CLINICAL ASPECTS OF GROWTH HORMONE THERAPY IN ADULTS

KLINISCHE ASPECTEN VAN GROEIHORMOON BEHANDELING BIJ VOLWASSENEN

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ABBREVIATIONS

Apase	alkaline phosphatase
APD	aminohydroxypropylidene
apo	apolipoprotein
AUCG	area under the curve glucose
AUCI	area under the curve insulin
BIA	bioelectrical impedance analysis
BMC	bone mineral content
BMD	bone mineral density
BMI	body mass index
BW	body weight
CRF	corticotrophin releasing factor
CT	computerized tomography
CV	coefficient of variation
DPA	dual-photon absorptiometry
DXA	dual-energy x-ray absorptiometry
FFA	free fatty acids
FFM	fat free mass
FM	fat mass
GH	growth hormone
GHBP	growth hormone binding protein
GHD	growth hormone deficiency
GHDA	growth hormone deficiency in the adult
GHRH	growth hormone releasing hormone
Ha	hydroxyapatite
HbA_1C	glycosylated hemoglobin
HDL	high-density lipoprotein
IGF-I	insulin-like growth factor I
IGFBP	insulin-like growth factor binding protein
IRMA	immuno radiometric assay
IU	international unit

kD	kilodalton
LBM	lean body mass
LDL	low-density lipoprotein
Lp(a)	lipoprotein (a)
MRI	magnetic resonance imaging
NHP	nottingham health profile
OGTT	oral glucose tolerance test
OH-proline	hydroxyproline
Pi	inorganic phosphate
PICP	carboxyterminal propeptide of type I collagen
PTH	parathyroid hormone
R	resistance
rhGH	recombinant derived human growth hormone
RIA	radio immunoassay
rmANOVA	repeated measures analysis of variance
SPA	single photon absorptiometry
TBW	total body water
TmP/GFR	renal tubular reabsorption maximum of phosphate corrected for glomerular filtration rate
UWW	under water weighing
YSM	years since menopause

In this thesis traditional units as well as SI units have been used.

For cholesterol measurements: to convert from traditional (mg/dL) to SI (mmol/L) multiply by: 0.02586

For IGF-I measurements: to convert from ng/mL to mmol/L multiply by: 0.1307

1.1 Introduction

At the beginning of this century, it was demonstrated that growth is regulated by a pituitary factor, currently called growth hormone (1). In 1921 for the first time growth promoting effects of crude anterior pituitary extracts were demonstrated in hypophysectomized and intact laboratory animals (2). Initially, growth hormone (GH) was prepared from animal sources, but it became evident that non primate GH was inactive in man (3). Early reports on the effects of human growth hormone administration date from the 1950s (3-10). It must be emphasized that the supply of human pituitary GH for clinical use was dependent on the availability of post-mortem pituitaries, the collection of which required cooperation of a large number of pathologists. Thus, save for short-term metabolic studies, between 1960 and 1985 the major use of pituitary GH was for the treatment of GH deficient (GHD) children. In 1964 the results were published of long-term human growth hormone administration for up to 2.5 years in 35 patients with short stature (11). The introduction of a radioimmunoassay (RIA) for the measurement of plasma growth hormone concentrations enabled quantitative assessment of GH secretion and restriction of GH therapy to (GHD) patients (12). Since the growth response was more pronounced in hypopituitary patients and supplies were scarce, it was decided that human GH therapy should be restricted to children with "classic" GHD. Between the late 1950s and 1985 thousands of children were treated with GH extracted from human pituitary glands.

In 1979 biosynthetic or recombinant human growth hormone (rhGH) was manufactured (13). In 1982 it was shown to be effective in man (14). Introduction of biosynthetic GH preceded the reports of several GHD patients who had contracted Creutzfeld-Jakob disease (15). Since it was suspected that the patients had been infected by human pituitary-derived GH the clinical use of pituitary GH was suspended (16-24). After 1985 there have been 51 cases of Creutzfeld-Jakob disease in patients previously treated with pituitary-derived human GH (25,26). As the incubation period of this disease can be as long as 35 years, new cases can be expected until the year 2020 (26).

Early short-term studies had been designed not only to document the efficacy of growth promotion, but also to assess the metabolic effects of GH therapy (8,10). Reports on the

physiological consequences of GHD in adults appeared since the 1960s (27). Systematic investigation of the characteristics of GHD in adults, however, began in the late 1980s with the introduction of rhGH and led to the description of the growth hormone deficiency syndrome (28).

The wide availability of rhGH has greatly expanded the therapeutic spectrum for its use. Also it stimulated the study of GH physiology and its actions.

1.2 The physiology of growth hormone secretion, modes of action and the assessment of growth hormone deficiency

Growth hormone (GH) is a single chain polypeptide of 191 amino acids, which is produced by the somatotropic cells of the anterior pituitary gland. Most of the naturally occurring GH is present in the 22 kD form (90%). About 10% is present in the 20 kD form. In addition to its role in promoting linear growth in children (29), GH is an important anabolic hormone with stimulatory effects on protein synthesis and on lipolysis (10,30-33).

Pituitary GH secretion is primarily regulated by the interaction of two hypothalamic peptides, GH-releasing hormone (GHRH) and somatostatin (somatotropin-release inhibitory factor), which are secreted into sinusoidal capillaries of the median eminence and reach the anterior pituitary gland via its portal veins. Naturally occurring hypothalamic GHRH binds to specific cell surface receptors on pituitary somatotropes and stimulates GH synthesis and release (34). The main physiologic action of somatostatin is to inhibit the synthesis and release of GH (35). GH is secreted in a pulsatile pattern (36). Human studies in which GH has been measured at frequent intervals have shown that most GH secretion occurs at night. Spontaneous GH release is primarily under tonic inhibitory control by somatostatin and increases when hypothalamic somatostatin secretion decreases (36,37). GH stimulates the liver and other peripheral tissues to produce insulin-like growth factor-I (IGF-I) (38-40), which in turn exerts a negative feedback in the hypothalamus and pituitary to suppress further GH release (41). Most metabolic effects of GH are mediated via IGF-I.

GH secretion increases transiently after exercise (42). In addition age, obesity, depression, and hyperglycemia reduce (stimulated) secretion of GH (43-47). However, depressed patients have elevated GH levels during daytime, but not during sleep (46). Basal GH levels in type I diabetes mellitus are increased in poorly controlled patients (47).

Human GH circulates in the blood partially bound to GH-binding protein (GHBP), which is synthesized in the liver (48,49). GH binds to transmembrane glycoprotein receptors of which the extramembraneous portion is identical to GHBP (50,51).

GH action

Direct effects of GH comprise the stimulation of longitudinal growth through direct effects on chondrocytes and osteoblasts (29,52,53), effects on carbohydrate and fat metabolism (54). GH decreases body fat by increasing the hydrolysis of triglycerides, releasing free fatty acids (FFA) and glycerol while decreasing FFA re-esterification (55,56). Also GH promotes a positive nitrogen balance (32,33,57-63). The mechanisms by which GH induces sodium retention are complex. Evidence exists for both a direct renal effect of GH and an indirect effect mediated by the renin-angiotensin system (64).

IGF-I

Indirect effects of GH are mediated mainly via IGF-I, most of which is of hepatic origin (38-40). Local tissue production of IGF-I, however, forms a large contribution to circulating IGF-I levels (65). Therefore IGF-I may act as a paracrine/autocrine factor, as well as as a hormone. IGF-I activates a specific cell-surface receptor which is structurally is very similar to the insulin receptor.

GH stimulates bone growth directly by stimulating the differentiation of epiphyseal growth plate precursor cells and indirectly by increasing the responsiveness to IGF-I and enhancing the local production of IGF-I (29,52). Thus, serum IGF-I levels do not completely account for, and may not be an accurate index of IGF-I tissue effects.

Circulating IGF-I levels are low in patients with GH-deficiency and increase during GH replacement therapy, reflecting the decrease or absence of spontaneous GH secretion (66,67).

IGF-binding proteins (IGFBPs)

IGF-I is present in blood and tissue both free and bound to at least six IGF-binding proteins (IGFBPs) of which IGFBP-3 (also previously known as GH-dependent BP) is the predominant circulating binding protein in postnatal life and accounts for nearly 95% of the total IGFBP activity (68-70). The physiological role of the IGFBPs is uncertain They may act as a reservoir for IGF-I, since they bind with 70-95% of the circulating IGF-I in plasma (68,71,72). IGFBP-3 levels are correlated with GH secretion; in GHD there are decreased levels, which normalize during GH replacement therapy (68,69,73). In acromegaly there are increased levels of IGFBP-3, which decrease after treatment with somatostatin (68).

Although IGFBP-1 levels have been reported to be elevated in GHD and depressed in acromegaly it appears that IGFBP-1 production is GH-independent and is more related to plasma insulin levels (74,75). IGFBP-1 may act as an inhibitor of IGF-I action.

Assessment of GH deficiency

So far little attention has been paid to diagnostic criteria of GHD in adults (GHDA). In children before the advent of radioimmunoassays, prerequisites for the diagnosis were retarded longitudinal growth and evidence of pituitary disease involving one or more additional pituitary hormones. After the introduction of the RIA method it was recommended that the diagnosis of GHD should be based on an absent or subnormal GH peak after stimulation.

In adults GHD has been defined to be present when maximum peak levels of GH after provocative testing do not exceed 0.5 to 5 μg/L depending on the cut-off values that have been (arbitrarily) chosen (66,67,76). When comparing stimulated GH release, spontaneous GH secretion, IGF-I, and IGFBP-3 in adults with suspected GHD, insulin-induced hypoglycemia is the only test which separates patients with organic hypothalamic/pituitary disease from their matched normal controls (77). However, this test is potentially dangerous and uncomfortable, which limits its use to experienced endocrine units under adequate supervision (78,79).

The acute GH response to an arginine infusion has been found comparable in young and old men and similar degree of GH stimulation was reported as that achieved by an

insulin induced hypoglycemia (80-82). Arginine induced GH release is mainly induced by a decrease in somatostatinergic tone, while GH responses to hypoglycemia are probably mediated by both an increase in hypothalamic GHRH and an inhibition of somatostatin release (83).

The use of GHRH to stimulate GH secretion is accurate, more easy to perform and has less side-effects (84-87).

Since IGF-I is GH-dependent it might also be used for screening patients with suspected GHD (88,89). It is important to realize, that IGF-I is also dependent on the nutritional status of the patient (90,91).

Also IGFBP-3 levels are regulated by GH. In children it was found a good (73) as well as a poor (92) indicator of the GH secretory status. In conclusion GHD is considered to be present, when there is a history of pituitary disease, in combination with a lowered GH response to a provocation test. Solely for screening purposes the assessment of IGF-I or IGFBP levels might be performed, but there seems to exist a considerable overlap between GHD individuals and their age- and sex-matched controls (77).

1.3 Nitrogen metabolism

Following infection, injury or surgical trauma, an obligatory loss of protein emerges. This is caused by the mobilization of amino acids from skeletal muscle, the major site of body protein. Whole body nitrogen balance becomes negative, as the amino acids are transported to the visceral organs, where they serve as precursors for acute phase proteins and glucose. The nitrogen released from the amino acids forms urea, which is excreted in the urine. The amount of nitrogen loss generally is proportional to the severity of the illness (Table 1). The protein losses are minimal and well tolerated, if the patient is healthy, body composition is normal, and the clinical course is uncomplicated. Prolonged protein losses lead to protein malnutrition. These patients will often develop delayed wound healing, are more sensitive to infections, and eventually their recovery may be jeopardized. Administration of adequate or even supraphysiologic quantities of protein and calories decrease, but do not abolish the negative nitrogen balance and muscle breakdown (94). Studies of protein turnover demonstrate that feeding of the critically ill does not alter the accelerated breakdown, but it increases protein synthesis resulting in a reduction of net protein loss (95). Since there is a strong association between a poor nutritional status and a poor outcome in the surgical patient, it is important to maximize efforts to improve nutritional efficiency (96).

Table 1. Estimates of cumulative nitrogen loss following catabolic illness (first ten days, ad libitum feeding)

Precipitating factor	Cumulative nitrogen loss (g)
Major burn	170
Multiple injury	150
Peritonitis	136
Single fracture	115
Major operation	50
Minor operation	24
Pneumonia	59

Adapted from ref. 93

Growth hormone has long been known for its anabolic effects (6). These include a positive nitrogen, phosphorus, potassium and magnesium balance. The ratio of potassium/proteinsparing is 3:1, which is comparable to that found in muscle (58). A reduction in blood urea concentration is an easily detectable biochemical change associated with the anabolic actions of GH (97). Since GH also liberates metabolic fuels by lipolysis it is the drug of choice as anabolic agent (50). The role of GH in nitrogen metabolism will be further discussed in chapter 1.6.

1.4. Body composition assessment

Before discussing body composition in GHD adults some general principles and methods of body composition assessment will be reviewed more extensively with special emphasis on the ones used in the studies that are presented in this thesis.

