed between men and women.

Paper IV

To compare the treatment response of three years of GH replacement in elderly and younger GHD adults.

Paper V

To investigate the importance of previous pituitary irradiation therapy on baseline characteristics and treatment response in GHD adults.

Subjects and study design

Patients

All patients included in *Papers I-V* were referred to and followed at the Centre for Endocrinology and Metabolism (CEM) at Sahlgrenska University Hospital, Göteborg, Sweden. CEM is an outpatient department that recruits patients from the western part of Sweden (Västra Götaland) whose 1.6 million inhabitants account for 17% of the Swedish population. Currently, there are data on around 500 GHD patients treated with GH at CEM. The studies included in this thesis are long-term studies and thus included patients with sufficient follow-up time. A total number of 207 patients (130 men) with adult onset GHD, aged 22-74 years, were included. Of these, 141 patients (68%) participated in more than one study. In 164 patients (80%), pituitary deficiency was caused by pituitary tumours and/or their treatment [non-functioning pituitary adenoma (NFPA) n=107, secreting pituitary adenoma n=39 and craniopharyngeoma n=18]. The patients had been treated with pituitary surgery (n=105), surgery and radiotherapy (n=52), radiotherapy alone (n=13), or no treatment (n=37).

Papers I-III included consecutive patients with adult onset GHD. In *Papers IV* and *V*, patients with previous acromegaly or Cushing's disease were excluded, because excess cortisol or GH possibly could affect baseline characteristics and response to GH replacement. *Paper IV* included 45 elderly GHD patients older than 65 years of age and 45 younger GHD patients with a mean age of 39.5 years. The two groups were comparable regarding the number of anterior pituitary hormonal deficiencies, gender, BMI and waist:hip ratio. *Paper V* included 18 GHD patients treated previously with pituitary irradiation (IRR group) and 18 non-irradiated patients (non-IRR group). All patients had NFPA as the cause of GHD and complete deficiency of anterior pituitary hormones at baseline. The groups were matched for age, gender, BMI and waist:hip ratio. In both study groups all patients had been treated with transsphenoidal pituitary surgery. In addition, all IRR patients had received conventional external fractionated irradiation therapy directed to the pituitary area (40 Gy).

In 196 patients (95%), the diagnosis of GHD was based on a peak GH <3 µg/L during a stimulation test [insulin (n=183), GH-releasing hormone (GHRH) (n=10) and glucagon (n=3)]. In nine patients, diagnosis was based on a 24-hour GH profile (sampling every 30 min). In two patients, both with a known anterior pituitary disease and three additional hormonal deficiencies, diagnosis was based on a low serum IGF-I level. The majority of patients had multiple anterior pituitary hormonal deficiencies; 62% had three additional hormonal deficiencies, and only 7% had isolated GHD. Possibly due to late effects of pituitary irradiation, several patients had more hormonal deficiencies at study end compared to baseline. When necessary, patients received adequate and stable therapy with glucocorticoids, thyroid hormone, and desmopressin. All testosterone-deficient men received testosterone therapy. At baseline, 60% of the oestrogen-deficient women received oestrogen replacement therapy.

Study protocols

All studies were prospective, single-centre, open-label studies of the effects of long-term GH replacement in patients with adult onset GHD. *Paper I* studied the effect of 10 years of GH replacement on muscle strength. *Papers II* and *III* studied the effects of 15 years of GH replacement. *Paper II* evaluated the effects on body composition and cardiovascular risk factors, and *Paper III* studied the effects on bone mass and density. *Paper IV* compared the effects of three years of GH replacement in elderly GHD adults (older than 65 years) with a control group of younger GHD patients (mean age = 39.5 years). *Paper V* compared baseline characteristics and the effects of 10 years of GH replacement between GHD patients treated with pituitary irradiation (IRR group) and a group of non-irradiated GHD patients (non-IRR group).

The initial target dose of GH in patients included before October 1993 was 11.9 μg/kg per day. This dose was lowered gradually and individualized when the weight-based dose regimen was abandoned. In the remaining patients, the GH dose was individualized from the beginning (16).

Dose titration and safety monitoring were performed every third month during the first year and every sixth month thereafter. Body weight was measured in the morning to the nearest 0.1 kg, and body height was measured to the nearest 0.01 m. BMI was calculated as the weight in kilograms divided by the height in meters squared. No effort was made to influence patients' physical activity level during the study period.

Physical and laboratory examinations were performed at baseline, after each year of GH replacement until 5 years, and then after 7, 10, 12 and 15 years, including measurements of muscle strength (*Paper I*), body composition (*Papers I, II, IV,* and *V*), bone mass, and bone density (*Papers III, IV,* and *V*).

Considerations on patient populations and study design

Papers I-III were long-term studies of different aspects of GH replacement. Due to the long duration of treatment, it was not possible to include an untreated control group. Therefore, we could not separate treatment effects from the effects of time. A control group of healthy age-matched subjects would have been valuable, but was not included. Comparisons with population-based reference values for IGF-I (all *Papers*), muscle strength (*Paper I*) and bone mineral density z-scores (*Papers III* and *IV*) may to some extent compensate for the lack of a control group.

Paper IV aimed to study the effects of GH replacement in elderly patients. Because ethical reasons precluded the inclusion of an untreated control group, the elderly patients were compared with a group of younger GHD patients. The groups were matched for anthropometric data and gender. Fully differentiating the effects of treatment and the effects of time would have required a control group of healthy age-matched individuals. *Paper V* was also a study comparing two groups – irradiated and non-irradiated GHD adults – that were comparable in terms of age, gender, BMI, and waist:hip ratio. All patients had complete deficiency of anterior pituitary hormones. The patients were included at a time when irradiation was gradually abandoned as a standard treatment after surgery. Because this was not a randomized study, we could not exclude the possibility that the patients who had received pituitary irradiation could have had a more aggressive pituitary disease. The irradiated patients had a longer duration of hypopituitarism before the start of GH replacement, which also could have affected the results. To study the effects of irradiation, a randomized study would have been preferred. However, because the negative effects of irradiation on the brain have been recognized (e.g. radiation-induced angiopathy, which could be a risk factor for cerebrovascular events), irradiation is now used only in patients with post-surgery tumour regrowth. Therefore, a randomized study was not possible due to ethical reasons.

In this thesis, 95% of the patients were diagnosed as having GHD based on a stimulation test, mainly an insulin tolerance test (ITT), and the studies included only severely GHD patients (peak GH of <3 µg/L). Patients who did not go through a stimulation test had a known pituitary disease and/or other hormonal deficiencies and were diagnosed based on a 24-hour GH profile or low IGF-I combined with a complete deficiency of anterior pituitary hormones. Those patients were diagnosed at a time when the diagnostic criteria for GHD had not been established. Because GHD is considered to be an early event in the development of hypopituitarism (129), most patients with pituitary disease and multiple hormonal deficiencies also have GHD, highly increasing the probability that all the patients were severely GHD.

Ethical considerations

Informed consent was obtained from all patients. All studies were approved by the Regional Ethical Review Board at the University of Gothenburg and the Swedish Medical Products Agency (Uppsala, Sweden).

Methods

Measurements of muscle function

Paper I measured muscle strength. Isometric knee-extensor and -flexor strength at knee angles of 60° (π/3 rad), and isokinetic muscle strength at angular velocities of 60°/sec (π/3 rad/sec) and 180°/sec (π rad/sec), were measured using a Kin-Com dynamometer (Chattecx Co., Chattanooga, TN, USA) (166). Gravity correction was used for isokinetic muscle strength (166). Right- and left-hand grip strength was measured using an electronic grip force instrument (Grippit®, AB Detector, Göteborg, Sweden), that measures maximum momentary force and mean force in Newtons over a set period of 10 seconds (167).

Local muscle endurance in the quadriceps muscle was measured as the percentage reduction (fatigue index) in peak torque between the first and last three knee extensions in a series of 50 maximal voluntary concentric contractions with an angle of velocity of 180°/sec (π rad/s) (168).

During isometric muscle contractions, superimposed single twitch electrical stimulation was given through percutaneous stimulation of the quadriceps muscle, as described by Rutherford et al. (169) and Thomeé et al. (170), to estimate the degree of activation of motor units at maximal voluntary contraction. An electrical stimulator monitored by a PC software program (AB Detektor, Göteborg, Sweden) was used, connected to 5 × 10-cm electrodes placed over the vastus medialis and rectus femoris muscles (170).

Because no control group was included, comparisons were made with a reference population of 144 healthy individuals aged 40-79 years from the Göteborg area who had undergone the same muscle function tests using the same equipment as in *Paper I* (171). The reference material was divided into 10-year cohorts. Applying predicted values for muscle function to each GHD patient allowed comparison with the reference population. The predicted values were obtained by calculating a mean value for each muscle test in each 10-year cohort of men and women in the reference population (171), and observed/predicted value ratios were then calculated. Twenty of the GHD patients were younger than 40 years of age at baseline, and six were younger than 40 years of age at study end. These patients were given a predicted value from the cohort of healthy controls aged 40-49, assuming no major change in muscle strength in previous adult age periods (172). This assumption may overestimate muscle strength in relation to normal in young GH-deficient men (173).

Dual-energy X-ray absorptiometry (DXA)

Papers I, II, IV, and *V* used DXA to measure lean soft tissue (LST) and body fat (BF) (174, 175). *Paper II* measured trunk fat (TF) (174), and appendicular lean soft tissue (ALST) was calculated as the sum of LST in the arms and legs and used to estimate skeletal muscle mass (176). *Papers III, IV,* and *V* used DXA to measure bone mineral content (BMC) and bone mineral density (BMD) in the total body, lumbar (L2-L4) spine, and femur neck (174). From the start of the study until the end of 1999, a LUNAR DPX-L scanner was used (Scanex, Helsingborg, Sweden), and a LUNAR Prodigy scanner (Scanex) was used from January 2000. Before changing equipment at the end of 1999, the old and new DXA machines were compared by measurements on the same day and on both machines in 30 subjects. No significant differences in body composition or bone parameters were found.