Indirect methods of body composition assessment are based on models in which the body is divided in several (two or four) distinct compartments or components. These methods involve measurements of a physical property of such a compartment, from which body composition subsequently is calculated. Most methods use the two compartment model in which the body is divided in fat mass (FM) and fat free mass (FFM) or lean body mass (LBM). The terms LBM and FFM are not equivalent, representing the nonadipose tissue mass and the nonlipid mass, respectively. Adipose tissue contains water and protein. However, LBM and FFM become functionally equivalent when estimated indirectly from total body water (TBW) measurements, because the procedure assumes that adipose tissue is anhydrous. This results in an overestimation of LBM (by approximately two percent), which differs from FFM by protein alone (98,99). Assessment of LBM is important because it consists for fifty percent of fat free muscle (100).

The components of the body have their specific physical properties. The FM is anhydrous, potassium free and has a density of 0.9 g/ml at 37 °C, while the FFM is considered to have a water content of 73% (101), a potassium content of approximately 66 mmol/kg and a relative constant density of 1.1 g/ml at 37 °C. The calculation of the compartments can be accomplished by using either a statistically derived equation (e.g. bioelectrical impedance analysis (BIA) for calculating TBW) or a model equation (e.g. calculating an unknown component from a property derived component, FFM = TBW/ 0.73).

Methods that use the two compartment model are assessment of TBW, total body potassium, urinary creatinine excretion, under water weighing (UWW) and anthropometry.

In the four compartment model the body is divided in water, protein, bone mineral and fat. Over the past several years, important refined or new techniques of body composition assessment have been introduced, which opened the possibility of estimating almost all the components of body composition. These include computerized tomography (CT), in vivo neutron activation analysis, dual-photon absorptiometry (DPA), dual energy X-ray absorptiometry (DXA) and magnetic resonance imaging (MRI).

Anthropometry

Anthropometric measurements of the human body are body height, weight, skinfold thickness, arm circumference, waist-hip ratio. Through rather empirically-derived equations body density, body fat and fat free mass can be calculated from a combination of these measurements. The major advantages of anthropometry over other methods of estimating body composition are that it is inexpensive and easy to perform.

Generalized skinfold equations that are mostly used were developed by Durnin and Womersley (102). Skinfolds are measured with calipers according standardized guidelines. The variables age, sex and sum of four skinfolds are used in regression equations that estimate body density (d). From this, FM can be calculated using the Siri equation (103).

$$FM = BW * (4.95/ d - 4.50)$$

Body mass index (BMI) or Quetelet's index (body weight/(body height)^2) is the most commonly used index of relative weight. However, because lean body mass generally decreases in advanced age, while fat mass increases, body mass index cannot be used as a measure of body fat mass in older age groups. When corrected for age and sex, BMI and skinfold measurements are strongly correlated (104) and can be used for the estimation of body fat in cross-sectional studies (105).

Bioelectrical impedance analysis

The principle of BIA is that lean tissues, which mainly consist of electrolyte containing water, readily conduct an applied current, whereas fat conducts little current. This current of 800 μA at a signal frequency of 50 kHz is generated by a bioelectrical impedance analyzer and is applied to the patient through electrodes that are placed on the wrist and the ankle at one side of the body. After correct positioning of the patient and the electrodes, the impedance (or resistance, R) of the body is controlled by the low impedance lean compartment and is a function of the specific resistance of lean tissue, the cross-sectional area and the length of the lean compartment. Since water is the major component of the lean body compartment, TBW and FFM can be calculated from the measured impedance in good accordance with other methods of body composition assessment (106-110). However, the validation of the BIA method under different circumstances (hydration status), in different patient groups (normal, obese, growth hormone deficient, elderly), using different body impedance analyzers, differences in electrode placement and using different prediction formulas, remains a matter of debate (111-116). Also the validity of the basic equation is discussed in that it cannot detect *changes* in body composition unless they are considerable (117). The bioelectrical impedance method predicts body composition with an error that is comparable with the error using anthropometric techniques, and thus seems to have little or no advantage over simpler and less expensive techniques (114).

Isotope dilution

TBW can be determined by isotope dilution using isotopes of water like deuterium oxide (D_2O) (118,119). The TBW tracer dilution method relies on administering an oral dose of labeled water and measuring its dilution in body water after equilibration. After equilibration urine samples are taken for measurement of dilution. Food intake shortly before and during equilibration must be avoided or corrected for, since this will expand the TBW pool. In the two-compartment model errors in the TBW measurement will be magnified when calculating fat mass (FM = BW - TBW/ 0.73). Another difficulty associated with this method is that the hydrogen dilution space is greater than the water

space because of the exchange with labile hydrogen of protein and other body constituents (98).

Dual energy x-ray absorptiometry (DXA)

DXA is a non-invasive method of measuring bone and soft tissue body composition. DXA directly measures fat mass, lean tissue mass and total body bone mineral content. This equipment uses an X-ray source which produces a dual-energy beam with energies of 38 and 70 keV by K-edge filtering using cerium. The DXA system performs rectilinear scans over the entire length of the patient's body, beginning at the top of the head and moving downwards towards the feet. A total body scan takes approximately 15-20 minutes and the subject receives less than 0.5 μSv radiation exposure. The analysis software calculates percent body fat based on the ratio of soft tissue attenuation of the two x-ray energies. Also lean tissue mass and bone mineral content are measured (120-124).

1.5 Incidence, causes and features of the growth hormone deficiency syndrome in adults

Most cases of hypopituitarism are acquired in adult life (Table 2). Most cases of adult GHD (GHDA) result from pituitary or peripituitary tumors or their treatment (126). The greater the number of anterior pituitary hormone deficiencies, the more severe GHD turns out to be (in terms of peak GH after GH-stimulation testing) (127). The incidence of GHDA is not known, but indirect estimates based on the number of patients with pituitary tumors suggest that GHDA may affect ten people per million annually (128). Apart from this, also childhood-onset GHDA is recognized. These children, who did demonstrate an insufficient growth, have been treated in most cases with hGH in order to reach their final height.

Table 2. Etiology of hypopituitarism in the adult

Congenital
Isolated
Multiple
Acquired
Pituitary and peripituitary tumors
Pituitary apoplexy
Surgery/ trauma
X-ray therapy
Infiltrations/ infections
Idiopathic

Adapted from ref. 125

GHDA has become recognized as a specific clinical syndrome (28). It is associated with changes in body composition (**1.5.1.**), impaired physical performance (**1.5.2.**), reduction in bone mineral content (**1.5.3.**), disorders in lipid metabolism, impaired cardiac function with increased cardiovascular morbidity and mortality (**1.5.4.**), and decreased psychological well-being (**1.5.5.**).

1.5.1 Body composition

GH-deficient children are relatively obese and a decrease in adipose tissue is one of the earliest noticeable changes after starting GH-replacement therapy (129-131). There are only a few studies in which body composition parameters of GHDA are more or less compared with controls (66,67,132-134). A control population should consist of patients with multiple anterior pituitary hormone deficits, without GHD, caused by a hypothalamic or pituitary disorder and its sequelae, such as operation and radiotherapy. However such a control population probably does not exist, since GH is the first and most commonly involved anterior pituitary hormone to become deficient in pituitary disease (127). The controls in the above mentioned studies, are therefore all healthy controls.

Fat mass

Body fat is increased and distributed more in a central than a peripheral pattern (67,133,134).

Lean body mass

There is a decrease in lean body mass (LBM) of approximately 4 kg as measured with whole body ^{40}K counting (67). Predicted values were based on age-, sex-, height-, and weight matched controls.

Skeletal muscle mass

Skeletal muscle normally comprises approximately 50% of LBM. Compared to controls GHD adults have a decreased muscle to fat ratio in the mid-thigh as assessed by computerized tomography (CT) (66). Also with anthropometric methods a decrease in muscle mass can be demonstrated (133).

1.5.2. Physical performance

Patients with GHD have lowered muscle size and strength, compared to normal controls (67,135). When GH replacement therapy is stopped in children, when adult height has been achieved, muscle size and strength decrease within several months (136). Initiation of GH replacement therapy in GHD adults causes an increase in lean body mass, 50% of which consists of muscle, while also exercise performance improves (137,138).

When studied in a metabolic ward situation an increase in whole body protein turnover, protein synthesis and breakdown were found resulting in a positive nitrogen balance (32,61). After a substantial increase of total body protein mass a new steady state will be reached.

1.5.3. Bone turnover and bone mineral mass

Prevalence of osteopenia in subjects with GHD of both childhood (139,140) and adult onset (141,142) is high. Susceptibility for fractures is increased, which is compatible with the inverse relationship between bone mineral density (BMD) and fracture risk (143-145). The mechanism of the reduction in BMD as observed in untreated patients with GHD is uncertain. There does not appear to be a relationship between the duration of GHD and the degree of reduction in BMD. Age of onset rather than duration seems to be important in determining the reduction in BMD (142). A positive correlation between circulating insulin-like growth factor (IGF-I) and BMD in individuals with acquired GHD has been reported (146,147).

GH has both direct and indirect actions on bone. Protein anabolic effects of GH result in increases in both muscle mass and strength. GH increases gastrointestinal calcium absorption and increases the renal phosphate threshold, thereby providing the substrate for bone formation. GH activates bone remodeling by stimulating chondrocyte, osteoblast and osteoclast activities (29,148,149). Based on these observations clinical studies were initiated to establish the potential effects of GH replacement therapy on bone turnover and bone mass. Several reports indicate that GH replacement results in a considerable increase of bone turnover as indicated by an increase of markers of bone resorption and bone formation (149-154). Long-term treatment with GH up to 30 months leads, after an initial downwards trend, to a sustained increase of bone mineral content (BMC) as measured in the axial and appendicular skeleton (155-158). In other words long-term GH replacement therapy appears to result in a favorable shift in the balance between bone resorption and bone formation.

1.5.4. Lipid metabolism, atherosclerosis and cardiac function

Altered serum concentrations of lipids and lipoproteins are well-recognized risk factors for cardiovascular and coronary artery disease (The Framingham study, (159)). Such changes include increased concentrations of serum total cholesterol (160), low-density lipoprotein (LDL) cholesterol, and apolipoprotein B (apo B), an elevated apo B:A1 ratio, and a decreased high-density lipoprotein (HDL) cholesterol concentration.

Atherosclerosis and cardiovascular morbidity and mortality seem more common in adults with hypopituitarism receiving routine hormone replacement but not growth hormone (128,143). In the case of multiple pituitary hormone deficiencies due to pituitary adenomas or after treatment with irradiation or surgery GHD is likely to be present (126,127). Therefore GHD might be a factor in this increased mortality from cardiovascular disease.

In the normal population as well as in GHD the role of GH in the control of lipoprotein metabolism and atherosclerosis has not been fully elucidated. Most studies have shown modest hypercholesterolemia and hypertriglyceridemia in GHD children and adults (161-165). GH treatment did not exert effects on lipids and lipoproteins in patients and volunteers without GHD (166-168). In severe GHD patients there was a lowering of serum cholesterol levels during GH therapy (67,169). In another study a favorable increase of HDL-cholesterol levels, but also an unfavorable increase in serum Lp(a) lipoprotein was observed during GH replacement therapy (170-172).

Apart from possible disturbances in lipid metabolism increased atherosclerosis was demonstrated among GHDA patients as established by high resolution ultrasonography of the carotid artery (173). Also, in GHDA a decrease in aortic distensibility was found (174). In animal experiments, this decrease in compliance could be reversed by the administration of growth hormone (175). Others found myocardial dysfunction as result of a reduction in left ventricular mass in GHDA patients (176,177). Myocardial contractility increased following short-term GH treatment in normal volunteers (178). In GHD patients an increase in left ventricular wall mass was found after six months GH replacement therapy (179).

1.5.5. Psychological well-being

Reliable tests have been developed to measure health status. One of these tests, which is used frequently, is the Nottingham Health Profile (NHP) developed in the early 1980s (180). It is a self-rating questionnaire by which perceived health problems and their impact on task performance in the physical, mental and social spheres are measured. Part I of the NHP consists of 38 statements covering six sections, each of which refers to a separate area of functioning. The sections comprise physical mobility, pain, sleep, energy, social isolation, and emotional reactions. Respondents are required to read each statement and to tick either 'yes' or 'no', depending on whether that problem applies to them at the time. The questionnaire takes between five and ten minutes to complete.

The sections contain between 3 and 9 items, each of which has received a certain scoring weight in order to make the maximum score per section 100. Thus a respondent affirming all the statements in one section would obtain a score of 100. Part II of the NHP consists of seven statements relating to the effect of health problems on occupation, jobs around the home, personal relationships, social life, sex life, hobbies and holidays.

Several studies have established the validity and reliability of the NHP (181,182). Validity and reliability of the Dutch adaptation were found good, with exception of the section social isolation (183).

When compared to several other questionnaires the NHP was the easiest to use and the most reliable in the sense of reproducibility and internal consistency.

NHP and GHD

Compared to controls GHD patients have a poorer quality of life. There were significant differences in the sections of physical mobility and social isolation. Interestingly no differences were found between patients with isolated GHD or GHD combined with deficiencies of anterior pituitary hormones (184).

In another study significant decreases were found for GHDA patients in the sections energy, social isolation, emotional reactions and sex life (185,186). Treatment for six

months with GH in a group of patients with childhood and adult onset GHD, showed improvement in the overall scores of the NHP and in the subsection of energy versus placebo (185).

1.6. Future uses of growth hormone

The proposed indications for GH therapy can largely be defined in three categories; the ones proven to be effective, the ones possibly effective and the speculative ones (187). Since restrictions due to scarcity of supply have been lifted, more and more fields to study the effect of GH therapy have been proposed. Some of these and the original indications are listed in Table 3.

Table 3. Uses of growth hormone

Conventional uses
classical GHD
chronic renal failure in children
Turner's syndrome
Nonconventional use
non GHD short stature
GHD adults
obesity
aging
critical illness or injury
glucocorticoid therapy
osteoporosis

Adapted from ref. 188

In none of the reports on the final outcome of GH therapy of **children with GHD**, a normal mean height has been reached. The average mean height achieved was in general slightly more than -2 SD (-12-14 cm) below the midparental mean height. Final outcome was negatively correlated with the degree of height loss at the onset of therapy, which implies that timing of the start of GH replacement is crucial (188,189). Growth failure is a major clinical complication of **chronic renal failure (CRF)**. GH therapy in CRF in patients without requiring dialysis, with dialysis or after renal transplant was proven to be effective (190-192). **Turner's syndrome** in girls is a fairly common disorder, which is, among other features, characterized by short stature. In a large study, treatment with GH was responsible for an estimated increase in final adult height of 8 cm (193). However, no final conclusions can be drawn at present concerning the actual gain in length due to GH treatment in this type of patients. In **non GHD short stature** data on the overall efficiency of GH therapy remain inconclusive.

GH replacement therapy in **GHD adults** will not be further discussed in this chapter. GH treatment in **obese patients** receiving hypocaloric nutrition, results in a nitrogen sparing effect which disappears after 5 weeks. Although cumulative weight loss, and body composition were not influenced by GH administration, the improvement in nitrogen retention may be beneficial in terms of fatigue (194).

Both GH deficiency in adulthood and **normal aging** are associated with decreases in protein synthesis and in the percent lean body and bone mass, and with increases in percent of body fat (28,66,195-198). Whole body protein turnover is decreased in these conditions (199). It is possible that a gradually reduced GH secretion and decreased IGF-I levels might account, at least in part, for one or more of the above effects of aging (44), and that some elderly people might benefit from treatment with GH (43). In several recent studies administration of GH to elderly subjects for periods varying from a few weeks to twelve months, resulted in improvements of nitrogen balance, in an increase in lean body mass, and in a decrease in percent body fat (60,200-205).

In a controlled study in patients undergoing major gastrointestinal **surgery**, after which they were not fed for six days, patients were randomized to receive placebo or GH (0.1 mg/kg.day). In the GH group there was a significant reduction in nitrogen excretion, and an increase in energy expenditure and fat oxidation. Protein turnover had increased, as reflected by an increased synthesis and breakdown resulting in an improved nitrogen balance (206). These latter findings were confirmed in patients who underwent elective cholecystectomy (33).

When in major gastrointestinal surgery GH is combined with **parenteral nutrition** even a positive nitrogen balance can be achieved (207). In normal individuals a positive nitrogen balance was reached during hypocaloric intravenous nutrition, when combined with GH (208). GH improves the efficiency of nutritional support in patients after major operation (209-211). This was recently also found in **patients after trauma** (212).

In patients with **severe sepsis** GH also did improve, but not reverse a negative nitrogen balance (213).

In animal studies GH was found to improve mechanical strength of the rat colon after operation (214), of rat tibial fractures (215), and of skin wounds (216). These observations support improved wound healing, induced by GH administration.

The positive effects of GH on nitrogen balance in **burn patients** were already demonstrated in the early 1960s (57,217), and have been confirmed since (58). It was not until recently, that it was demonstrated, that GH therapy also leads to a significant decrease in donor-site healing time in severely burned children and a reduction in the length of hospital stay by more than 25% (218,219).

Other positive effects on nitrogen balance were seen in patients that were catabolic due to treatment with **glucocorticosteroids** (62), **cancer** (220), and patients with infection of the **human immunodeficiency virus** and **AIDS** (221,222).

GH increases bone turnover (29). However, trials in **postmenopausal osteoporosis** so far have been disappointing. This subject will be discussed more extensively in chapter 6.

Other potential indications for GH administration are **ovulation induction** and **lactation failure**. These will not be further discussed.

1.7 Safety of human growth hormone therapy

Human GH extracted from cadaveric pituitaries has been shown in some instances to be contaminated with an infectious agent causing Creutzfeld-Jacob disease. Since the introduction of recombinant human GH this potential hazard has become eradicated in newly treated patients and will not be further discussed (see chapter 1.1). However, at the time it came unexpectedly and it reminds us of being alert to possible long-term, side effects of GH therapy.

There is a fundamental pharmacological difference in the treatment of patients with pituitary disease as compared to most other medical treatments, in the fact that in these patients deficiencies are replaced by hormonal substitutes, which represent normal hormone secretion. In patients with hypopituitarism, there is a necessity to replace thyroid, adrenocortical, gonadal and posterior lobe function in a way that mimics physiology as closely as possible. GH replacement therapy in GHD adults causes remarkably few side-effects, most of which are minor or transient and improve after dose reduction, suggesting that they are dose-dependent. The most frequently reported side-effects are related to water retention, like edema, arthralgia and carpal tunnel syndrome. Another reported complaint is transient muscle ache.

Long-term safety reports concerning GH replacement therapy are not available in adults. In 1988, there have been reports from Japan of an increased risk of leukemia in children (223). In a subsequent large survey, however, it was found that the risk of acquiring leukemia during GH therapy is not higher than that in the general population (224). In 1993 there was a report that GH therapy was associated with benign intracranial hypertension in 22 children and one adult (225). All patients had developed papilledema, while 21 of them presented with headache and/or visual changes. Cerebrospinal fluid pressure was increased in 11 of 12 patients in whom values were reported. Papilledema and symptoms of intracranial hypertension resolved in all patients after treatment was stopped. The fact that these reported cases began to appear only in 1986 may be due to the trend toward the use of higher and more frequent doses of GH and the expansion of the indication for use beyond GH replacement, which followed the

introduction of recombinant GH in 1985. So far, there have been no such reports in adults during GH replacement therapy.

GH dose in adult replacement therapy has decreased lately, in comparison with the initial studies (66,67,154,158). There is no good parameter to define the optimal GH dose. However, individual GH dose adjustment, with careful monitoring of side effects, until high normal IGF-I levels are reached, might prove a good strategy to obtain maximum biological effects of GH therapy in GHD patients.

In elderly men with lowered IGF-I levels, GH treatment caused carpal tunnel syndrome, gynaecomastia and hyperglycemia (226). Apart from the fact that these men were probably not GH deficient, the GH doses used were supraphysiological (30 μg/kg three times a week, 0.243 IU/kg.week) even for GHD. GH decreases the sensitivity to insulin. Therefore an individual, who is in a pre-diabetic state, with impaired insulin release, might develop clinically recognizable insulin deficiency after GH treatment is started. GH does not impair insulin secretion. Thus GH may reveal diabetes mellitus, but does not cause it.

In conclusion no deleterious side-effects of long-term GH therapy have been observed or are to be expected as long as the GH dose is within the physiological range.

1.8 Scope of the thesis/ Aims of the studies

Several aspects of the GHD syndrome, GH replacement therapy, and GH therapy were further investigated in this thesis.

At first several aspects of the GHD syndrome will be studied in a large group of patients with childhood and adult onset GHD. Is there a difference in the clinical symptomatology among these two groups of GHD patients, other than their length and the age of onset of GHD? **(chapter 2)**.

Thereafter the acute, initial effects of short-term GH replacement therapy will be studied in GHDA patients on nitrogen balance, sodium and potassium balance, IGF-I and IGFBP-3 and body composition. What is the degree of nitrogen and sodium retention and how long does it last? Does measurement of IGFBP-3 help in finding the optimal dose of GH replacement therapy? Are the changes in fat mass as measured by bioelectrical impedance analysis real or misleading? **(chapter 3)**.

Subsequently the effects of GH replacement are studied on renal phosphate handling, bone turnover and bone mineral content. These effects are compared to GH replacement therapy in combination with pamidronate, an inhibitor of bone resorption. Does pamidronate influence these parameters; is the combination of GH and pamidronate better with regard to the initial effects in bone mineral content? **(chapter 4)**.

In **chapter 5** the usefulness of GH as an adjuvant to pamidronate is studied in patients with postmenopausal osteoporosis. Elaborating on the impressive effects of GH on bone in GHD adults, can GH also play a role in the treatment of postmenopausal osteoporosis?

Finally the effects of long-term GH replacement therapy are studied in patients with *adult onset* GH-deficiency. Special emphasis is directed at several aspects of body composition assessment. Which method of body composition measurement is most suitable in the follow up of GHD patients on long-term GH replacement therapy? Will initial beneficial effects be maintained during long-term treatment? Are side-effects clinically important and persistent? **(chapter 6)**.

In the concluding chapter the results obtained are summarized and a synthesis is formulated on the current use of GH replacement therapy in GHD adults **(chapter 7)**.

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REFERENCES

1. **Crowe SJ, Cushing H.** 1910 Experimental hypophysectomy. Bull Johns Hopkins Hosp. 21:127-169.

2. **Evans HM, Long JA.** 1921 The effect of the anterior lobe administered intraperitoneally upon growth, maturity, and oestrous cycles of the rat. Anat Rec. 21:62-63.

3. **Beck JC, McGarry EE, Dyrenfurth I, Venning EH.** 1957 Metabolic effects of human and monkey growth hormone in man. Science. 125:884-885.

4. **Ikkos D, Luft R, Gemzell CA.** 1958 The effect of human growth hormone in man. Lancet. 1:720-721.

5. **Raben MS.** 1958 Treatment of a pituitary dwarf with human growth hormone. J Clin Endocrinol Metab. 18:901-903.

6. **Henneman PH, Forbes AP, Moldawer M, Dempsey EF, Carroll EL.** 1960 Effects of human growth hormone in man. J Clin Invest. 39:1223-1238.

7. **Batts AA, Bennett LL, Garcia J, Stein J.** 1954 The effect of growth hormone on muscle potassium and on extracellular fluid. Endocrinology. 55:456-465.

8. **Raben MS, Hollenberg CH.** 1959 Effect of growth hormone on plasma fatty acids. J Clin Invest. 38:484-488.

9. **Raben MS.** 1962 Clinical use of human growth hormone. N Engl J Med. 266:82-86.

10. **Henneman DH, Henneman PH.** 1960 Effects of human growth hormone on levels of blood and urinary carbohydrate and fat metabolites in man. J Clin Invest. 39:1239-1245.

11. **Soyka LF, Ziskind A, Crawford JD.** 1964 Treatment of short stature in children and adolescents with human pituitary growth hormone (Raben). N Engl J Med. 271:754-764.

12. **Glick SM, Roth J, Yalow RS, Berson SA.** 1963 Immunoassay of human growth hormone in plasma. Nature. 199:784.

13. **Goeddel DV, Heyneker HL, Hozumi T, et al.** 1979 Direct expression in Escherichia coli of a DNA sequence coding for human growth hormone. Nature. 281:544-548.

14. **Hintz RL, Rosenfeld RG, Wilson DM, et al.** 1982 Biosynthetic methionyl human growth hormone is biologically active in adult man. Lancet. 1:1276-1279.

15. **Raiti S.** 1985 Human growth hormone and Creutzfeld-Jakob disease. Ann Intern Med. 103:288-289.

16. 1985 Ban on growth hormone. Lancet. 1:1172.

17. **Laron Z, Josefsberg Z.** 1985 Withdrawal of human pituitary growth hormone. Lancet. 1:1324.

18. **Powell-Jackson J, Weller RO, Kennedy P, Preece MA, Whitcombe EM, Newsom-Davis J.** 1985 Creutzfeld-Jakob disease after administration of human growth hormone. Lancet. 2:244-246.

19. **Brown P, Gajdusek DC, Gibbs CJ, Asher DM.** 1985 Potential epidemic of Creutzfeld-Jakob disease from human growth hormone therapy. N Engl J Med. 313:728-731.

20. **Underwood LE, Fisher DA, Frasier SD, et al.** 1985 Degenerative neurologic disease in patients formerly treated with human growth hormone. J Paed. 107:10-12.

21. **Brown P.** 1988 Human growth hormone therapy and Creutzfeld-Jakob disease: a drama in three acts. Pediatrics. 81:85-92.

22. **Koch TK, Berg BO, De Armond SJ, Gravina RF.** 1985 Creutzfeld-Jakob disease in a young adult with idiopathic hypopituitarism. N Engl J Med. 313:731-734.