Daily quality control was performed according to manufacturer's protocol. A spine phantom was measured at least once a week. Every single spine phantom measurement was compared to a baseline value, based on a mean of 10 repeated measurements. A maximum 1.5% deviation from baseline value was accepted. A European phantom (COMAC-BME Quantitative Assessment of Osteoporosis Study Group) was measured once a year.

BMD z-score (i.e., the difference in SD of age- and sex-matched healthy subjects) and t-score, (i.e., the difference in SD of sex-matched young [20-39 years of age] healthy subjects) were determined using the Lunar DPX-L software program. The reference database used was the LUNAR USA reference population for the region examined.

DXA is a non-invasive, widely used method to evaluate body composition and BMD. It has the advantage of giving a low X-ray exposure. The DXA technique can distinguish between three compartments: fat, lean soft tissue and bone mineral. The classification of osteopenia and osteoporosis by the World Health Organisation (WHO) is based on measurements with DXA. In terms of BMD measurements one limitation of the DXA technique is that DXA is a two-dimensional technique (174, 175). Therefore, increases in bone size perpendicular to the DXA image are not taken into account, which may result in an overestimation of BMD in a large bone compared to a small bone. Furthermore, it cannot differentiate between cortical and trabecular bone. Such differentiation requires a computed tomography (CT) technique, which is more expensive and gives a higher X-ray exposure, limiting its use in clinical studies.

In terms of body composition, DXA can separate fat mass from lean soft tissue and also perform regional measurements. The main fat compartment of interest as a cardiovascular risk factor is visceral fat. DXA measures trunk fat, but does not separate subcutaneous fat from visceral fat. This would need CT or magnetic resonance imaging (MRI), both of which are expensive and time-consuming, and CT is associated with a higher X-ray exposure.

DXA measures LST but does not measure muscle separately. However, ALST has shown good correlation with skeletal muscle mass (176). The main potential confounding factor is the fluid retention and change in extracellular water (ECW) associated with GH treatment.

Four-compartment model

Papers I and *V* used a four-compartment model. Both 15-year studies (*Papers II* and *III*) could not use the four-compartment model because the whole body counter for measurements of total body potassium (TBK) was no longer in use. In the four-compartment model used, body weight (BW) is the sum of the four compartments: body cell mass (BCM), extracellular water (ECW), fat-free extracellular solids (FFECS) and body fat (BF). These compartments were calculated based on assessments of BW, TBK and total body water (TBW) (177, 178). TBK was determined in a whole body counter by counting the gamma radiation from the naturally present ^{40}K, which is a constant fraction (0.012%) of all natural potassium (177, 178). TBW was determined by the isotope dilution of tritiated water (177, 178). BCM was calculated from TBK with the formula BCM (kg) = TBK (mmol) × 0.0833, assuming cellular tissue has an average potassium-nitrogen ratio of 3 mmol/g and a protein content of 25% (177, 179). Intracellular water (ICW) was assumed to be 75% of BCM; thus ICW (kg) = 0.75 × BCM (kg). ECW (kg) was estimated as ECW (kg) = TBW (kg) – ICW (kg) (177, 178). FFECS is mainly the extracellular solids of bone and collagen, and was assumed to be a constant fraction (12%) of normal body weight: FFECS = 0.12 x BWnorm, where BWnorm is the "normal" BW for the body height (177, 178). The BWnorm for each patient was taken from Swedish population reference tables as described previously (178). Finally BF was calculated as: BF (kg) = BW (kg) – (FFECS+BCM+ECW).

There are two main sources of errors when using the four-compartment model. One lies in the weaknesses of the methods used to measure TBK and TBW, and the other lies in the assumptions used in the calculations of BCM, BF and ECW from TBK and TBW. Concerning the method used for measuring TBK possible contamination with certain isotopes used in medical examinations as well as the possible presence of disintegration products of radon-222 must be considered (177). The TBW method may have variations in absorption and time for equilibration. Another limitation of the TBW method is that the biological variation in hydration coefficient, even in normal healthy individuals, in non-negligible (180). The calculations of BCM and ECW are based on assumptions of the relation between certain components of cellular tissue, as described above – assumptions that may not be correct under all circumstances (177, 179). The calculation of FFECS relies on an estimated "normal" weight for height (177). This assumes constant FFECS with increasing age, although it is likely that FFECS decreases with age. The probable overestimation of FFECS in elderly persons results in underestimation of BF in the magnitude of 1-3 kg (177).

Biochemical analysis

Until June 2004, serum IGF-I concentration was determined by a hydrochloric acid-ethanol extraction radioimmunoassay (RIA) (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). Inter- and intra-assay coefficients of variation (CVs) were 5.4% and 6.9%, respectively, at a mean serum IGF-I level of 126 µg/L, and 4.6% and 4.7%, respectively, at a mean serum IGF-I level of 327 µg/L. From June 2004, serum IGF-I concentration was determined using a chemiluminescence immunoassay (Nichols Advantage®; Nichols Institute Diagnostics). Throughout the study period, the standard used for calibration of the IGF-I assays was the WHO NIBSC 1st IRR 87/518. After comparing individual serum IGF-I values with age- and sex-adjusted values obtained from a reference population of 197 men and 195 women (181), individual IGF-I SD scores were calculated (182).

Serum levels of total cholesterol (TC), HDL-C and TG concentrations were determined using enzymatic methods (42, 65). LDL-C was calculated according to Friedewald's formula adjusted to SI units (183). Serum insulin was determined by RIA (Phadebas, Pharmacia, Sweden). Until April 1998, blood glucose was measured with the glucose-6-phosphate dehydrogenase method (Kebo Lab, Stockholm, Sweden). From May 1998, plasma glucose was measured with a hexokinase method (Roche Diagnostics Scandinavia AB, Bromma, Sweden). In *Papers II* and *V*, blood glucose values obtained before May 1998 were converted to plasma glucose values using a multiplication factor of 1.11. Blood HbA1c was determined by high-pressure liquid chromatography (Waters, Millipore AB, Sweden).

Statistical methods

All descriptive statistical results are presented as the mean and SEM. For all variables, within-group differences were calculated using a repeated measures analysis of variance (ANOVA), with all data obtained from all time points and with time as the independent variable. Post-hoc analysis was performed using the Student-Newman-Keuls test. Between-group differences were calculated by a two-way ANOVA, with all data obtained from all time points. To eliminate baseline differences, data were transformed into per cent change or change from baseline before the between-group analyses. All analyses were performed according to the intention-to-treat principle (using the carry-forward principle). Correlations were calculated using Pearson's linear regression coefficient. A two-tailed $p<0.05$ was considered significant.

Results

GH dose, serum IGF-I and BMI

In *Papers I-III* and *V*, the dose of GH prescribed at the baseline visit was 0.41-0.88 mg/day. The GH dose was gradually reduced to 0.33-0.47 mg/day at study end. Most patients in *Paper IV* started their GH replacement in later years than patients in the long-term studies (*Papers I-III* and *V*). The initial GH dose in *Paper IV* was 0.15 (0.01) mg/day in elderly patients and 0.24 (0.02) mg/day in younger patients. The dose was increased in both groups to 0.24 (0.02) and 0.33 (0.02) mg/day, respectively in elderly and younger patients. Mean serum IGF-I and IGF-I SD scores increased in all *Papers*. In *Papers I-III* and *V*, the serum IGF-I SD score (adjustment for age and gender) was above the normal range during the first three years, but within the normal range (± 2 SD) after that. In *Paper IV* serum IGF-I SD score was within the normal range throughout the study period.

In *Paper III* mean body height decreased 0.5 cm during the 15 years of GH replacement, but remained unchanged in all other *Papers*. BMI increased in both 15-year studies (*Papers II and III*), but was stable in the shorter studies (*Papers I*, *IV*, and *V*).

Muscle strength

Paper I measured upper leg muscle strength and handgrip strength. There was a sustained increase in isometric knee flexor strength throughout the study period. Concentric knee flexor strength (60°/sec and 180°/sec) and concentric knee extensor strength (180°/sec) increased transiently during the early years of GH replacement, but subsequently decreased to values below baseline at study end. Also, isometric knee extensor strength and concentric knee extensor strength (60°/sec) were lower at study end compared to baseline. Right-hand grip strength increased transiently at 3-7 years of GH replacement; left-hand grip strength was unaffected. Upper leg local muscle endurance decreased transiently (the fatigue index increased transiently) at 3–7 years of GH replacement.

As estimated from the superimposition of single twitches on isometric contractions, GH replacement did not alter the estimated torque at maximal motor unit activation during the first 7 years of GH replacement. After 10 years, the estimated value increased compared to baseline, suggesting decreased voluntary motor unit activation at study end.

After correction for age and gender using observed/predicted value ratios, there were sustained increases in all variables reflecting muscle performance except for isometric knee extensor strength (Figure 1).

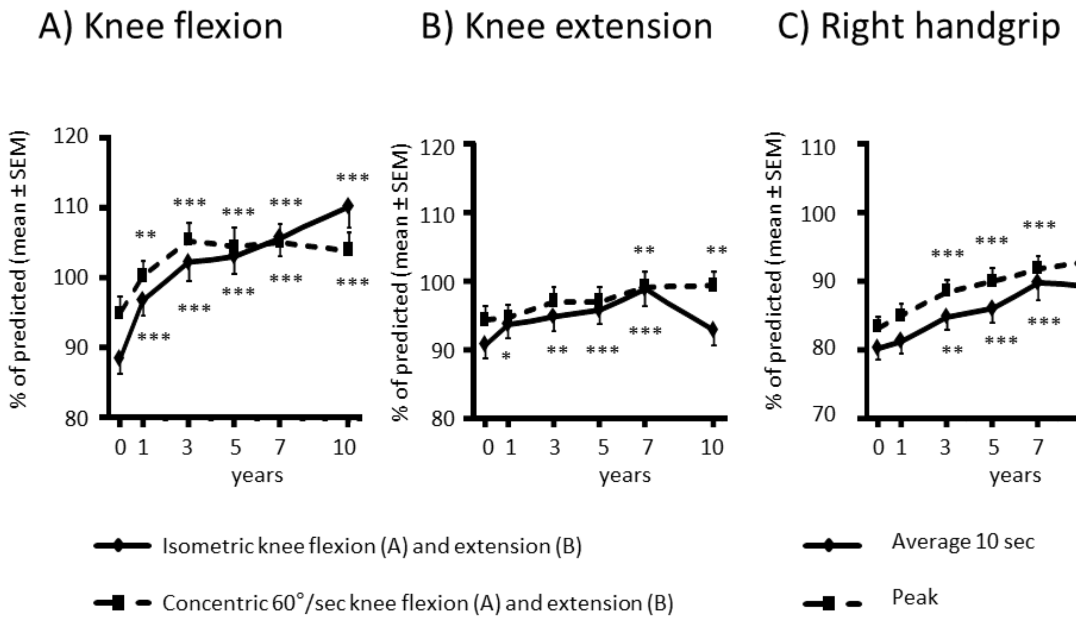

Figure 1. The effects of 10 years of GH replacement in 109 hypopituitary adults on muscle strength; A) Knee flexion, B) Knee extension and C) Handgrip strength (*Paper I*). Results are shown as per cent of predicted. Predicted values were obtained from the mean value in each 10-year cohort of men and women, respectively, in a reference population. The vertical bars indicate the SEM for the mean values shown. The statistical analyses are based on a repeated measures ANOVA followed by Student-Newman-Keuls post hoc test. *p<0.05; **p<0.01; ***p<0.001 vs. baseline.

Body composition

Total body LST, as measured by DXA, increased 2-5% during the first year of GH replacement (*Papers I, II, IV*, and *V*) and was then increased at a stable level (Figure 2). Using the four-compartment model BCM increased (*Papers I* and *V*) and ECW increased in *Paper I*, but in *Paper V* the increase was not statistically significant.

Total BF, as measured by DXA, decreased 8-9% during the first year of treatment (*Papers I, II, IV*, and *V*). Using the four-compartment model BF decreased 8-13% during the first year (*Papers I* and *V*). In *Paper I*, BF, as measured by DXA, remained below the baseline level up to 5 years and then returned to the baseline level, whereas in *Paper II* BF stayed below the baseline level up to 10 years of GH replacement (Figure 2). Using the four-compartment model, BF stayed below baseline throughout the 10-year follow-up period (*Papers I* and *V*). In *Paper II,* although body fat had returned to the baseline level after 15 years of GH replacement, body fat expressed as a percentage of body weight was still below the baseline level at study end.

TF, as measured using DXA in *Paper II*, decreased 10% during the first year (p<0.001), stayed below the baseline level up to 5 years (p<0.001), and then increased to 6% above the baseline level after 15 years (p<0.001). ALST, (*Paper II*) used as an estimate of skeletal muscle mass, increased up to 10 years of GH replacement (p<0.05) and then decreased toward the baseline value (Figure 2).

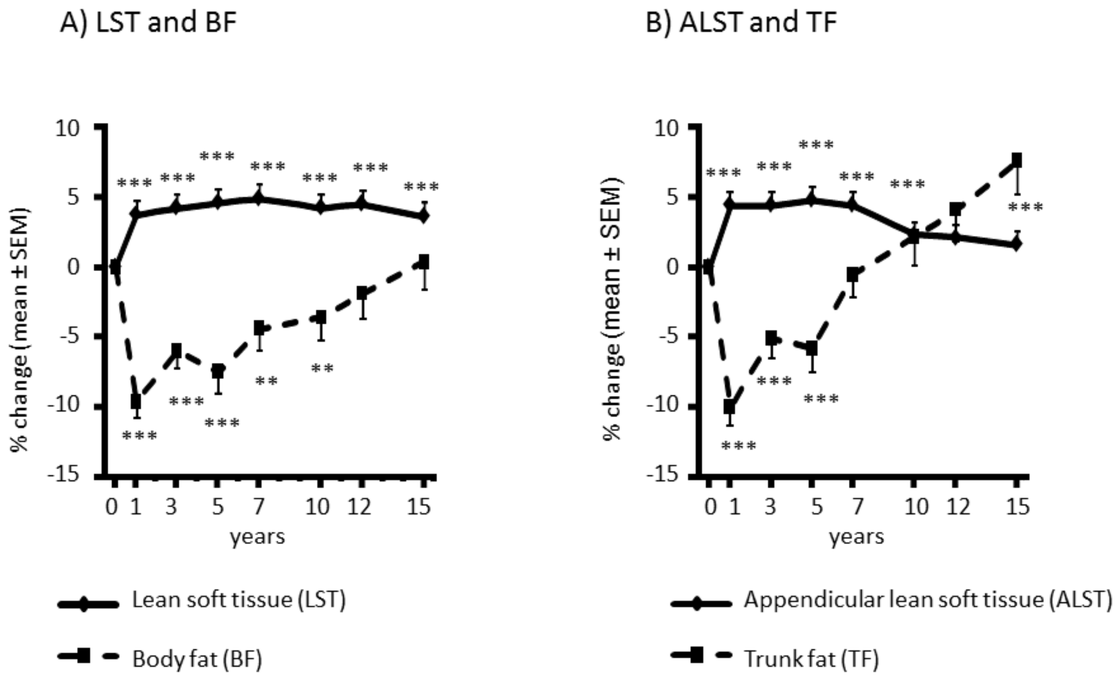

Figure 2. The effects of 15 years of GH replacement, on body composition as measured by DXA, in 156 hypopituitary adults. The results are shown as per cent change from baseline. The vertical bars indicate the SEM for the mean values shown. LST, Lean soft tissue; BF, Body fat; ALST, Appendicular lean soft tissue; TF, Trunk fat. The statistical analyses are based on a repeated measures ANOVA followed by Student-Newman-Keuls post hoc test. *p<0.05; **p<0.01; ***p<0.001 vs. baseline.

Lipids and glucose

There were sustained decreases in serum levels of TC and LDL-C (both p<0.001 vs. baseline) up to 15 years of GH replacement (*Paper II*; Figure 3). In *Paper V*, the decrease in LDL-C was significant in both IRR and non-IRR patients. The decrease in TC was significant only in the IRR group, but we observed no difference in the treatment response between groups (*Paper V*). Serum HDL-C concentration increased up to 15 years (p<0.001 vs. baseline; *Paper II*) and up to 10 years in both groups (*Paper V*). Serum TG level did not change (*Paper II* and *V*). Fasting plasma glucose increased (p<0.001) and blood HbA1c decreased (p<0.001) throughout the 15 years of GH replacement (*Paper II*; Figure 3). *Paper V* showed similar results for plasma glucose and HbA1c in both groups. In *Paper V*, fasting serum insulin remained unchanged throughout the study period in both groups.

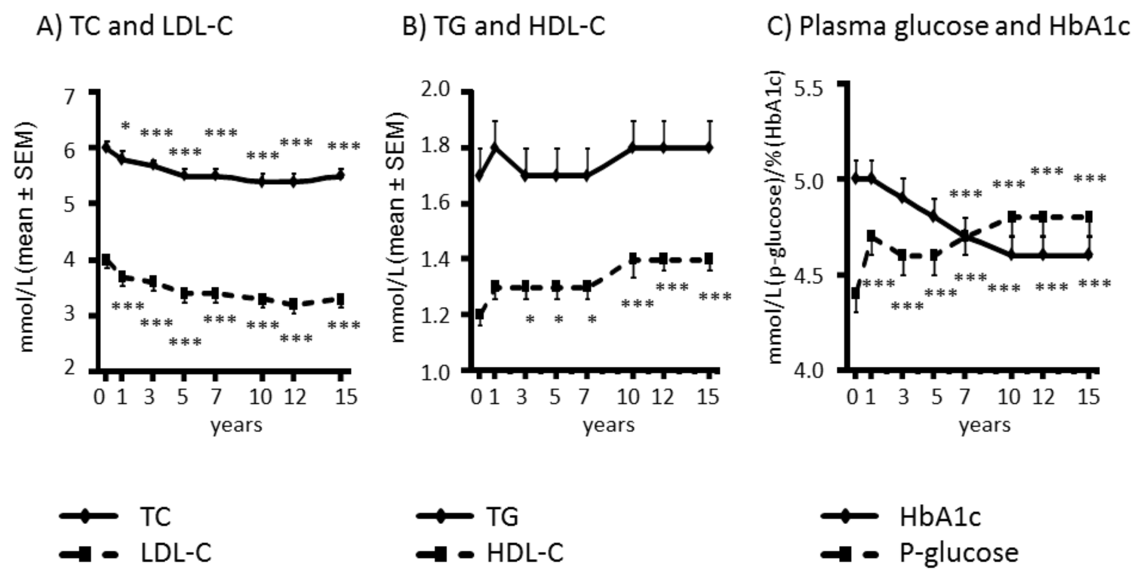

Figure 3. The effects of 15 years of GH replacement, in 156 hypopituitary adults, on lipid profile and glucose metabolism. TC, Total cholesterol; LDL-C, LDL-cholesterol; TG, Triglycerides; HDL-C, HDL-cholesterol. The vertical bars indicate the SEM for the mean values shown. The statistical analyses are based on a repeated measures ANOVA followed by Student-Newman-Keuls post hoc test. *p<0.05; **p<0.01; ***p<0.001 vs. baseline.