23. **Gibbs CJ, Joy A, Heffner R, et al.** 1985 Clinical and pathological features and laboratory confirmation of Creutzfeld-Jakob disease in a recipient of pituitary-derived human growth hormone. N Engl J Med. 313:734-739.

24. **Job JC, Maillard F, Goujard J.** 1992 Epidemiologic survey of patients treated with growth hormone in France in the period 1959-1990; preliminary results. Horm Res. 38(Suppl 1):35-43.

25. **Preece M.** 1993 Human pituitary growth hormone and Creutzfeld-Jakob disease. Horm Res. 39:95-98.

26. **Frasier SD, Foley TP.** 1994 Clinical review 58. Creutzfeldt-Jakob disease in recipients of pituitary hormones. J Clin Endocrinol Metab. 78:1277-1279.

27. **Bojs G, Falkheden T, Sjögren B.** 1961 Studies on glomerular filtration rate and renal plasma flow in pituitary insufficiency in man including measurements of the renal extraction of para-aminohippuric acid. Acta Endocrinol (Copenh). 37:616-622.

28. **Cuneo RC, Salomon F, McGauley GA, Sönksen PH.** 1992 The growth hormone deficiency syndrome in adults. Clin Endocrinol (Oxf). 37:387-397.

29. **Isaksson OGP, Lindahl A, Nilsson A, Isgaard J.** 1987 Mechanism of the stimulatory effect of growth hormone on longitudinal bone growth. Endocrin Rev. 8:426-438.

30. **Fryburg DA, Gelfand RA, Barrett EJ.** 1991 Growth hormone acutely stimulates forearm muscle protein synthesis in normal humans. Am J Physiol. 260:E499-E504.

31. **Lundeberg S, Belfrage M, Wernerman J, von der Decken A, Thunell S, Vinnars E.** 1991 Growth hormone improves muscle protein metabolism and whole body nitrogen economy in man during a hyponitrogenous diet. Metabolism. 40:315-322.

32. **Binnerts A, Swart GR, Wilson JH, et al.** 1992 The effect of growth hormone administration in growth hormone deficient adults on bone, protein, carbohydrate and lipid homeostasis, as well as on body composition. Clin Endocrinol (Oxf). 37:79-87.

33. **Hammarqvist F, Strömberg C, von der Decken A, Vinnars E, Wernerman J.** 1992 Biosynthetic human growth hormone preserves both muscle protein synthesis and the decrease in muscle-free glutamine, and improves whole-body nitrogen economy after operation. Ann Surg. 216:184-191.

34. **Cronin MJ, Thorner MO.** 1989 Basic studies with growth hormone-releasing factor. In: DeGroot L.J. ed. Endocrinology, Philadelphia:W.B.Saunders Company; 183-191.

35. **Reichlin S.** 1983 Somatostatin. N Engl J Med. 309:1495-1501.

36. **Winer LM, Shaw MA, Baumann G.** 1990 Basal plasma growth hormone levels in man: new evidence for rhythmicity of growth hormone secretion. J Clin Endocrinol Metab. 70:1678-1686.

37. **Vance ML, Kaiser DL, Evans WS, et al.** 1985 Pulsatile growth hormone secretion in normal man during a continuous 24-hour infusion of human growth hormone releasing factor (1-40). Evidence for intermittent somatostatin secretion. J Clin Invest. 75:1584-1590.

38. **Salmon WD, Daughaday WH.** 1957 A hormonally controlled serum factor which stimulates sulfate incorporation by cartilage in vitro. J Lab Clin Med. 49:825-836.

39. **Schwander JC, Hauri C, Zapf J, Froesch ER.** 1983 Synthesis and secretion of insulin-like growth factor and its binding protein by the perfused rat liver: dependence on growth hormone status. Endocrinology. 113:297-305.

40. **Clemmons DR, Van Wyk JJ.** 1984 Factors controlling blood concentration of somatomedin C. Clin Endocrinol Metab. 13:113-143.

41. **Berelowitz M, Szabo M, Frohman LA, Firestone S, Chu L, Hintz RL.** 1981 Somatomedin-C mediates growth hormone negative feedback by effects on both the hypothalamus and the hypopituitary. Science. 212:1279-1281.

42. **Hagberg JM, Seals DR, Yerg JE, et al.** 1988 Metabolic responses to exercise in young and older athletes and sedentary men. J Appl Physiol. 65:900-908.

43. **Rudman D, Kutner MH, Rogers CM, Lubin MF, Fleming GA, Bain RP.** 1981 Impaired growth hormone secretion in the adult population. Relation to age and adiposity. J Clin Invest. 67:1361-1369.

44. **Zadik Z, Chalew SA, McCarter RJ, Meistas M, Kowarski AA.** 1985 The influence of age on the 24-hour integrated concentration of growth hormone in normal individuals. J Clin Endocrinol Metab. 60:513-516.

45. **Williams T, Berelowitz M, Joffe SN, et al.** 1984 Impaired growth hormone respons to growth hormone-releasing factor in obesity. N Engl J Med. 311:1403-1407.

46. **Mendlewicz J, Linkowski P, Kerkhofs M, et al.** 1985 Diurnal hypersecretion of growth hormone in depression. J Clin Endocrinol Metab. 60:505-512.

47. **Press M, Tamborlane WV, Thorner MO, et al.** 1984 Pituitary response to growth hormone-releasing factor in diabetes. Failure of glucose mediated suppression. Diabetes. 33:804-806.

48. **Baumann G, Shaw MA, Amburn K.** 1989 Regulation of plasma growth hormone-binding proteins in health and disease. Metabolism. 38:683-689.

49. **Baumann G, Shaw MA.** 1990 Plasma transport of the 20,000-dalton variant of human growth hormone (20K): evidence for a 20K-specific binding site. J Clin Endocrinol Metab. 71:1339-1343.

50. **Thorner MO, Vance ML.** 1988 Growth hormone, 1988. J Clin Invest. 82:745-747.

51. **De Vos AM, Ultsch M, Kossiakoff AA.** 1992 Human growth hormone and extracellular domain of its receptor: crystal structure of the complex. Science. 255:306-312.

52. **Isaksson OGP, Jansson J-O, Gause IAM.** 1982 Growth hormone stimulates longitudinal bone growth directly. Science. 216:1237-1238.

53. **Kassem M, Blum WF, Ristelli J, Mosekilde L, Eriksen EF.** 1993 Growth hormone stimulates proliferation and differentiation of normal human osteoblast-like cells in vitro. Calc Tissue Int. 52:222-226.

54. **Davidson MB.** 1987 Effect of growth hormone on carbohydrate and lipid metabolism. Endocrin Rev. 8:115-131.

55. **Moskowitz J, Fain JN.** 1970 Stimulation by growth hormone and dexamethasone of labeled cyclic adenosine 3',5'-monophosphate accumulation by white fat cells. J Biol Chem. 245:1101-1107.

56. **Van Vliet G, Bosson D, Craen M, Du Caju MVL, Malvaux P, Vanderschüren-Lodeweyckx M.** 1987 Comparative study of the lipolytic potencies of pituitary-derived and biosynthetic human growth hormone in hypopituitary children. J Clin Endocrinol Metab. 65:876-879.

57. **Liljedahl S-O, Gemzell CA, Plantin L-O, Birke G.** 1961 Effect of human growth hormone in patients with severe burns. Acta Chir Scand. 122:1-14.

58. **Wilmore DW, Moylan JA, Bristow BF, Mason AD, Pruitt BA.** 1974 Anabolic effects of human growth hormone and high caloric feedings following thermal injury. Surg Gynecol Obstet. 138:875-884.

59. **Manson JM, Wilmore DW.** 1986 Positive nitrogen balance with human growth hormone and hypocaloric intravenous feeding. Surgery. 100:188-197.

60. **Binnerts A, Wilson JH, Lamberts SWJ.** 1988 The effects of human growth hormone administration in elderly adults with recent weight loss. J Clin Endocrinol Metab. 67:1312-1316.

61. **Russell-Jones DL, Weissberger AJ, Bowes SB, et al.** 1993 The effects of growth hormone on protein metabolism in adult growth hormone deficient patients. Clin Endocrinol (Oxf). 38:427-431.

62. **Horber FF, Haymond MW.** 1990 Human growth hormone prevents the protein catabolic side effects of prednisone in humans. J Clin Invest. 86:265-272.

63. **Valk NK, van der Lely AJ, de Herder WW, Lindemans J, Lamberts SWJ.** 1994 The effects of human growth hormone (GH) administration in GH-deficient adults: a 20-day metabolic ward study. J Clin Endocrinol Metab. 79:1070-1076.

64. **Ho KY, Kelly JJ.** 1991 Role of growth hormone in fluid homeostasis. Horm Res. 36(Suppl 1):44-48.

65. **D'Ercole AJ, Stiles AD, Underwood LE.** 1984 Tissue concentrations of somatomedin C: further evidence for multiple sites of synthesis and paracrine or autocrine mechanisms of actions. Proc Natl Acad Sci. 81:935-939.

66. **Jørgensen JOL, Pedersen SA, Thuesen L, Jørgensen J, Skakkebæk NE, Christiansen JS.** 1989 Beneficial effects of growth hormone treatment in GH-deficient adults. Lancet. 1:1221-1225.

67. **Salomon F, Cuneo RC, Hesp R, Sönksen PH.** 1989 The effects of treatment with recombinant human growth hormone on body composition and metabolism in adults with growth hormone deficiency. N Engl J Med. 321:1797-1803.

68. **Hardouin S, Gourmelin M, Noguiez P, et al.** 1989 Molecular forms of serum insulin-like growth factor (IGF)-binding proteins in man: relationships with growth hormone and IGFs and physiological significance. J Clin Endocrinol Metab. 69:1291-1301.

69. **Blum WF, Ranke MB.** 1990 Insulin-like growth factor binding proteins (IGFBPs) with special reference to IGFBP-3. Acta Paediatr Scand. 367(Suppl):55-62.

70. **Drop SLS.** 1992 Report on the nomenclature of the IGF binding proteins. J Clin Endocrinol Metab. 74:1215-1216.

71. **Zapf J, Hanin C, Waldrogel M, Froesch ER.** 1986 Acute metabolic effects and half-lives of intravenously administered insulin-like growth factors I and II in normal and hypophysectomized rats. J Clin Invest. 77:1768-1775.

72. **Villaudy J, Blat C, Drop SLS, Goldé A, Harel L.** 1994 Difference in biological effects between insulin-like growth factor binding protein 1 and 3. Growth Factors. 10:107-114.

73. **Blum WF, Ranke MB, Kietzmann K, Gauggel E, Zeisel HJ, Bierich JR.** 1990 A specific radioimmunoasssay for the growth hormone (GH)-dependent somatomedin-binding protein: its use for diagnosis of GH deficiency. J Clin Endocrinol Metab. 70:1292-1298.

74. **Suikkari A-M, Koivisto VA, Rutanen E-M, Yki-Järvinen H, Karonen S-L, Seppälä M.** 1988 Insulin regulates the serum levels of low molecular weight insulin-like growth factor-binding protein. J Clin Endocrinol Metab. 66:266-272.

75. **Suikkari A-M, Koivisto VA, Koistinen R, Seppälä M, Yki-Järvinen H.** 1989 Dose-response characteristics for suppression of low molecular weight plasma insulin-like growth factor-binding protein by insulin. J Clin Endocrinol Metab. 68:135-140.

76. **Sönksen PH, Cuneo RC, Salomon F, et al.** 1991 Growth hormone therapy in adults with growth hormone deficiency. Acta Paediatr Scand. 379:139-146.

77. **Hoffman DM, O'Sullivan AJ, Baxter RC, Ho KKY.** 1994 Diagnosis of growth-hormone deficiency in adults. Lancet. 343:1064-1068.

78. **Shah A, Stanhope R, Matthew D.** 1992 Hazards of pharmacological tests of growth hormone secretion in childhood. Br Med J. 304:173-174.

79. **Jones SL, Trainer PJ, Perry L, Wass JAH, Besser GM, Grossman A.** 1994 An audit of the insulin tolerance test in adult subjects in an acute investigation unit over one year. Clin Endocrinol (Oxf). 41:123-128.

80. **Merimee TJ, Lillicrap DA, Rabinowitz D.** 1965 Effect of arginine on serum-levels of human growth-hormone. Lancet. 2:668-670.

81. **Merimee TJ, Rabinowitz D, Riggs L, Burgess JA, Rimoin DL, McKusick VA.** 1967 Plasma growth hormone after arginine infusion. Clinical experiences. N Engl J Med. 276:434-439.

82. **Blichert-Toft M.** 1975 Stimulation of the release of corticotropin and somatotrophin by metyrapone and arginine. Acta Endocrinol (Copenh). 195(Suppl):69-85.

83. **Koppeschaar HPF, ten Horn CD, Thijssen JHH, Page MD, Dieguez C, Scanlon MF.** 1992 Differential effects of arginine on growth hormone releasing hormone and insulin induced growth hormone secretion. Clin Endocrinol (Oxf). 36:487-490.

84. **Schriock EA, Lustig RH, Rosenthal SM, Kaplan SL, Grumbach MM.** 1984 Effect of growth hormone (GH)-releasing hormone (GRH) on plasma GH in relation to magnitude and duration of GH Deficiency in 26 children and adults with isolated GH deficiency or multiple pituitary hormone deficiencies: evidence for hypothalamic GRH deficiency. J Clin Endocrinol Metab. 58:1043-1049.

85. **Gelato MC, Pescovitz OH, Cassoria F, Loriaux DL, Merriam GR.** 1984 Dose-response relationships for the effects of growth hormone-releasing factor-(1-44)-NH_2 in young adult men and women. J Clin Endocrinol Metab. 59:197-201.

86. **Lang I, Kurz R, Geyer G, Tragl KH.** 1988 The influence of age on human pancreatic growth hormone releasing hormone stimulated growth hormone secretion. Horm metab Res. 20:574-578.

87. **Albini CH, Reiter EO, Mills BJ, MacGillivray MH.** 1988 Diagnostic value of the growth hormone-releasing factor stimulation test. Clin Pharmacol Ther. 43:696-700.

88. **Rosenfeld RG, Wilson DM, Lee PDK, Hintz RL.** 1986 Insulin-like growth factors I and II in evaluation of growth retardation. J Paediatr. 109:428-433.

89. **Hasegawa Y, Hasegawa T, Aso T, et al.** 1993 Comparison between insulin-like growth factor-I (IGF-I) and IGF binding protein-3 (IGFBP-3) measurement in the diagnosis of growth hormone deficiency. Endocr J. 40:185-190.

90. **Donahu SP, Phillips LS.** 1989 Response of IGF-I to nutritional support in malnourished hospital patients: a possible indicator of short-term changes in nutritional status. Am J Clin Nutr. 50:962-969.

91. **Furlanetto RW.** 1990 Insulin-like growth factor measurements in the evaluation of growth hormone secretion. Horm Res. 33(Suppl 4):25-30.

92. **Phillip M, Chalew SA, Avinoam Kowarski A, Stene MA.** 1993 Plasma IGFBP-3 and its relationship with quantitative growth hormone secretion in short children. Clin Endocrinol (Oxf). 39:427-432.

93. **Wilmore DW.** 1977 The metabolic management of the critically ill. New York:Plenum Medical Book Company; 149.

94. **Streat SJ, Beddoe AH, Hill GL.** 1987 Aggressive nutritional support does not prevent protein loss despite fat gain in septic intensive care patients. J Trauma. 27:262-266.

95. **Herrman VM, Clarke D, Wilmore DW, Moore FD.** 1980 Protein metabolism: effect of disease and altered intake on the stable ^{15}N curve. Surg Forum. 31:92-94.

96. **Dempsey DT, Mullen JL, Buzby GP.** 1988 The link between nutritional status and clinical outcome: can nutritional intervention modify it? Am J Clin Nutr. 47:352-356.

97. **Dahms WT, Owens RP, Kalhan SC, Kerr DS, Danish RK.** 1989 Urea synthesis, nitrogen balance, and glucose turnover in growth-hormone deficient children before and after growth hormone administration. Metabolism. 38:197-203.

98. **Sheng HP, Huggins RA.** 1979 A review of body composition studies with emphasis on total body water and fat. Am J Clin Nutr. 32:630-647.

99. **Cunningham JJ.** 1991 Body composition as a determinant of energy expenditure: a synthetic review and proposed general prediction equation. Am J Clin Nutr. 54:963-969.

100. **Forbes GB, Bruining GJ.** 1976 Urinary creatinine excretion and lean body mass. Am J Clin Nutr. 29:1359-1366.

101. **Pace N, Rathbun EN.** 1945 Studies on body composition. III. The body water and chemically combined nitrogen content in relation to fat content. J Biol Chem. 158:685-691.

102. **Durnin JVGA, Womersley J.** 1974 Body fat assessed from total body density and its estimation from skinfold thickness : measurements on 481 men and women aged from 16 to 72 years. Br J Nutr. 32:77-97.

103. **Siri WE.** 1961 Body composition from fluid spaces and density: analysis of methods. In: Brozek J, Henschel A.. eds. Techniques for measuring body composition. Washington, DC: National Academy of Sciences; 223-244.

104. **Must A, Dallal GE, Dietz WH.** 1991 Reference data for obesity: 85th and 95th percentiles of body mass index (wt/ht^2) and triceps skinfold thickness. Am J Clin Nutr. 53:839-846.

105. **Garrow JS, Webster J.** 1985 Quetelet's index(W/H^2) as a measure of fatness. Int J Obesity. 9:147-153.

106. **Lukaski HC, Johnson PE, Bolonchuk WW, Lykken GI.** 1985 Assessment of fat-free mass using bioelectrical impedance measurements of the human body. Am J Clin Nutr. 41:810-817.

107. **Lukaski HC, Bolonchuk WW, Hall CB, Siders WA.** 1986 Validation of tetrapolar bioelectrical impedance method to assess human body composition. J Appl Physiol. 60:1327-1332.

108. **Kushner RF, Schoeller DA.** 1986 Estimation of total body water by bioelectrical impedance analysis. Am J Clin Nutr. 44:417-424.

109. **Segal KR, Van Loan M, Fitzgerald PI, Hodgdon JA, Van Itallie TB.** 1988 Lean body mass estimation by bioelectrical impedance analysis: a four-site cross-validation study. Am J Clin Nutr. 47:7-14.

110. **Stewart SP, Bramley PN, Heighton R, et al.** 1993 Estimation of body-composition from bioelectrical impedance of body segments: comparison with dual-energy x-ray absorptiometry. Br J Nutr. 69:645-655.

111. **Runge VM, Eisenmann PA, Johnson SC.** 1987 Effects of exercise induced dehydration on BIA to determine body composition. Med Sci Sports Exerc. 19:S38.

112. **Zabetakis GW, Gleim GW, Vitting KE, Gardenswartz MH.** 1987 Volume changes effect electrical impedance measurements of body composition. Med Sci Sports Exerc. 19:S40.

113. **Svendsen OL, Haarbo J, Heitman HL, Gotfredsen A, Christiansen C.** 1991 Measurement of body fat in elderly subjects by dual-energy x-ray absorptiometry, bioelectrical impedance and anthropometry. Am J Clin Nutr. 53:1117-1123.

114. **Deurenberg P, van der Kooij K, Leenen R, Weststrate JA, Seidell JC.** 1990 Sex and age specific prediction formulas for estimating body composition from bioelectrical impedance: a cross validation study. Int J Obesity. 15:17-25.

115. **Mazess RB.** 1991 Do bioimpedance changes reflect weight, not composition? Am J Clin Nutr. 53:178-179 (letter).

116. **Deurenberg P, Weststrate JA, Hautvast JGAJ, van der Kooij K.** 1991 Is the bioelectrical-impedance method valid? Am J Clin Nutr. 53:179 (letter).

117. **Forbes GB, Simon W, Amatruda JM.** 1992 Is bioimpedance a good predictor of body composition change? Am J Clin Nutr. 56:4-6.

118. **Stansell MJ, Mojica Jr L.** 1968 Determination of body water content using trace levels of deuterium oxide and infrared spectrophotometry. Clin Chem. 14:1112-1124.

119. **Lukaski HC, Johnson PE.** 1985 A simple inexpensive method of determining total body water using a tracer dose of D_2O and infrared absorption of biological fluids. Am J Clin Nutr. 41:363-370.

120. **Mazess RB, Barden HS, Bisek JP, Hanson J.** 1990 Dual-energy x-ray absorptiometry for total-body and regional bone-mineral and soft-tissue composition. Am J Clin Nutr. 51:1106-1112.

121. **Haarbo J, Gotfredsen A, Hassager C, Christiansen C.** 1991 Validation of body composition by dual energy x-ray absorptiometry (DEXA). Clin Physiol. 11:331-341.

122. **Fuller NJ, Laskey MA, Elia M.** 1992 Assessment of the composition of major body regions by dual-energy x-ray absorptiometry (DEXA), with special reference to limb muscle mass. Clin Physiol. 12:253-266.

123. **Svendsen OL, Haarbo J, Hassager C, Christiansen C.** 1993 Accuracy of measurements of body composition by dual-energy x-ray absorptiometry in vivo. Am J Clin Nutr. 57:605-608.

124. **Pritchard JE, Nowson CA, Strauss BJ, Carlson JS, Kaymakci C, Wark JD.** 1993 Evaluation of dual-energy x-ray absorption as a method of measurement of body fat. Eur J Clin Nutr. 47:216-228.

125. **Sönksen PH.** 1990 Replacement therapy in hypothalamo-pituitary insufficiency after childhood: management in the adult. Horm Res. 33(Suppl 4):45-51.

126. **Lindholm J, Rasmussen P, Korsgaard O.** 1976 Endocrine function in patients with pituitary adenoma before and after hypophysectomy. Acta Endocrinol (Copenh). 82:52-61.

127. **Toogood AA, Beardwell CG, Shalet SM.** 1994 The severity of growth hormone deficiency in adults with pituitary disease is related to the degree of hypopituitarism. Clin Endocrinol (Oxf). 41:511-516.

128. **Rosén T, Bengtsson B-Å.** 1990 Premature mortality due to cardiovascular disease in hypopituitarism. Lancet. 336:285-288.

129. **Tanner JM, Whitehouse RH.** 1967 The effect of human growth hormone on subcutaneous fat thickness in hyposomatotrophic and panhypopituitary dwarfs. J Endocrinol. 39:263-268.

130. **Parra AP, Argote RM, García G, Cervantes C, Alatorre S, Pérez-Pasten E.** 1979 Body composition in hypopituitary dwarfs before and during human growth hormone therapy. Metabolism. 28:851-857.

131. **Gregory JW, Greene SA, Jung RT, Scrimgeour CM, Rennie MJ.** 1991 Changes in body composition and energy expenditure after six weeks' growth hormone treatment. Arch Dis Child. 66:598-602.

132. **Binnerts A, Deurenberg P, Swart GR, Wilson JH, Lamberts SWJ.** 1992 Body composition in growth hormone-deficient adults. Am J Clin Nutr. 55:918-923.

133. **de Boer H, Blok GJ, Voerman HJ, de Vries PM, van der Veen EA.** 1992 Body composition in adult growth hormone-deficient man, assessed by anthropometry and bioimpedance analysis. J Clin Endocrinol Metab. 75:833-837.

134. **Rosén T, Bosaeus I, Tölli J, Lindstedt G, Bengtsson B-Å.** 1993 Increased body fat mass and decreased extracellular fluid volume in adults with growth hormone deficiency. Clin Endocrinol (Oxf). 38:63-71.

135. **Cuneo RC, Salomon F, Wiles CM, Sönksen PH.** 1990 Skeletal muscle performance in adults with growth hormone deficiency. Horm Res. 33(Suppl 4):55-60.

136. **Rutherford OM, Jones DA, Round JM, Buchanan CR, Preece M.** 1991 Changes in skeletal muscle and body composition after discontinuation of growth hormone treatment in growth hormone deficient young adults. Clin Endocrinol (Oxf). 34:469-475.

137. **Cuneo RC, Salomon F, Wiles CM, Hesp R, Sönksen PH.** 1991 Growth hormone treatment in growth hormone-deficient adults. I. Effects on muscle mass and strength. J Appl Physiol. 70:688-694.

138. **Cuneo RC, Salomon F, Wiles CM, Hesp R, Sönksen PH.** 1991 Growth hormone treatment in growth hormone-deficient adults.II. Effects on exercise performance. J Appl Physiol. 70:695-700.

139. **Kaufman J-M, Taelman P, Vermeulen A, Vandeweghe M.** 1992 Bone mineral status in growth hormone-deficient males with isolated and multiple pituitary deficiencies of childhood onset. J Clin Endocrinol Metab. 74:118-123.

140. **Hyer SL, Rodin DA, Tobias JH, Leiper A, Nussey SS.** 1992 Growth hormone deficiency during puberty reduces adult bone mineral density. Arch Dis Child. 67:1472-1474.

141. **Elgindy N, Grunditz R, Thorén M, Degerblad M, Sjöberg HE, Ringertz H.** 1991 Long term follow-up of metacarpal cortical thickness and bone mineral density in panhypopituitarism. Radiol Diagn. 32:326-330.

142. **Holmes SJ, Economou G, Whitehouse RW, Adams JE, Shalet SM.** 1994 Reduced bone mineral density in patients with adult onset growth hormone deficiency. J Clin Endocrinol Metab. 78:669-674.

143. **Wüster C, Slencka E, Ziegler R.** 1991 Erhöhte prävalenz von osteoporose und arteriosklerose bei konventionell substituierter hypophysenvorderlappeninsuffizienz: bedarf einer zusätzlichen wachstumshormonsubstitution? Klin Wochenschr. 69:769-773.