Diabetes mellitus

At baseline in *Paper II*, four (two men) of the 156 patients had DM type 2. During the 15-year study period, 12 patients (9 men) were diagnosed with and treated for DM type 2 (within 5 years, n=2; after 5-10 years, n=7; after >10 years, n=3). At baseline, patients who later developed DM type 2 had higher BW (96.4 vs. 81.3 kg; p<0.01), BF (35.3 kg vs. 26.6 kg; p<0.01), TF (18.1 vs. 14.1 kg; p<0.01), fasting plasma glucose (5.1 vs. 4.4 mmol/L; p<0.001) and serum TG (2.3 vs. 1.7 mmol/L; p<0.05). In *Paper V* (18 patients in each group) one patient in the IRR group had DM type 2 at baseline and one IRR patient developed DM type 2 during the 10-year study period. In the non-IRR group no patient had DM.

BMC and BMD

The effects of GH replacement on BMC and BMD, as measured using DXA, were evaluated in *Papers III-V*. Mean total body BMC increased in *Papers III-V*, except in elderly patients in *Paper IV*, where total body BMC remained constant during three years. In *Papers III* and *V*, total body BMC increased to 5-7% above baseline value after 10 years, and we observed no further increase between 10 and 15 years in *Paper III*. Total body BMD remained constant during the first 3-7 years (*Papers III-V*), increased 2-3% after 10 years (*Papers III* and *V*), and stayed at 2% above the baseline level up to 15 years (*Paper III*; Figure 4).

In *Papers III-V*, Lumbar (L2-L4) spine BMC and BMD increased throughout the study periods. After 10 years, lumbar (L2-L4) spine BMC was 9-11% and BMD was 6-9% above baseline (*Papers III* and *V*), with no further increase between 10 and 15 years of treatment (*Paper III*).

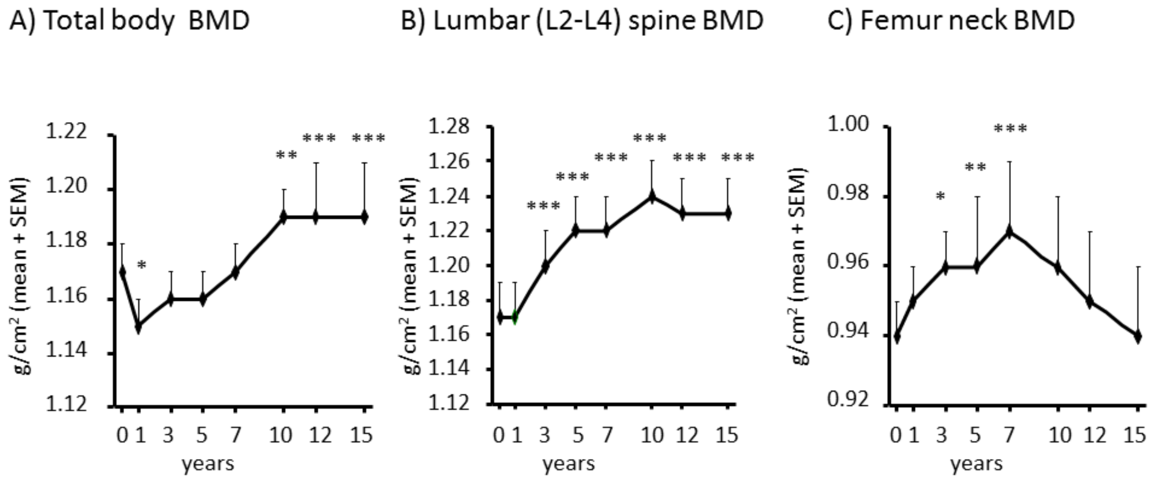

Figure 4. The effects of 15 years of GH replacement, in 126 hypopituitary adults, on bone mineral density (BMD) in the total body (A), lumbar (L2-L4) spine (B) and femur neck (C). The vertical bars indicate the SEM for the mean values shown. The statistical analyses are based on a repeated measures ANOVA followed by Student-Newman-Keuls post hoc test. *p<0.05; **p<0.01; ***p<0.001 vs. baseline.

In *Paper IV* femur neck BMC and BMD increased in both elderly and younger patients during three years of GH replacement. In the long-term studies, femur neck BMC peaked at 6-9% above baseline after 7-10 years (*Papers III* and *V*). After 10 years, femur neck BMC decreased and was 5% above baseline after 15 years (*Paper III*). Femur neck BMD peaked at 3-4% above baseline after 7 years of treatment (*Papers III* and *V*), then decreased and returned to baseline after 15 years (Figure 4; *Paper III*). Femur neck z-score remained significantly elevated above the baseline level after 15 years.

Fractures

During 15 years of GH replacement in *Paper III*, no fractures were reported in men. One woman suffered a hip fracture and one woman had a symptomatic vertebral fracture, both after seven years of GH replacement. X-ray examinations were not performed to determine asymptomatic vertebral fractures. No patient lost >5 cm of height but two men and four women had a height loss of 3-4.5 cm. In *Paper IV* no fractures were reported in any group.

Gender differences

Papers I-III evaluated gender differences, and found that women received a higher GH dose than men (both p<0.001) except during the first year of treatment. *Paper I* showed no dose difference between men and women, but women received a higher dose per kg BW than men. Mean IGF-I SD score increased more in women than in men (*Papers I-III*).

After correction for age and gender, baseline muscle strength was lower in women in all measurable variables except isometric knee flexor strength and the fatigue index (*Paper I*). Men and women demonstrated a similar response in all variables reflecting muscle performance (*Paper I*).

Paper II evaluated gender differences in lipid profile, glucose metabolism and body composition. At baseline, women had higher serum TC (6.3 (1.4) vs. 5.8 (0.13) mmol/L, $p<0.05$) and higher serum HDL-C (1.4 (0.06) vs. 1.1 (0.03) mmol/L, $p<0.001$) than men. Similar gender differences in serum lipid profile were seen after 15 years of GH replacement. The decrease in serum LDL-C was marginally greater in women than in men ($p<0.05$). There was no gender difference in the treatment effect in terms of other serum lipid levels, plasma glucose, blood HbA1c, body composition or blood pressure.

Paper III evaluated gender differences in BMC and BMD. At all skeletal locations measured, BMD, t-scores and z-scores increased more in men compared to women. Similar gender differences, with men being more responsive, were seen for BMC except for femur neck BMC, where there was no significant gender difference.

Gonadal status

All gonadotropin deficient men received testosterone replacement therapy. Therefore it was not possible to perform comparisons between hypogonadal men with and without testosterone.

Younger women received oestrogen replacement therapy more often than older women. At baseline, the mean age of hypogonadal women receiving oestrogen replacement was 45-47 years compared to 58-60 years in women without oestrogen replacement (all $p<0.001$; *Papers I-III*). At study start 52-60% of gonadotropin deficient women received oestrogen replacement therapy in the two 15-year studies compared to 26-31% at study end (*Papers II* and *III*). Fewer women received oestrogen replacement at study end compared to baseline because some discontinued treatment due to age. Women on oestrogen replacement therapy received a higher dose of GH than women without oestrogen replacement ($p<0.001$; *Papers II* and *III*). Baseline level and treatment response did not differ regarding age- and sex-corrected muscle performance, body composition, lipid profile, glucose metabolism, BMC, or BMD between women with or without oestrogen replacement at baseline.

Elderly vs. younger patients

Paper IV compared elderly and younger patients. Younger patients received a higher dose of GH than elderly patients, and the absolute level of serum IGF-I concentration was lower in elderly patients compared to younger patients. Mean IGF-I SD score (adjustment for age and gender) was similar and within the normal range (± 2 SD) in both study groups.

Body height, BW and BMI remained constant and did not differ significantly between groups. There were sustained reductions in waist circumference, waist:hip ratio and DXA-measured

BF in both groups without any between-group difference. LST increased to a similar extent in both groups.

At baseline, no differences in total body and lumbar (L2-L4) spine BMC, BMD or t-score were seen between groups (Table 1). However, elderly patients had a higher mean lumbar (L2-L4) spine z-score compared to younger patients at baseline ($p<0.05$), and z-score values were approximately zero (approximately that predicted based on age and gender) in the elderly GHD group. Femur neck BMC, BMD and t-score were lower in elderly compared to younger GHD patients ($p<0.05$ and $p<0.001$, respectively), but we found no difference in femur neck z-score between groups. There was no statistically significant difference in responsiveness to three years of GH replacement in BMC or BMD between groups, except for femur neck BMC, where the increase was more marked in the younger patients ($p<0.05$ vs. elderly group). At study end femur neck BMC, BMD and t-score remained lower in elderly patients ($p<0.05$), and lumbar (L2-L4) spine z-score remained higher in elderly patients compared to younger patients.

Table 1. Total body, lumbar (L2-L4) spine and femur neck BMD during three years of GH replacement in 45 GHD adults >65 years and 45 younger control GHD adults with a mean age of 39.5 years.