144. **Black DM, Cummings SR, Genant HK, Nevitt MC, Palermo L, Browner W.** 1992 Axial and appendicular bone density predicts fractures in older women. J Bone Miner Res. 7:633-638.

145. **Cummings SR, Black DM, Nevitt MC, et al.** 1993 Bone density at various sites for prediction of hip fractures. Lancet. 341:72-75.

146. **Johansson AG, Burman P, Westermark K, Ljunghall S.** 1992 The bone mineral density in acquired growth hormone deficiency correlates with circulating levels of insulin-like growth factor I. J Intern Med. 232:447-452.

147. **Bing-You RG, Denis M-C, Rosen CJ.** 1993 Low bone mineral density in adults with previous hypothalamic-pituitary tumors: Correlations with serum growth hormone responses to GH-releasing hormone, insulin-like growth factor I, and IGF binding protein 3. Calc Tissue Int. 52:183-187.

148. **Barnard R, Ng KW, Martin TJ, Waters MJ.** 1991 Growth hormone (GH) receptors in clonal osteoblast-like cells mediate a mitogenic response to GH. Endocrinology. 128:1459-1464.

149. **Johansen JS, Pedersen SA, Jørgensen JOL, et al.** 1990 Effects of growth hormone (GH) on plasma bone gla protein in GH-deficient adults. J Clin Endocrinol Metab. 70:916-919.

150. **Brixen K, Nielsen HK, Mosekilde L, Flyvbjerg A.** 1990 A short course of recombinant human growth hormone treatment stimulates osteoblasts and activates bone remodeling in normal human volunteers. J Bone Miner Res. 5:609-618.

151. **Nielsen HK, Jørgensen JOL, Brixen K, Christiansen JS.** 1991 Serum osteocalcin and bone isoenzyme alkaline phosphatase in growth hormone-deficient patients: dose-response studies with biosynthetic human GH. Calc Tissue Int. 48:82-87.

152. **Schlemmer A, Johansen JS, Pedersen SA, Jørgensen JOL, Hassager C, Christiansen C.** 1991 The effect of growth hormone (GH) therapy on urinary pyridinoline cross-links in GH-deficient adults. Clin Endocrinol (Oxf). 35:471-476.

153. **Whitehead HM, Boreham C, McIlrath EM, et al.** 1992 Growth hormone treatment of adults with growth hormone deficiency: results of a 13-month placebo controlled cross-over study. Clin Endocrinol (Oxf). 36:45-52.

154. **Bengtsson B-Å, Edén S, Bosaeus I, et al.** 1993 Treatment of adults with growth hormone deficiency with recombinant human growth hormone. J Clin Endocrinol Metab. 76:309-317.

155. **Degerblad M, Elgindy N, Hall K, Sjöberg H-E, Thorén M.** 1992 Potent effect of recombinant growth hormone on bone mineral density and body composition in adults with hypopituitarism. Acta Endocrinol (Copenh). 126:387-393.

156. **Vandeweghe M, Taelman P, Kaufman J-M.** 1993 Short and long-term effects of growth hormone treatment on bone turnover and bone mineral content in adult growth hormone-deficient males. Clin Endocrinol (Oxf). 39:409-415.

157. **O'Halloran DJ, Tsatsoulis A, Whitehouse RW, Holmes SJ, Adams JE, Shalet SM.** 1993 Increased bone density after recombinant human growth hormone (GH) therapy in adults with isolated GH deficiency. J Clin Endocrinol Metab. 76:1344-1348.

158. **Rosén T, Johansson G, Hallgren P, Caidahl K, Bosaeus I, Bengtsson B-Å.** 1994 Beneficial effects of 12 months replacement therapy with recombinant human growth hormone to growth hormone deficient adults. Endocrinol Metab. 1:55-66.

159. **Kannel WB, Castelli WP, Gordon T, McNamara PM.** 1971 Serum cholesterol, lipoproteins and the risk of coronary artery disease. Ann Intern Med. 74:1-12.

160. **Neaton JD, Wentworth D.** 1992 Serum cholesterol, blood pressure, cigarette smoking, and death from coronary artery disease. Arch Intern Med. 152:56-64.

161. **Blackett PR, Weech PK, McConathy WJ, Fesmire JD.** 1982 Growth hormone in the regulation of hyperlipidaemia. Metabolism. 31:117-120.

162. **Winter RJ, Thompson RG, Green OC.** 1979 Serum cholesterol and triglycerides in children with growth hormone deficiency. Metabolism. 28:1244-1249.

163. **Merimee TJ, Hollander W, Fineberg SE.** 1972 Studies of hyperlipidemia in the HGH-deficient state. Metabolism. 21:1053-1061.

164. **Merimee TJ, Fineberg SE, Hollander W.** 1973 Vascular disease in the chronic HGH-deficient state. Diabetes. 22:813-819.

165. **de Boer H, Blok GJ, Voerman HJ, Phillips M, Schouten JA.** 1994 Serum lipid levels in growth hormone-deficient men. Metabolism. 43:199-203.

166. **Aloia JF, Zanzi I, Cohn SH.** 1975 Absence of an effect of chronic administration of growth hormone on serum lipids. Metabolism. 24:795-798.

167. **Møller A, Rasmussen LM, Thuesen L, Christiansen JS.** 1989 Impact of human growth hormone on plasma lipoprotein concentrations. Horm metab Res. 21:207-209.

168. **Seng G, Louisy P, Toussain P, Drouin P, Debry G.** 1989 Metabolic effects of a single administration of growth hormone on lipid and carbohydrate metabolism in normal-weight and obese subjects. Am J Clin Nutr. 50:1348-1354.

169. **Cuneo RC, Salomon F, Watts GF, Hesp R, Sönksen PH.** 1993 Growth hormone treatment improves serum lipids and lipoproteins in adults with growth hormone deficiency. Metabolism. 42:1519-1523.

170. **Mbewu AD, Durrington PN.** 1990 Lipoprotein(a): structure, properties and possible involvement in thrombogenesis and atherogenesis. Atherosclerosis. 85:1-14.

171. **Keller U, Miles JM.** 1991 Growth hormone and lipids. Horm Res. 36(Suppl 1):36-40.

172. **Edén S, Wiklund O, Oscarsson J, Rosén T, Bengtsson B-Å.** 1993 Growth hormone treatment of growth hormone-deficient adults results in a marked increase in Lp(a) and HDL cholesterol concentrations. Arterioscler Thromb. 13:296-301.

173. **Markussis V, Beshyah SA, Fisher C, Sharp P, Nicolaides AN, Johnston DG.** 1992 Detection of premature atherosclerosis by high-resolution ultrasonography in symptom-free hypopituitary adults. Lancet. 340:1188-1192.

174. **Lehman ED, Hopkins KD, Weissberger AJ, Gosling RG, Sönksen PH.** 1993 Aortic distensibility in growth hormone deficient adults. Lancet. 341:309.

175. **Brüel A, Oxlund H.** 1991 Biosynthetic growth hormone changes the collagen and elastin contents and biomechanical properties of the rat aorta. Acta Endocrinol (Copenh). 125:49-57.

176. **Shahi M, Beshyah SA, Hackett D, Sharp PS, Johnston DG, Foale RA.** 1992 Myocardial dysfunction in treated adult hypopituitarism: a possible explanation for increased cardiovascular mortality. Br Heart J. 67:92-96.

177. **Merola B, Cittadini A, Colao A, et al.** 1993 Cardiac structural and functional abnormalities in adult patients with growth hormone deficiency. J Clin Endocrinol Metab. 77:1658-1661.

178. **Thuesen L, Christiansen JS, Sørensen KE, Jørgensen JOL, Ørskov H, Henningsen P.** 1988 Increased myocardial contractility following growth hormone administration in normal man. Dan Med Bull. 35:193-196.

179. **Cuneo RC, Salomon F, Wilmshurst P, et al.** 1991 Cardiovascular effects of growth hormone treatment in growth-hormone-deficient adults: stimulation of the renin-aldosterone system. Cli Sci. 81:587-592.

180. **Hunt SM, McEwen J, McKenna SP.** 1986 Measuring health status. London:Croom Helm.

181. **Hunt SM, McEwen J, McKenna SP, Backett EM, Pope C.** 1984 Subjective health assessment and the perceived outcome of minor surgery. J Psychosom Res. 28:105-114.

182. **Hunt SM, McKenna SP, McEwen J, Williams J, Papp E.** 1981 The Nottingham Health Profile: subjective health status and medical consultations. Soc Sci Med. 15A:221-229.

183. **Essink-Bot M-L, van Agt HME, Bonsel GJ.** 1992 NHP of SIP: een vergelijkend onderzoek onder chronisch zieken. T Soc Gezondheidsz. 70:152-159.

184. **Björk S, Jönsson B, Westphal O, Levin J-E.** 1989 Quality of life of adults with growth hormone deficiency: a controlled study. Acta Paediatr Scand. 356:55-59.

185. **McGauley GA.** 1989 Quality of life assessment before and after growth hormone treatment in adults with growth hormone deficiency. Acta Paediatr Scand. 356:70-72.

186. **Rosén T, Wirén L, Wilhelmsen L, Wiklund I, Bengtsson B-Å.** 1994 Decreased psychological well-being in adult patients with growth hormone deficiency. Clin Endocrinol (Oxf). 40:111-116.

187. **Wilson DM.** 1992 Clinical actions of growth hormone. Endocrinol Metab Clin North Am. 21:519-537.

188. **Lippe BM, Nakamoto JN.** 1993 Conventional and nonconventional uses of growth hormone. Rec Prog Horm Res. 48:179-235.

189. **Frasier SD.** 1983 Human pituitary growth hormone (hGH) therapy in growth hormone deficiency. Endocrin Rev. 4:155-170.

190. **Hokken-Koelega ACS, Stijnen T, de Muinck Keizer-Schrama SMPF, et al.** 1991 Placebo-controlled, double blind, cross-over trial of growth hormone treatment in prepubertal children with chronic renal failure. Lancet. 338:585-590.

191. **Hokken-Koelega ACS, Stijnen T, de Jong MJCW, et al.** 1994 Double-blind trial comparing the effects of two doses of growth hormone in prepubertal patients with renal insufficiency. J Clin Endocrinol Metab. 79:1185-1190.

192. **Hokken-Koelega ACS, Stijnen T, de Ridder MAJ, et al.** 1994 Impressive growth with growth hormone therapy in severely growth-retarded adolescents after renal transplantation. Lancet. 343:1313-1317.

193. **Rosenfeld RG.** 1990 Non-conventional growth hormone therapy in Turner syndrome: the United States experience. Horm Res. 33:137-143.

194. **Snyder DK, Clemmons DR, Underwood LE.** 1988 Treatment of obese , diet restricted subjects with growth hormone for 11 weeks: effects on anabolism, lipolysis, and body composition. J Clin Endocrinol Metab. 67:54-61.

195. **Young VR, Steffee WP, Pencharz PB, Winterer JC, Scrimshaw NS.** 1975 Total human body protein synthesis in relation to protein requirements at various ages. Nature. 253:192-194.

196. **Forbes GB, Reina JC.** 1970 Adult lean body mass declines with age: some longitudinal observations. Metabolism. 19:653-663.

197. **Forbes GB.** 1976 The adult decline in lean body mass. Hum Biol. 48:161-173.

198. **Rudman D.** 1985 Growth hormone, body composition, and aging. J Am Geriatr Soc. 33:800-807.

199. **Young VR.** 1990 Protein and amino acid metabolism with reference to aging and the elderly. Prog Clin Biol Res. 326:279-300.

200. **Rudman D, Feller AG, Nagraj HS, et al.** 1990 Effects of human growth hormone in man over 60 years old. N Engl J Med. 323:1-6.

201. **Rudman D, Feller AG, Cohn L, Rudman IW, Draper MW.** 1991 Effects of human growth hormone on body composition in elderly man. Horm Res. 36(Suppl 1):73-81.

202. **Marcus R, Butterfield G, Holloway L, et al.** 1990 Effects of short term administration of recombinant human growth hormone to elderly people. J Clin Endocrinol Metab. 70:519-527.

203. **Holloway L, Butterfield G, Hintz RL, Gesundheit N, Marcus R.** 1994 Effects of recombinant human growth hormone on metabolic indices, body composition, and bone turnover in healthy elderly women. J Clin Endocrinol Metab. 79:470-479.

204. **Kaiser FE, Silver AJ, Morley JE.** 1991 The effect of recombinant human growth hormone on malnourished older individuals. J Am Geriatr Soc. 39:235-240.

205. **Borst SE, Millard WJ, Lowenthal DT.** 1994 Growth hormone, exercise, and aging: the future of therapy for the frail elderly. J Am Geriatr Soc. 42:528-535.

206. **Ward HC, Halliday D, Sim AJW.** 1987 Protein and energy metabolism with biosynthetic human growth hormone after gastrointestinal surgery. Ann Surg. 206:56-61.

207. **Ponting GA, Halliday D, Teale JD, Sim AJW.** 1988 Postoperative positive nitrogen balance with intravenous hyponutrition and growth hormone. Lancet. 1:438-440.