		Baseline	1 year	2 years	3 years	P value (0-3 yrs)
Total body BMD (g/cm^2)	Elderly	1.19 (0.02)	1.18 (0.02)	1.19 (0.02)	1.19 (0.02)	0.21
	Young	1.23 (0.01)	1.22 (0.01)	1.22 (0.01)	1.24 (0.01)	
Lumbar (L2-L4) spine BMD (g/cm^2)	Elderly	1.19 (0.03)	1.20 (0.03)	1.22 (0.03)[b]	1.23 (0.04)[d]	0.22
	Young	1.20 (0.02)	1.21 (0.02)	1.25 (0.03)	1.26 (0.03)[d]	
Femur neck BMD (g/cm^2)	Elderly	0.91 (0.02)[a]	0.92 (0.02)[a]	0.92 (0.02)[a]	0.93 (0.02)[a,b]	0.22
	Young	1.02 (0.02)	1.03 (0.02)	1.05 (0.02)[c]	1.05 (0.02)[d]	

All values are shown as the mean (SEM). The statistical analyses are based on a repeated measures ANOVA followed by Student-Newman-Keuls post-hoc test. P-values (0-3 years) are based on an analysis of the percent change from baseline, whereas other p-values are based on an analysis of the absolute values. [a] $p<0.001$ vs. younger patients; [b] $p<0.05$ vs. baseline; [c] $p<0.01$ vs. baseline; [d] $p<0.001$ vs. baseline

After using an analysis of covariance to correct for the longer duration of hypopituitarism, the more marked reduction in the elderly patients in terms of femur neck BMC at baseline and study end lost statistical significance ($p=0.18$ and $p=0.06$, respectively). However, the between-group differences at baseline and study end in terms of femur neck BMD and t-score remained significant ($p<0.001$).

After accounting for higher GH dose in younger GHD patients, the between-group response difference for femur neck BMC lost statistical significance ($p=0.22$).

Effects of previous pituitary irradiation therapy

Paper V compared patients who previously received pituitary irradiation therapy (IRR group) with patients who had not received pituitary irradiation (non-IRR group). All patients had adult onset NFPA as the cause of hypopituitarism, and complete deficiency of anterior pituitary hormones. The two groups were matched on the group level for age, gender, BMI, and waist:hip ratio, but the duration of hypopituitarism was longer in the IRR group.

At baseline, blood pressure, anthropometric data, body composition, and bone mass were similar in both groups. The mean daily dose of GH as well as serum IGF-I concentration did not differ significantly between groups. Systolic and diastolic blood pressure did not change in either group. BW tended to increase, and waist:hip ratio tended to decrease, without any between-group differences. As measured by DXA and assessed by the four-compartment model, both groups showed similar BF, LST and BCM throughout the study period. DXA-measured BMC and BMD in total body, lumbar (L2-L4) spine, and femur neck were similar in both study groups.

At baseline, IRR patients had lower serum HDL-C and higher serum TG and insulin (Table 2). Baseline levels of TC, LDL-C, fasting glucose, and HbA1c were similar in both study groups. Regarding serum TG level, response to 10-year GH replacement was more prominent in the IRR patients, but there was no significant within-group difference in any group. Although serum TC decreased significantly only in the non-IRR group, it did not differ between groups. In both groups, circulating LDL-C and HbA1c decreased in both groups and circulating HDL-C and fasting glucose increased without any between-group difference. At study end, serum HDL-C remained lower in the IRR group and other metabolic indices were similar in both groups.

After using an analysis of covariance to correct for the longer duration of hypopituitarism in IRR patients, increased serum insulin and reduced serum HDL-C at baseline in the IRR group remained significant (both $p<0.05$ vs. non-IRR group), and the difference in serum TG level lost statistical significance ($p=0.06$). At study end, the reduced concentration of serum HDL-C in IRR patients remained statistically significant after correction for duration of hypopituitarism ($p<0.05$).

During 10-year GH replacement, two IRR patients died due to fatal cardiac events, whereas no patient died in the non-IRR group. Two IRR patients had nonfatal myocardial infarctions and one IRR patient had a nonfatal cerebral infarction after 2 years. Only one nonfatal vascular event (a cerebral infarction) occurred among non-IRR patients.

Table 2. Circulating total cholesterol (TC), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), triglycerides (TG), fasting glucose, insulin, and HbA1c during 10-year GH replacement in 18 GHD adults that had previously received pituitary irradiation therapy (IRR group) and 18 non-irradiated GHD patients (non-IRR group). At baseline, the IRR patients had lower serum HDL-C level ($P < 0.05$) and higher serum TG and insulin level (both $P < 0.05$). At study end, serum HDL-C level was still lower in the IRR group ($P < 0.05$), whereas other metabolic indices were similar in both groups. Values are presented as the mean (SEM).

	Group	Baseline	1 year	5 years	10 years	P-value[§]
TC	**IRR**	5.9 (0.3)	5.6 (0.3)	5.2 (0.2)*	5.1 (0.2)**	0.86
(mmol/L)	**Non-IRR**	6.1 (0.2)	6.0 (0.1)	5.8 (0.1)	5.6 (0.2)	
LDL-C	**IRR**	4.1 (0.3)	3.7 (0.3)	3.4 (0.2)*	3.2 (0.2)***	0.98
(mmol/L)	**Non-IRR**	4.3 (0.2)	4.0 (0.1)	3.7 (0.2)*	3.4 (0.2)***	
HDL-C	**IRR**	1.0 (0.1)[#]	1.0 (0.0)	1.1 (0.1)	1.1 (0.0)*[, #]	0.91
(mmol/L)	**Non-IRR**	1.2 (0.1)	1.3 (0.1)	1.4 (0.1)*	1.4 (0.1)***	
TG	**IRR**	1.9 (0.2)[#]	1.9 (0.2)	1.6 (0.2)	1.7 (0.2)	<0.05
(mmol/L)	**Non-IRR**	1.4 (0.1)	1.4 (0.2)	1.7 (0.2)	1.6 (0.2)	
Glucose	**IRR**	4.2 (0.1)	4.4 (0.1)	4.6 (0.3)	4.7 (0.3)*	0.96
(mmol/L)	**Non-IRR**	4.3 (0.1)	4.5 (0.1)	4.7 (0.1)	4.7 (0.1)*	
Insulin	**IRR**	11.3 (1.4)[#]	13.5 (1.9)	9.9 (1.2)	11.9 (1.7)	0.08
(mU/L)	**Non-IRR**	7.4 (1.1)	10.3 (0.8)	9.3 (1.2)	8.1 (1.8)	
HbA1c	**IRR**	5.0 (0.2)	5.2 (0.2)	4.9 (0.2)	4.6 (0.2)**	0.18
(%)	**Non-IRR**	5.0 (0.1)	5.0 (0.1)	4.6 (0.1)**	4.5 (0.1)***	

* $p<0.05$; ** $p<0.01$; *** $p<0.001$ (vs. baseline); [#] $p<0.05$ (vs. non-IRR at baseline or at study end)
[§] Between-group p-value (0-10 years)

General discussion

This thesis focuses on long-term effects of GH replacement in hypopituitary patients with adult onset GHD. Since GH replacement in adults started in the early 1990s, the effects of GH replacement up to 5-10 years have been investigated in previous studies. Only a few studies have investigated GH replacement lasting up to 10 years. *Paper I* is the first published study on muscle strength during ten years of GH replacement. In most patients, GH replacement will continue over decades, adding to the importance of GH studies that comprise more than 10 years. To our knowledge, *Papers II* and *III* represent the first published studies on 15 years of GH replacement in adults. *Papers IV* and *V* aimed to study the effects of GH replacement in elderly patients and in patients previously treated with pituitary irradiation, respectively. These are two groups of patients where there is insufficient knowledge about the effects of GH replacement.

A limitation of the studies is that there was no untreated control group. For ethical reasons, the studies could not investigate a control group of GHD patients without GH replacement. In *Paper I,* we compared data on muscle strength with data from a reference population. In *Papers III* and *IV* BMD z-scores were used, which may to some extent compensate for the lack of control group. *Papers IV* and *V* compared baseline characteristics and treatment effects in two different groups of GHD patients. *Paper IV* compared elderly and younger GHD patients, and *Paper V* compared patients previously receiving pituitary irradiation with non-irradiated patients. An untreated control group would have been of value in all five *Papers*.

As in many long-term studies, there were changes of assays and equipment. At the end of 1999, the DXA machine used was changed. The new DXA, however, yielded similar estimates of body composition parameters as the old one. In terms of t-scores and z-scores, we used the LUNAR USA reference population as the reference database throughout the study period. Further, the assays for measurements of glucose and IGF-I were changed during the study period. Based on measurements in the local laboratory, we used a conversion factor of 1.11 to transfer blood glucose values to plasma glucose values. For IGF-I measurements, the WHO NIBSC 1st IRR 87/518 standard was used for calibration throughout the study period. However, changing the assays and DXA equipment could have influenced the results. Furthermore, the level of physical activity was not recorded. However, no effort was made to influence the patients' physical activity level during the study period.

Dose of GH

The GH dose in the patients included before October 1993 was based on BW. Therefore, the GH dose prescribed at the baseline visit in *Papers I-III* and *V* was relatively high, and then gradually individualized and reduced. Consequently, the IGF-I SD score was supraphysiological during the first years in *Papers I-III* and *V*. *Paper IV* included a higher proportion of patients who started GH replacement in later years when the GH dose was individualized from the start. In these patients, the initial GH dose at baseline was relatively low and then gradually increased. The IGF-I SD score was within the normal range (± 2 SD) throughout the study period in *Paper IV*. The initial high doses of GH in the early GH treatment trials caused side-effects, mainly related to fluid retention (12-14). Starting with a low, individualized dose has shown similar efficacy and fewer side effects (15, 16). Furthermore, the results of the *Papers* presented in this thesis show that supraphysiological IGF-I values can be avoided using individualized GH dose titration starting with a low initial GH dose.

Muscle strength

Paper I included measurements of muscle strength. Absolute values of both isometric and isokinetic knee flexor strength increased during the first half of the study. Studies with a duration of one year or less showed no effect of GH on isokinetic muscle strength (184, 185), whereas isometric strength increased in one study (184). In studies investigating 1-5 years of GH replacement, both isometric and isokinetic knee flexor strength increased (32, 51, 53, 54, 56, 186, 187) in line with the results reported in *Paper I*. After 10 years of GH replacement, only isometric knee flexor strength was still increased. The reason why GH replacement, in both *Paper I* and a previous study (188), had a more marked effect on isometric than on isokinetic knee flexor strength remains unknown, but it could be hypothesized that isometric strength might be more related to the intramuscular metabolic adaptations after increased muscle mass, i.e. augmented protein synthetic rate (184, 185).

During the second half of the study (years 5-10), several measures of absolute muscle strength decreased toward baseline values whereas the increase in age- and sex-adjusted muscle strength was sustained and in some variables was even progressive. This suggests that after the initial increase in absolute muscle strength, GH replacement partly protected against the normal age-related decrease in muscle strength. This finding concurs with the results of an earlier 10-year study of 10 GHD adults, in which GH attenuated the age-related decline in muscle strength measured as the mean of elbow flexion, and shoulder and hip abduction (187).

To investigate neuromuscular function, superimposed single twitch electrical stimulations were performed in *Paper I*. The level of activation of motor units at voluntary maximal muscle effort did not change during the first 7 years of therapy, but voluntary motor unit activation decreased after 10 years. This study did not include a control placebo group, but it has been suggested that the voluntary motor unit activation decreases with increasing age (189). Therefore, the present results may suggest that GH replacement cannot fully protect against age-related decline in motor neuron activation.

In an earlier 5-year trial GH replacement normalized knee flexor strength and almost normalized knee extensor strength, after correction for age and sex, whereas handgrip strength did not normalize (53). In *Paper I*, handgrip strength was almost normalized (88-93% of predicted values), suggesting that longer treatment periods are necessary to achieve normalization in distal muscle groups. GH replacement increases distal muscle mass to a lesser extent than proximal muscle mass; therefore a less marked and slower increase in handgrip strength than upper leg strength could be anticipated (190). Activity level correlates positively with both handgrip (171, 190) and upper leg muscle strength (191, 192). Therefore, it could be hypothesized that the use of handgrip muscles in modern daily activities is inferior to the use of leg muscles even in relatively active patients.

In agreement with previous trials of shorter duration (51-53, 186), GH replacement transiently impaired local muscle endurance (i.e., the fatigue index transiently increased), although it returned to baseline values after 10 years. GH replacement increased aerobic endurance (193, 194). Although not measured in the present study, a possible general increase in cardiorespiratory performance might explain the reversal of the initial decrease in local muscle endurance. However, local intramuscular changes over time cannot be excluded.

The initially relatively high GH dose was gradually reduced. Despite this reduction, the increase in age- and sex-corrected muscle strength was sustained and even progressive until approximately 7 years of therapy. This might suggest that a lower dose of GH than that given at study start could be beneficial for muscle performance possibly because it could reduce fluid-related side effects that hamper muscle function (e.g., as muscle and joint pain). On the other hand, reducing GH dosage over time might have contributed to the reduction in absolute values of muscle strength during the last years of the study.

We cannot exclude that a possible general increase in physical activity might have contributed to the increase and normalization of patients' muscle strength. Baseline measurements of muscle strength were performed approximately 6-8 months after the patients with pituitary disease had been completely treated. It is plausible that impairments resulting from initial treatment might affect lower leg muscle strength at baseline to some extent. A substantial placebo effect cannot be excluded either. There are, however, data from a placebo controlled randomized study showing increased muscle strength after 12 months in the GH treated group compared to the placebo group (195).

In conclusion, GHD adults receiving 10 years of GH replacement showed increased muscle strength during the first half of the study and partial protection against the normal age-related decline in muscle strength and neuromuscular function thereafter. GH therapy normalized knee flexor and extensor strength, and almost normalized hand-grip strength in both genders. Subanalyses demonstrated that patients in the younger half of the study population showed a greater treatment response in age- and sex-corrected values of isometric and isokinetic (180°/sec) knee extensor strength and right handgrip strength compared to those in the older half.

Body composition

Studies up to 10 years (*Papers I*, *IV* and *V*) showed no significant increase in BMI. In the two 15-year studies (*Papers II* and *III*), BMI increased as observed in several other long-term studies (65, 110, 111). This suggests that BMI increases during long-term GH replacement as in the general population during a 15-year period (196, 197).

There was a sustained increase in total body LST as measured using DXA, in shorter and longer studies (*Papers I*, *II*, *IV* and *V*). In contrast, ALST, used as an estimate of skeletal muscle mass (176), returned to the baseline level after 10 years of GH replacement (*Paper II*).

This concurs with the results on absolute values of muscle strength (*Paper I*), which increased initially and then decreased with age as in the background population. Previous studies showed a greater effect of GH on proximal compared to distal lean mass (190), which might concur with our results of increased total body LST but unchanged ALST after 15 years of GH replacement. However, DXA cannot determine whether the increase in proximal (mainly trunk) lean mass after 15 years represents increases in muscle tissue, visceral organs, and/or connective tissue.

During the first year of GH replacement, total BF decreased, both as measured by DXA (*Papers I, II, IV* and *V*) and as estimated using the four-compartment model (*Papers I* and *V*), concurring with the results of previous short-term studies (60, 62) and a recent meta-analysis (61). As observed in previous studies from our centre (42, 65), the decrease in total BF was more marked using the four-compartment model compared to DXA (*Papers I* and *V*). The four-compartment model could underestimate BF in elderly patients (178), which might partly explain the discrepancy in results between DXA and the four-compartment model in a long-term study. However, this discrepancy was also observed in a 1-year study (178), and the reason for this difference between the methods remains unknown (178). In *Paper IV*, total BF stayed below the baseline level throughout the 3-year study period. In the studies with longer follow-up time, total body fat as measured by DXA gradually increased and returned to the baseline level (Papers *I, II,* and *V*). This result concurs with age-related increase in BF observed in the background population (198). Although BF returned to the baseline level, BF expressed as a percentage of body weight was still below the baseline level after 15 years (*Paper II*), indicating that GH replacement exerted favourable effects on body fat between 10 and 15 years of therapy. Using the four-compartment model, total BF stayed below the baseline level up to 10 years (*Papers I* and *V*). Because the whole body counter is no longer in use we did not have data on TBK and could not use the four-compartment model in the 15-year study (*Paper II*).

TF, as measured by DXA, decreased during the first year and then increased and exceeded the baseline level after 15 years of GH replacement (*Paper II*). Because DXA does not differentiate between subcutaneous and visceral fat we could not determine whether the initial decrease in TF represents subcutaneous or visceral fat. Increased abdominal fat mass in relation to peripheral fat mass during increased duration of GH replacement might be explained by the redistribution of body fat seen with normal ageing (199-201).

Lipid profile

Improvements in serum lipid profile (i.e., increased serum HDL-C concentration and decreased serum levels of TC and LDL-C) were sustained during up to 10 and 15 years of GH replacement, respectively (*Papers II* and *V*). Because most of the changes in body composition had returned to baseline after 15 years of GH replacement, it is less likely that these improvements were only explained by the transient changes in body composition. Previous studies support direct effects of GH on lipid metabolism. GH increases the expression of LDL receptors in the liver (112) and enhances the catabolism of LDL (113). GH administration may yield a higher degree of LDL turnover than indicated by changes in serum

LDL-C concentrations (114) and increases very low density lipoprotein (VLDL)-apolipoprotein B (apoB) turnover (115). The level of TG was unchanged in both studies in line with the results of some previous studies (48, 64, 65).

Glucose metabolism and diabetes mellitus

As observed previously in studies of shorter duration (65), fasting plasma glucose concentration increased and blood HbA1c decreased (*Papers II* and *V*). Although the meaning of this finding is unclear, it could suggest that insulin sensitivity was approximately unchanged. As further support of unchanged insulin sensitivity, serum TG level, which inversely correlates with insulin sensitivity (114), was unchanged.

At baseline, four patients had DM type 2. GHD adults not receiving GH replacement are insulin resistant (18, 83), and they may be at increased risk of developing DM type 2. In two recent studies based on international surveillance databases, patients with obesity and disturbed metabolic profile at baseline had an increased risk of diabetes (104, 106). One study showed a higher overall risk of diabetes compared to the background population, particularly during the first year of GH replacement (106); the other study showed a diabetes incidence that was similar or slightly above that of the background population (104). In our study, most patients received a low dose of GH at initiation of therapy. Only two patients developed DM type 2 during the first 5 years of GH replacement, but another 9 patients developed DM type 2 during the remaining study period, suggesting that a proportion of GHD patients will develop DM type 2 with increasing duration of GH replacement and increasing age of the patients. In line with the results of the aforementioned studies (104, 106), the patients who developed DM type 2 were more obese and had more disturbed glucose metabolism at baseline. In a general population in Sweden aged 20-100 years, the incidence of DM type 2 was 378 cases/100,000 people/year (202). With this incidence, eight GHD patients would be expected to develop DM type 2, indicating an increased risk in our patients compared to the normal population. In a Swedish multi-centre study, the prevalence of diabetes increased in GHD women but not in GHD men receiving GH replacement, partly attributed to reduced physical activity and increased BMI in the GHD women (105). Determining whether increased risk of DM type 2 in adult GHD is due to impaired metabolic baseline status and sedentary life style or whether the risk is accelerated by GH replacement requires further studies.