208. **Manson JM, Smith RJ, Wilmore DW.** 1988 Growth hormone stimulates protein synthesis during hypocaloric parenteral nutrition. Ann Surg. 208:136-142.

209. **Jiang Z-M, He G-Z, Zhang S-Y, et al.** 1989 Low-dose growth hormone and hypocaloric nutrition attenuate the protein-catabolic respons after major operation. Ann Surg. 210:513-525.

210. **Ziegler TR, Rombeau JL, Young LS, et al.** 1992 Recombinant human growth hormone enhances the metabolic efficacy of parenteral nutrition: a double-blind, randomized controlled study. J Clin Endocrinol Metab. 74:865-873.

211. **Ziegler TR, Young LS, Ferrari-Baliviera E, Demling RH, Wilmore DW.** 1990 Use of human growth hormone combined with nutritional support in a critical care unit. J P E N. 14:574-581.

212. **Petersen S, Holaday NJ, Jeevanandam M.** 1994 Enhancement of protein synthesis efficiency in parenterally fed trauma victims by adjuvant recombinant hunan growth hormone. J Trauma. 36:726-733.

213. **Voerman HJ, Strack van Schijndel RJM, Groeneveld ABJ, de Boer H, Nauta JP, van der Veen EA.** 1992 Effects of recombinant human growth hormone in patients with severe sepsis. Ann Surg. 216:648-655.

214. **Christensen H, Andreassen TT, Oklund H.** 1992 Increased mechanical strength of left colon in old rats treated with growth hormone. Gerontol. 38:245-251.

215. **Jørgensen PH, Bak B, Andreassen TT.** 1991 Mechanical properties and biochemical composition of rat cortical femur and tibia after long-term treatment with biosynthetic human growth hormone. Bone. 12:353-359.

216. **Seyer-Hansen M, Andreassen TT, Jørgensen PH, Oxlund H.** 1992 Influence of biosynthetic human growth hormone on the biomechanical strength development in skin incisional wounds of diabetic rats. Eur Surg Res. 25:162-168.

217. **Soroff HS, Pearson E, Green NL, Artz CP.** 1960 The effect of growth hormone on nitrogen balance at various levels of intake in burned patients. Surg Gynecol Obstet. 111:259-273.

218. **Herndon DN, Barrow RE, Kunkel KR, Broemeling L, Rutan RL.** 1990 Effects of recombinant human growth hormone on donor-site healing in severely burned children. Ann Surg. 212:424-431.

219. **Gilpin DA, Barrow RE, Rutan RL, Broemeling L, Herndon DN.** 1994 Recombinant human growth hormone accelerates wound healing in children with large cutaneous burns. Ann Surg. 220:19-24.

220. **Wolf RF, Pearlstone DB, Newman E, et al.** 1992 Growth hormone and insulin reverse net whole body and skeletal muscle protein catabolism in cancer patients. Ann Surg. 216:280-290.

221. **Mulligan K, Grunfeld C, Hellerstein MK, Neese RA, Schambelan M.** 1993 Anabolic effects of recombinant human growth hormopne in patients with wasting associated with human immunodeficiency virus infection. J Clin Endocrinol Metab. 77:956-962.

222. **Krentz AJ, Koster FT, Crist DM, et al.** 1993 Anthropometric, metabolic, and immunological effects of recombinant human growth hormone in AIDS and AIDS-related complex. J Acquir Immune Defic Syndr. 6:245-251.

223. **Watanabe S, Tsunematus Y, Fujimoto J, Komiyama A.** 1988 Leukemia in patients treated with growth hormone. Lancet. 1:159.

224. **Stahnke N.** 1992 Leukemia in growth hormone treated patients: an update. Horm Res. 38(Suppl 1):56-62.

225. **Malozowski S, Tanner LA, Wysowski D, Fleming GA.** 1993 Growth hormone, insulin-like growth factor I, and benign intracranial hypertension. N Engl J Med. 329:665-666.

226. **Cohn L, Feller AG, Draper MW, Rudman IW, Rudman D.** 1993 Carpal tunnel syndrome and gynaecomastia during growth hormone treatment of elderly men with low circulating IGF-I concentrations. Clin Endocrinol (Oxf). 39:417-425.

2 Adult Growth Hormone Deficiency Syndrome Patients Demonstrate Heterogeneity Between Childhood Onset and Adult Onset

ABSTRACT

Background and Methods .The recognition of a clinical syndrome of growth hormone deficiency (GHD) in adulthood is now gaining acceptance. Its onset may be during either childhood (CO) or adulthood (AO) but potential differences in patient characteristics and symptoms, as well as biochemical parameters have not previously been explored. In this study the baseline data from CO (idiopathic; N=74; mean age 29 yrs) and AO (acquired; N=99; mean age 44 yrs) patients enrolled in a trial of GH administration in GHD adults have been compared.

Results. Final height in CO patients was considerably less than in AO patients (161 vs. 171 cm; *P<0.001*), while their body weight was less (64 vs. 85 kg). Body mass index was lower in CO patients (24 ± 5 vs. 29 ± 5 kg/m^2; *P<0.001*) and lean body mass corrected by height was also lower (263 ± 65 vs. 333 ± 72 g/cm; *P<0.001*). Percent body fat was similar for both groups, waist/hip ratio was significantly less in CO than AO (0.90 ± 0.09 vs. 0.96 ± 0.06; *P<0.001*), but triceps skinfold thickness was not different. Serum IGF-I levels in CO were lower than in AO (55 ± 57 vs. 71 ± 35 ng/ml, *P<0.001*) and 92 % of CO and 85 % of AO patients had values below the normal range. The IGFBP-3 concentration was significantly lower in CO patients (1534 ± 1007 vs. 2393 ± 953 ng/ml, *P<0.001*) and 76% of CO but only 35% of AO had values below the normal range. The correlation between IGF-I and IGFBP-3 was much stronger in CO (r=0.93) than in AO (r=0.57). Osteocalcin levels were elevated in CO and normal in AO GHD patients (8.6 ± 3.3 vs. 5.6 ± 3.0 ng/ml; *P<0.001*; normal adult range 1.8 - 6.6). Serum lipid concentrations were much closer to normal values in CO than in AO patients: elevated cholesterol levels were observed in 52 % of CO, and 78 % of AO patients, while lowered HDL-cholesterol was found in 54 % of CO, and 74 % of AO patients. The quality of life (QoL), evaluated with the Nottingham Health Profile questionnaire, showed abnormal dimension scores for all 6 subscales both in CO and AO patients, but the degree of distress in the AO patients was significantly higher for physical mobility and energy levels in comparison to CO patients.

Conclusions. The results demonstrate considerable differences in the clinical and biochemical presentation of the adult GHD syndrome. Adult patients with idiopathic CO GHD show somatic underdevelopment similar to pituitary dwarfism: they remain too small, have generalized obesity and a lowered muscle mass, elevated osteocalcin levels, marginal abnormalities in their lipid metabolism and are relatively content with their life. However, somatic development is complete in AO GHD patients, who have previously experienced the full advantage of health and physical maturation. With GHD most of them become obese, with a central abdominal localization, and the majority have lowered HDL-cholesterol levels indicating the risk of premature atherosclerosis. Subjectively they experience a greater loss in their quality of life. These metabolic and body composition abnormalities as well as the subjective psychological distress caused by the lack of GH in previously healthy adult individuals suggests a beneficial effect of hGH replacement therapy in AO GHD. For CO GHD individuals hGH therapy might in retrospect have been better continued into adulthood after the end of skeletal growth in order to induce full somatic maturation.

INTRODUCTION

The estimated prevalence of growth hormone deficiency (GHD) in children in the western world is about 1 in 12,000, which amounts to 85 per million inhabitants (1). The additional prevalence of hypopituitarism acquired in adulthood, due to pituitary and peri-sellar tumors and/or after radiotherapy is unknown. It seems reasonable, however, to assume that a total of about 250 adults per million are GHD.

GHD in children can be easily recognized as a failure to grow and the diagnosis and therapy of childhood "hypopituitary dwarfism" have historically been focused on height assessment. The main goal of hGH therapy in children has always been to achieve a normal adult height. Other symptoms of childhood GHD such as lowered muscle mass/strength, fat accumulation, lowered bone density, and poor social adaptation, have not played an important role so far.

The concept of a clinically recognizable syndrome of GH deficiency in adulthood has gained widespread acceptance after the initial reports on the beneficial effects of long-term GH replacement therapy in GHD adults (2-4). Decreases in quality of life, muscle strength, bone mass, as well as changes in body composition indicating an increased amount of body fat were reported to be reversible with GH treatment in most patients (2,3,5-8). In addition, recent epidemiological studies indicate that there is an increased (doubled) incidence of cardiovascular mortality in patients with hypopituitarism who are receiving optimal replacement therapy with cortisol, thyroxin and sex steroids (8). This increased mortality seems to be directly related to the development of premature atherosclerosis in hypopituitary patients as indicated by a study with high resolution ultrasonic imaging of the carotid and femoral arteries (9). Measurements of serum lipids point to abnormalities in total cholesterol (increased) and HDL-cholesterol concentrations (decreased) in a considerable proportion of untreated GHD adults (2,10-12).

No studies have previously been designed to investigate whether the clinical syndrome related to GHD in adulthood might present with signs, symptoms and biochemical parameters which differ between patients who became GHD in childhood (childhood onset: CO) and patients who became GHD in adulthood (adult onset: AO). In the present study we examined a number of clinical and biochemical aspects of the GHD syndrome in adulthood in relation to time of onset.

METHODS

Patients

The results presented were obtained from the baseline assessment of 173 adult patients (18-60 years of age), who were enrolled to participate in a randomized, double-blind, placebo-controlled study on the safety and efficacy of hGH replacement therapy in adults with GHD. The study was sponsored and monitored by Lilly Research Centre Ltd. (Windlesham, Surrey, UK). It was a multinational, multicentre study with 12 participating institutions in 10 European countries.

Patients to be included in the study had GHD arising during adult life (AO) and present for at least one year, or had GHD arising and treated during childhood (CO) but had not been treated with hGH in the previous two years. For the diagnosis of GHD a peak GH value less than 5 g/l in a standard stimulation test was required as an inclusion criterion. IGF-I and/or IGFBP-3 values were not taken into account for the diagnosis. Replacement therapy with cortisol, thyroxin, sex steroids or vasopressin had to be stable for at least six months prior to the start of the study, and no patients with hypertension were included.

Anthropometric measurements

Height was measured for each patient and the distribution within each group was calculated and compared to the distribution of reference values measured for a normal, fully-grown population (13). Total body weight was also measured and the distribution for AO patients was compared to weight for height reference standards from a normal population (14). A similar comparison for the CO patients could not be carried out since the height distribution for this group was predominantly low and did not match a normal adult population. Weight ranges were also evaluated using body mass index (BMI; Quetelet's index) calculated from weight/height^2 (15). Waist circumference was measured at the level of the umbilicus and hip circumference at the level of the greater trochanter, and the waist/hip ratio was calculated for each patient. Skinfold thicknesses were measured with calipers, at the triceps, biceps, subscapular and suprailiac sites and for each patient these values were added together to give a sum of skinfolds value.

Body composition

Bioelectrical-impedance was measured and total body water was calculated from the formula provided by the manufacturer of the body composition analyzer (Holtain, Dyfed, UK) as:

$$\text{Total body water} = ((\text{Height}^2 / \text{Bioelectrical impedance}) \times 0.585) + 1.625$$

Assuming that fat mass is anhydrous and that lean body mass contains 730 g water/kg, then:

$$\text{Lean body mass} = \text{Total body water} / 0.73$$

Fat mass was calculated from the difference between total body weight and lean body mass (16,17).

Serum Biochemistry

Serum samples were taken from each patient in a fasting state. Insulin-like growth factor-I (IGF-I) was measured by an IGFBP-blocked RIA (18) using a kit supplied by BioMerieux (Nurtingen, Germany) and insulin-like growth factor binding protein-3 (IGFBP-3) was measured by a two-site immunoassay (19). Osteocalcin levels and insulin concentrations were measured by standard radioimmunoassays. Total cholesterol, high density lipoprotein (HDL) cholesterol, apolipoproteins A1 and B, triglyceride and glucose concentrations were each measured by standard techniques. Glycosylated hemoglobin (HbA_1C) was assessed by high-performance liquid chromatography using routine methods.

Quality of life assessment

QoL was assessed from the Hospital Anxiety and Depression scale (20) and from the Nottingham Health Profile (NHP) developed by Galen Research (Manchester, UK). The HAD scale is a self-rating scale which provides separate measures for the two constructs, anxiety and depression. For each construct a score below 8 is normal, a score between 8 and 10 is "borderline" and a score above 10 indicates a probable disorder of the relevant mood. The NHP data was analyzed by Galen Research for each of the subsections and these were compared for each group with scores found in other chronic illnesses. NHP scores are inversely related to the patient's QoL, i.e. a higher score indicates a worse QoL.