Bone

Total body and lumbar (L2-L4) spine BMC, BMD, t-score, and z-score values increased and significantly exceeded baseline levels after 15 years of GH replacement (*Paper III*). The main increase occurred during the first 7-10 years. In the shorter studies, all variables reflecting bone mass and density in lumbar (L2-L4) spine increased throughout the study periods (*Papers IV-V*). Taken together, the results of previous studies suggest that GH replacement increases BMC and BMD during the first 5-10 years of therapy, and absolute values of BMC and BMD plateau after that time frame (40, 42, 46, 47, 49). No previous studies were conducted for longer time period than 10 years. In *Paper III*, no further gain in absolute values of total body and lumbar (L2-L4) spine BMC and BMD occurred between 10 and 15 years of GH replacement. However, lumbar (L2-L4) spine z-score was higher at 15 years compared to the 10-year value. In the femur neck, the response to 15 years of GH replacement differed from to the responses in total body and lumbar (L2-L4) spine. Femur neck BMC and BMD reached maximum levels after 7 years and then started to decrease. After 15 years, femur neck BMD and t-score had returned to the baseline value. In the two shorter studies, femur neck BMC, BMD, t-score and z-score exceeded the baseline level after 3 and 10 years, respectively, of GH replacement (*Papers IV-V*).

In *Paper III*, it is unclear why bone mass and density decreased in the femur neck but not in the lumbar (L2-L4) spine between years 10-15 of GH replacement. Femur neck is composed of more cortical bone, whereas lumbar spine is composed of more trabecular bone (203). It is well known that the trabecular bone in the lumbar spine is sensitive to sex steroids, which is noticed for instance in postmenopausal women (203). In the femur neck, with its predominantly cortical bone, BMD decreases with increasing age, resulting in senile osteoporosis that affects both elderly men and women (203). The sex steroid replacement used in this study may have contributed to increased bone density at lumbar (L2-L4) spine but had only a small effect on bone density in femur neck. GH dosage was gradually reduced during the study period, and we cannot exclude the possibility that the GH dose used at study end was not high enough to maintain the increase in femur neck bone mass and density. In some support of this assumption, the per cent change in serum IGF-I correlated positively with the per cent change in femur neck BMC after 15 years. This indicates that patients with the highest increase in serum IGF-I and likely the highest dose of GH had the greatest treatment response in terms of femur neck BMC. On the contrary, in *Paper IV*, in which a higher percentage of the patients received a low GH dose from the start, femur neck BMC and BMD still increased significantly. However, long-term treatment might require higher doses of GH to overcome age-related decrease in femur neck bone mass and density.

A main question is whether GH replacement reduces the risk of fractures. GHD patients not receiving GH replacement have an increased risk of fractures (35-37). Some evidence suggests that GH replacement can reduce the incidence of fractures (37, 204). Although an increased number of falls resulting from visual impairment caused by pituitary tumours or their treatment might be important, BMD t-score is an important predictor of fracture risk (205-207). Therefore, increased bone mass and density likely means that 15 years of GH replacement can reduce fracture risk in GHD patients (*Paper III*), and that 3 years of GH replacement possibly had a beneficial effect on the risk of fractures both in elderly and younger patients (*Paper IV*). *Paper III* reported two fractures – one hip fracture and one symptomatic vertebral fracture – and *Paper IV* reported none. We did not X-ray patients to determine asymptomatic vertebral fractures. Some estimates suggest that two thirds to three quarters of vertebral fractures are asymptomatic and, therefore, undiagnosed (208). Patients with vertebral fractures show a mean height loss of around 5 cm (209, 210). In *Paper III* no patient had a height loss of 5 cm or more and six patients (2 men) had a height loss of 3-4.5 cm. We cannot exclude the possibility that a few GHD patients in *Paper III* had asymptomatic vertebral fractures, and none of the studies included in this thesis was large enough to estimate fracture risk. Estimating fracture risk would require large, multi-centre studies because GHD is a relatively rare condition and most centres do not have enough patients to evaluate fracture risk.

Gender differences

In line with previous studies (48, 65, 110), women received a higher GH dose than men (*Papers II* and *III*). In *Paper I* there was no gender difference absolute GH dose, but women received a higher GH dose adjusted for body weight. IGF-I SD scores increased more in women than in men in *Papers II* and *III*, but in *Paper I* the increase in IGF-I SD score was greater in men. Men in *Paper I* had supraphysiological IGF-I SD scores during the first 3-5 years of GH replacement.

As in previous studies of shorter duration (51-53), women had lower age- and sex-corrected muscle strength than men at baseline (*Paper I*). However, there were no gender differences in the treatment responses in muscle function (*Paper I*). In terms of body composition, women responded to treatment less markedly than men in *Paper I*. *Paper II* showed no gender difference in treatment response in any variable reflecting body composition. In *Paper II*, women had a marginally more beneficial reduction of serum LDL-C level, but there were no gender differences in treatment response in any other variable reflecting lipid profile or glucose metabolism. In conclusion, our data suggest similar responsiveness to long-term GH replacement in variables reflecting body composition, lipid profile, and glucose metabolism when women receive a higher dose of GH than men.

At all skeletal sites, except for femur neck BMC, treatment response to GH replacement was greater in men than in women in terms of bone mass and density (*Paper III*). Several previous studies observed similar gender differences in responsiveness (40, 42, 43, 45, 46). The mechanisms underlying these gender differences are not fully understood, but sex hormones might play a role (46). In terms of oestrogens, there was no difference in treatment responses in BMD or BMC at any skeletal site measured between gonadotropin-deficient women receiving or not receiving oestrogen replacement at baseline. A smaller number of women in *Paper III* received oestrogen replacement at study end (n=15) than at baseline (n=25), which could have contributed to the lack of difference between women with and without oestrogen therapy. Further studies are required to clarify the importance of oestrogen replacement during long-term GH replacement. Androgens may interact with GH, resulting in increased bone mass (46). We cannot exclude the possibility that testosterone replacement in *Paper III* contributed to increased bone mass and density in GHD men. This aspect could not be evaluated in more detail because most men were gonadotropin-deficient and all gonadotropin-deficient men received testosterone replacement.

In conclusion, our data suggest a similar treatment response for body composition, lipid profile, and glucose metabolism in men and women when women receive a higher dose of GH. Variables reflecting bone mass and density showed a greater treatment response among men. Taken together, our data suggest that interactions between GH and sex steroids play a greater role in bone mass and density.

Gonadal status

Because all gonadotropin-deficient men received testosterone replacement therapy we could not compare testosterone-deficient and testosterone-sufficient men (*Papers I-III*). Around 60% of the women received oestrogen replacement therapy at baseline (*Papers I-III*), and more of the younger women received oestrogen than did the older women. At study end, fewer women were on oestrogen replacement because oestrogen was discontinued due to age (*Papers I-III*). The only observed difference between women on oestrogen vs. hypogonadal women without oestrogen was that women on oestrogen received a higher GH dose than women without oestrogen (*Papers I-III*). There was no difference in treatment response between the two groups in any variable reflecting bone mass and density, body composition, muscle strength, or metabolic indices (*Papers I-III*), possibly because many women discontinued their treatment and did not get the effect of oestrogen throughout the study period. In addition small groups limit the power of the analysis.

Elderly patients

There is insufficient knowledge about elderly patients' responsiveness to GH replacement, especially in terms of the effect of GH replacement on bone mass and density. *Paper IV* investigated the effects of GH in elderly patients compared to younger patients. In the majority of patients, the dose of GH was individualized from study start (*Paper IV*). In agreement with previous observations (121, 211), individualized GH replacement resulted in a lower dose of GH in elderly compared to younger GHD patients (121, 211). Mean IGF-I SD score was within the normal physiological range (± 2 SD) throughout the 3 years of GH replacement in both groups. Although the younger patients tended to have more marked increases in serum IGF-I concentration and IGF-I SD score than the elderly patients, there were no statistical differences between groups, suggesting that elderly GHD patients are sensitive to GH and that a relatively low dose of GH can produce a significant increase in serum IGF-I concentration in this group of patients (*Paper IV*).

The 3-year GH replacement regimen improved body composition in both study groups (*Paper IV*), including sustained reductions in waist circumference, waist:hip ratio, and total body fat without any between-group difference. Lean soft tissue increased throughout the study period in both groups. Our results concurred with previous studies, which demonstrated that GH replacement has approximately similar efficacy regarding improved body composition in younger and elderly GHD patients (52, 121, 122).

There was no between-group difference in total body and lumbar (L2-L4) spine BMC at baseline or in the response to 3-year GH replacement. Elderly patients had lower femur neck BMC than the younger control GHD patients at baseline, and younger patients showed a more marked increase in femur neck BMC in response to treatment. After using an analysis of covariance to correct for the longer duration of hypopituitarism in elderly patients, femur neck BMC no longer differed between groups. The more marked increase in femur neck BMC in younger patients lost statistical significance when correcting for the higher dose of GH in that group. Taken together, these findings indicate that BMC is approximately similar in elderly and younger GHD patients and that there is no major difference in responsiveness to GH replacement therapy (*Paper IV*).

There was no significant difference between groups regarding total body BMD, t-score, or z-score at baseline. The absolute levels of femur neck BMD and t-score were lower in the elderly patients. However, there was no difference between groups in femur neck z-score (BMD corrected for gender and age), suggesting that lower femur neck BMD in elderly patients resulted from normal age-related decline in BMD. In the lumbar (L2-L4) spine, there was no between-group difference in BMD or t-score at baseline. However, lumbar (L2-L4) spine z-score was higher in elderly compared to younger GHD patients. These results confirm that BMD, after correcting for normal age-related decline, is higher in elderly than in younger GHD patients (52, 121, 122). For BMD at all skeletal sites measured, responsiveness to 3-year GH replacement was similar in both study groups. In both groups, total body BMD was unchanged after 3 years, whereas lumbar (L2-L4) spine and femur neck BMD increased, demonstrating that GH replacement improves lumbar (L2-L4) spine and femur neck BMD in younger as well as elderly GHD patients. Whether the mechanism is direct effects of GH on bone or indirect effects such as increased physical activity, possibly related to improved QoL, cannot be determined in this study.

GH replacement is motivated in elderly patients with impaired quality of life, body composition and serum lipid pattern (6). Several studies have shown that GH replacement is similarly efficient in elderly and younger GHD adults in terms of improvements in these variables (52, 120-122). The results of *Paper IV* also showed approximately similar efficacy of GH replacement regarding increased bone mass and density. Because elderly GHD patients do not have reduced BMD compared to age-matched healthy subjects, this will not be an indication for GH therapy in most elderly GHD patients. However, BMD increases in elderly GHD patients receiving GH replacement for other reasons, further supporting the notion that GH replacement is useful in elderly patients.

Effects of previous pituitary irradiation therapy

Paper V evaluated the effects of previous pituitary irradiation therapy on baseline characteristics and responsiveness to 10 years of GH replacement. In this single-centre, open-label, prospective study, IRR patients displayed a more severely impaired cardiovascular risk profile at baseline, with increased serum levels of insulin and TG and reduced serum HDL-C concentration, compared to non-IRR patients. Ten-year GH replacement improved body composition, bone mass, and serum lipid profile in both groups and partly eliminated the baseline differences although serum HDL-C level was still reduced in the IRR patients at study end. Vascular events and DM type 2 were more common in the IRR patients during GH replacement.

Patients were included in 1990-1996 and most IRR patients had received the pituitary irradiation therapy in the late 1980s, when the use of irradiation therapy as a standard therapy was gradually abandoned. However, this study was not randomized and we cannot fully exclude the possibility that IRR patients had more aggressive pituitary tumours than non-IRR patients. Because irradiation therapy gradually diminishes the secretion of anterior pituitary hormones (127, 128), we included only patients with complete anterior pituitary hormonal deficiency with the purpose of having comparable study groups.

GH dose, IGF-I, or IGF-I SD scores did not differ significantly between groups. There was no significant difference at baseline in any variable reflecting body composition or bone mass, likely because both groups were matched for BMI and waist:hip ratio. Ten-year GH replacement improved body composition and increased BMC and BMD levels at all skeletal sites measured, without any between-group difference.

Baseline serum levels of insulin and TG were increased and HDL-C was decreased in the IRR patients compared to the non-IRR patients. These data suggests that previous conventional pituitary irradiation is associated with insulin resistance because serum TG level generally correlates inversely with insulin sensitivity (212). One patient in the IRR group had treated DM type 2 at study start and one patient developed DM type 2 during GH replacement; the non-IRR group had no patients with DM type 2. Two IRR patients had treated hypertension at baseline. Reduced insulin sensitivity in IRR patients might be associated with increased risk of DM type 2 and hypertension although this needs to be confirmed in larger studies than the present one.

Response to GH replacement was similar between groups in terms of circulating concentrations of TC, HDL-C and LDL-C, and levels of fasting glucose, insulin, and HbA1c. There was a significant between-group difference in serum TG response to GH replacement but there was no within-group change in serum TG concentration in any of the study groups. Although serum lipid profile improved in both study groups, serum HDL-C levels were still lower in the IRR group at study end. This suggests that GH replacement only partly reverses baseline differences between IRR and non-IRR patients.

There were five vascular (four cardiac) events in the IRR group (i.e., approximately one vascular event per 35 patient years) during the 10-year GH replacement. An earlier study performed at our centre, which included 289 GHD adults with previous non-functioning hypopituitary disease, reported nine vascular events (two myocardial infarctions and seven cerebrovascular events) during the 1443 patient-years (i.e., approximately one vascular event per 160 patient years) (139). The present study is too small to statistically evaluate vascular morbidity and mortality, but suggests that IRR patients with complete deficiency of anterior pituitary hormones have an increased rate of vascular events during GH replacement compared to other groups of GHD patients. Radiation-induced angiopathy is a risk factor for stroke (135, 136), and some studies suggested that previous radiotherapy predicts increased cerebrovascular mortality in hypopituitary patients not receiving GH therapy (137, 138, 140). Our results suggest that pituitary irradiation may also be a risk factor for increased rate of cardiac events, possibly at least to some extent due to the more severely impaired cardiovascular risk factors observed at baseline in IRR patients.

Previous radiotherapy is associated with reduced QoL (131-133). We did not measure QoL or physical activity, but we cannot exclude the possibility that more severely impaired QoL and reduced physical activity contributed to the more severely disturbed cardiovascular risk profile in the IRR group. However, no attempt was made to influence patients' physical activity level during the study period. Furthermore, the baseline difference in serum TG level lost, marginally, statistical significance when the longer duration of hypopituitarism in the IRR patients was accounted for using an analysis of covariance. This might suggest that the impaired cardiovascular risk status in the IRR patients was to some extent caused by the longer duration of disease. The extent to which early GH replacement can reduce vascular events in IRR patients more efficiently requires further study.

In conclusion, *Paper V* shows that IRR patients with adult onset GHD have a more impaired cardiovascular risk profile than non-IRR control GHD patients. Ten-year GH replacement improved body composition, bone mass, and serum lipid profile in both groups and partly eliminated baseline differences. Vascular events and DM type 2 occurred more commonly in IRR patients during the GH replacement, requiring confirmation in larger studies. Future studies are also needed to explore whether early GH replacement more efficiently reduces vascular events in IRR patients than the GH replacement given in the *Paper V*.

Safety aspects

In *Paper II*, which was the largest study included in this thesis, 21 patients died during the 15-year study period (i.e., 1 death/94 patient years). Another study from our centre reported 1 death/180 patient years (139). One study based on the global KIMS database and one Dutch national study observed a mortality rate of 1 death/130-140 patient years, which was slightly higher than that expected in the background population (155, 156). Thus, the mortality rate was higher in the present study, but our patients were older at baseline and the mean follow-up time in the other studies was shorter, 4-6 years (139, 155, 156), compared to that in *Paper II*. However, none of the studies presented in this thesis is large enough to evaluate mortality. Determining the extent to which GH replacement can reduce the increased overall and cardiovascular mortality seen in untreated GHD requires larger studies.

Conclusions

Since recombinant human GH became available 25 years ago, numerous studies have shown that GH in adults has effects on body composition, bone mass and density, muscle strength, and glucose and lipid metabolism. The studies included in this thesis evaluated the effects of GH over periods up to 15 years and increased the knowledge of GH replacement in two subgroups of patients (i.e., elderly patients and patients previously treated with pituitary irradiation). The main conclusions can be summarized as follows:

- Ten years of GH replacement increased muscle strength during the first half of the study. Thereafter, GH replacement partly protected against the normal age-related decline in muscle strength and neuromuscular function, resulting in approximately normalized muscle strength after 10 years (*Paper I*).

- There was a sustained increase in lean soft tissue during 15 years of GH replacement. Other changes in body composition were transient, probably due to normal ageing (*Paper II*).

- The lipid profile improved (*Papers II and V*). Considering the relatively small changes in body composition at study end, this indicates direct effects of GH on lipid metabolism.

- The effects of GH on glucose metabolism were conflicting, but likely glucose homeostasis was approximately unchanged (*Paper II* and *V*). However, GH likely increases the risk of DM type 2 in obese GHD adults with impaired glucose homeostasis at baseline (*Paper II*).

- Fifteen years of GH replacement induced a sustained increase in total body and lumbar (L2-L4) spine BMD and BMC. Femur neck BMC and BMD peaked after 7 years of treatment and then decreased toward baseline values, possibly due to normal ageing or relatively low level of mean GH dose during the last years of the study (*Paper III*).

- Three years of GH replacement increased lumbar (L2-L4) spine and femur neck BMC and BMD in elderly GHD patients to the same extent as in younger patients. This gives further support for the notion that GH replacement is useful in elderly GHD patients (*Paper IV*).

- Patients previously treated with pituitary irradiation displayed a more severely impaired cardiovascular risk profile compared with non-irradiated patients. This could be of importance for the more marked cardiovascular morbidity observed in this group. GH replacement only partly reversed these metabolic aberrations (*Paper V*).

- Although women received a higher GH dose (*Papers II* and *III*) or a higher GH dose adjusted for body weight (*Paper I*), women had a less favourable response in bone mass and density (*Paper III*). Muscle strength (*Paper I*), body composition (*Paper II*), glucose metabolism (*Paper II*) and lipid profile (*Paper II*) measurements showed no gender differences. Taken together, our data suggest that interactions between GH and sex steroids are more important for bone than for the other variables measured.

Future perspectives

- Because GH replacement may continue over decades, further larger long-term studies (20-25 years) of GH replacement are needed and preferably should include an age-matched healthy control group to differentiate between treatment effects and effects of time and ageing.

- It remains to be determined whether increased BMC and BMD in response to GH replacement will reduce the risk of fractures in the total group of GHD adults as well as in subgroups of patients. This will require large, probably multi-centre studies, since GHD is a rare condition and individual centres will not have enough patients to evaluate fracture risk.

- The effects of pituitary irradiation on cardiovascular risk factors require further study. Our centre no longer uses irradiation as a standard treatment after pituitary surgery, but still uses irradiation for tumour recurrence or regrowth when a reoperation is not possible.

- Men had a greater treatment response in bone variables, but not in body composition or cardiovascular risk factors. Further studies that look in more detail into the effect of sex hormones are needed to clarify the mechanisms behind these gender differences.

- Future, larger studies are needed to investigate whether GH replacement affects the increased mortality seen in hypopituitary adults not receiving GH.

- Safety aspects during long-term GH replacement, especially the risk of diabetes mellitus type 2 and cancer incidence, need to be further investigated in large-scale studies.

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