Statistical Methods

For the comparison of CO and AO, the analysis was performed on rank transformed data using an analysis of variance model, incorporating effects of onset, gender, and the onset-by-gender interaction. The following model was used:

$$Y_{ij}k = \mu + O_i + G_j + OG_{ij} + e_{ij}k$$

where: $Y_{ij}k$= measurement for patient k within onset group i and gender j, μ = overall mean, O_i = onset group i, G_j = gender j, OG_{ij} = onset-by-gender interaction, $e_{ij}k$= error term. Tests for differences between genders, for AO and CO patients separately, were made by using the overall mean square error from the full model using the CONTRAST statement in SAS (21). Spearman correlation coefficients were used to examine the linear relationship between two variables. In addition to the formal hypothesis tests, the distributions of certain parameters were summarized (using percentiles of the distribution), and compared to normative data available from the published literature.

RESULTS

Etiology and diagnostic criteria

The spectrum of causes of GHD (Table I; in 7 CO and 1 AO patients no etiological diagnosis was available) was entirely different between the two groups of patients. Idiopathic GHD, isolated or multiple, was reported in 89.5%, tumor in 4.5%, and other causes in 6% of CO GHD subjects. Concomitant medication of thyroxin, cortisol or sex steroids was used in 54%, 28%, and 60% respectively of all CO patients. The duration of GHD could not be determined, but in all CO patients the diagnosis had been made during early childhood, and all patients had been previously treated with hGH to final height. A minority of CO GHD adults had become GHD around puberty, mainly due to craniopharyngioma (4.4%).

(Peri-)pituitary tumors were reported as the cause of GHD in 82 % of AO cases with pituitary adenomas predominant, 56%, followed by

(continued page 60)

Table I. Etiology of Growth Hormone Deficiency (GHD) in Childhood and Adult Onset Patients.

Childhood Onset	n	%
Idiopathic		
Isolated growth hormone deficiency	19	28.4
Growth hormone plus TSH deficiency	7	10.4
Growth hormone plus LH/FSH deficiency	4	6.0
Multiple deficiency	30	44.8
Trauma, Empty sella, Post-tubercular condition	4	6.0
Craniopharyngioma, Dysgerminoma	3	4.4
Adult Onset		
Active Adenoma	30	30.6
Inactive Adenoma	25	25.5
Craniopharyngioma	19	19.4
Dysgerminoma, Pinealoma, Epidermoid Cyst	6	6.1
Post-Tubercular Condition, Histiocytosis	2	2.0
Trauma, Sheehan Syndrome, Empty Sella	9	9.2
Idiopathic, Hypothalamic Origin	7	7.2

Table II. Demographic, anthropometric and body composition data, and circulating IGF-I and IGFBP-3 levels in 173 adult GHD patients.

	Total		Males		Females	
	CO (n=74)	AO (n=99)	CO (n=55)	AO (n=61)	CO (n=19)	AO (n=38)
A. Demographic and anthropometric data						
Age (years)	28.8 ± 8	43.5 ± 9[a]	28.6 ± 8	43.5 ± 10	29.5 ± 8	43.4 ± 7
Height (cm)	160.8 ± 10.0	171.0 ± 6.9[a]	163.9 ± 10.0	176.1 ± 6.9[b]	151.9 ± 7.6	162.4 ± 5.5
Weight (kg)	63.7 ± 17	84.9 ± 17[a]	66.6 ± 17	88.7 ± 15[b]	55.4 ± 13	78.6 ± 19
BMI (kg/m^2)	24.5 ± 5.5	28.9 ± 5.2[a]	24.7 ± 6.0	28.5 ± 4.0[b]	23.9 ± 5.0	29.6 ± 6.0
Waist/Hip ratio	0.90 ± 0.09	0.96 ± 0.05[a]	0.91 ± 0.06	0.97 ± 0.06[b]	0.86 ± 0.15	0.93 ± 0.05[c]
B. Body composition						
Bio-impedance (ohm)	530 ± 90	447 ± 88[a]	506 ± 93	418 ± 80[b]	599 ± 86	493 ± 80[c]
Body fat mass (kg)	20.2 ± 10	27.6 ± 12[a]	19.8 ± 11	24.8 ± 11[b]	21.5 ± 10	32.3 ± 14[c]
Lean body mass (kg)	43.5 ± 12	57.2 ± 14[a]	46.8 ± 12	64.0 ± 13[b]	33.8 ± 5	46.3 ± 7[c]
Percent body fat	31.1 ±11	31.9 ± 13	28.9 ± 11	27.4 ± 12	37.5 ± 10	39.0 ± 11[c]
Sum of skinfolds (mm)	83.3 ± 37	87.5 ± 31	83.2 ± 41	80.3 ± 76	83.5 ± 28	99.3 ± 34[b,c]
C. Serum IGF-I and IGFBP-3 concentrations						
IGF-I (ng/ml) (normal 91-340)	56 ± 58	71 ± 35[a]	61 ± 62	82 ± 37	41 ± 42	53 ± 23[c]
IGFBP-3 (ng/ml) (normal 1950-3800)	1534 ± 1007	2393 ± 953	1564 ± 991	2462 ± 904	1446 ± 1073	2283 ± 1028
Osteocalcin (ng/ml) (normal 1.8-6.6)	8.6 ± 3.3	5.6 ± 3.0[a]	8.8 ± 3.5	6.1 ± 2.6	7.8 ± 2.5	4.9 ± 3.4

[a] *P<0.001* vs. CO
[b] *P<0.001* vs. CO Males
[c] *P<0.001* vs. AO Males

Table III Height distribution in CO and AO patients

	Percentile						
Height (cm)	**3%**	**10%**	**25%**	**50%**	**75%**	**90%**	**97%**
Adult Onset							
Males	165.0	167.0	171.9	175.5	182.0	186.5	188.0
Females	155.0	155.8	158.0	162.0	166.3	170.6	172.0
Childhood Onset							
Males	142.2	150.0	157.4	163.0	173.2	177.2	181.0
Females	137.8	141.5	145.2	153.0	158.3	163.2	164.0
Reference[a]							
Males	166.6	167.7	172.2	177.5	182.5	187.9	192.1
Females	151.6	156.0	160.8	165.1	168.2	171.9	175.1

[a]Reference values from Prader Swiss Growth Standard (13).

craniopharyngiomas, 19%. Concomitant medication of thyroxin, cortisol, and sex steroids was used in 83%, 79% and 86% of all AO patients, indicating a high incidence of other anterior pituitary hormonal insufficiencies. The duration of GHD in AO subjects, was 8.9 ± 7.1 years (range 0.45 - 32.1).

For the biochemical diagnosis of GHD, standardized GH provocation tests were used. The most frequently performed tests were the arginine (AO: n=31; CO: n=24), the insulin (AO: n=42; CO: n=3) and the clonidine test (AO: n=1; CO: n=31). Other test substances used were L-dopa, GHRH and glucagon. The highest reported peak GH values were 4.3 and 4.6 g/l in AO and CO patients respectively.

Demographic and Anthropometric Data

CO patients were significantly younger (*P<0.001*, Table IIA) and shorter (*P<0.001*) than AO GHD patients, and male patients were taller than females in both groups. The height distribution of the AO GHD patients was almost identical to that of the reference standard for both male and females. However, for the CO GHD patients approx. 50% of both male and females had height values below the 2 SD range for the normal adult reference population (Table III).

Body weights were significantly higher in AO than in CO GHD patients (*P<0.001*; Table IIA) and in both groups males were heavier than females (*P<0.001*). For AO subjects the distribution of body weights indicated that more than 25% of both males and females had values higher than the upper 2SD range of the reference population (14). No adult weight reference standard can be used for a height distribution similar to that of the CO subjects.

CO patients had a significantly (*P<0.001*) lower BMI compared to AO GHD patients (Table IIA), although again it is difficult to compare BMI values between the groups due to the short stature of CO subjects. In AO, almost 40% of the patients, males as well as females, had a BMI value above 30 kg/m^2 (> 85% for a normal population = obesity grade II), (22) while more than 10% of them had a BMI value higher than 36 (> 95% for a normal population obesity grade III). In CO subjects, only 10% of the patients had a value higher than 30 kg/m^2. Three of 74 CO patients (4.1%) and 21/99 AO GHD patients (21.2%) had a body weight above 100 kg.

The waist/hip ratio, shown in Table IIA, was significantly (*P<0.001*) lower in CO individuals compared to AO patients, though both CO and AO subjects had a waist/hip ratio significantly (*P<0.001*) higher than the value reported in normal individuals, 0.85 ± 0.04 (22,23). There was also a significant gender difference in waist/hip ratio for both CO and AO groups, males having significantly higher values than females (*P=0.022* and *P=0.007* respectively).

Body Composition

Mean values of body composition measurements are presented in Table IIB. Although on a weight basis CO subjects had less body fat than AO GHD patients (*P<0.001*), the values for percentage body fat were very similar between the two groups, females having a higher amount than males in both CO and AO (Table IV). Compared with values from a normal age- and sex-matched population assessed with the same methodology, (24-28) CO as well as AO patients have significantly more body fat mass and percent body fat.

CO subjects also had significantly less lean body mass than AO on a net weight basis (Table IIB) and males had significantly (*P<0.001*) higher values than females

in each group. Total lean body mass in CO GHD individuals was decreased (*P<0.01*) in comparison with reference standards, (56.5 ± 2.3 kg) (24-26) but in AO GHD patients lean body mass was not different from the average of the reference population. Calculation of the lean body mass /height, in g/cm, (29) also indicated that CO patients had significantly less lean body mass per unit height than AO patients (Table IV), males having a higher value than females in both groups. On the percentile distribution, the 90% value for both sexes in CO, 337 g/cm, lies between the 50% and 75% value for both sexes in AO, which indicates that per unit height AO subjects may have up to 20% more lean mass. In AO, the range of values of g/cm is not different from that of an adult age-matched reference population in which lean body mass was assessed by hydrodensitometry (28).

Additional information on body fat distribution was obtained from skinfold measurements. There were no significant differences between AO and CO for sum of skinfolds (Table IIB) or for the distribution of triceps skinfold thickness.

IGF-I and IGFBP-3

Mean IGF-I levels (Table IIC) were lower in CO than in AO GHD patients (*P<0.001*) although the level for each group was below the normal adult reference range (18). The individual IGF-I values in AO and CO plotted versus age are presented in Figure 1 in relation to the 5% and 95% range of a normal population (18). IGF-I levels were below the 2SD range in 92% of the CO patients and in 85% of the AO patients. Interestingly IGF-I levels in males were significantly higher than in females in AO (*P<0.001*) but not in CO (*P=0.172*). In CO but not in AO patients IGF-I levels were significantly and negatively correlated with age (Table V). The decline with age in CO subjects is dramatic: at age 40 to 50 IGF-I levels are in the range measured in normal subjects of age 80 years and beyond (18). Although in AO there was no correlation with age, there was a significant negative correlation with duration of GHD (Table V).

Table IV Distributions of body composition parameters

		Percentile				
Percent body fat (%)		**10%**	**25%**	**50%**	**75%**	**90%**
Total	CO	19.0	27.1	32.0	37.7	41.7
	AO[a]	17.8	26.1	33.4	40.5	45.7
Males	CO	17.5	26.0	30.3	34.3	40.1
	AO	15.7	23.0	30.0	35.1	39.0
Females	CO[b]	23.6	30.8	37.7	43.3	53.1
	AO[c]	27.2	34.3	41.6	45.7	51.4
Lean body mass / Height (g/cm)						
Total	CO	202	233	258	297	337
	AO[a]	250	281	315	376	439
Males	CO	221	247	266	324	342
	AO	276	308	354	415	464
Females	CO[b]	169	201	227	250	263
	AO[c]	225	257	281	310	338
Triceps skinfold thickness (mm)						
Total	CO	10.0	13.5	19.0	26.0	34.0
	AO	10.1	12.2	16.2	21.2	33.0
Males	CO	9.2	11.5	15.6	19.2	34.6
	AO	9.7	12.4	16.0	23.0	25.2
Females	CO[b]	12.0	16.4	19.0	27.8	32.4
	AO[c]	15.0	19.0	26.0	32.2	42.2

[a] *P<0.05*, CO vs AO;
[b] *P<0.05*, CO male vs CO female
[c] *P<0.05*, AO male vs AO female.

Table V Correlation of IGF-I and IGFBP-3 levels with demographic and body composition data

	Childhood Onset				Adult Onset			
	IGF-I		IGFBP-3		IGF-I		IGFBP-3	
	r	*P*	r	*P*	r	*P*	r	*P*
Duration	-	-	-	-	-0.35	<0.001	-0.29	0.007
Age	-0.53	<0.001	-0.53	<0.001	-0.18	0.078	- 0.05	0.650
Height	0.30	0.010	0.32	0.005	0.52	<0.001	0.23	0.035
Weight	0.34	0.003	0.37	0.001	0.16	0.111	0.21	0.035
Lean body mass	0.46	<0.001	0.47	<0.001	0.36	<0.001	0.29	0.004

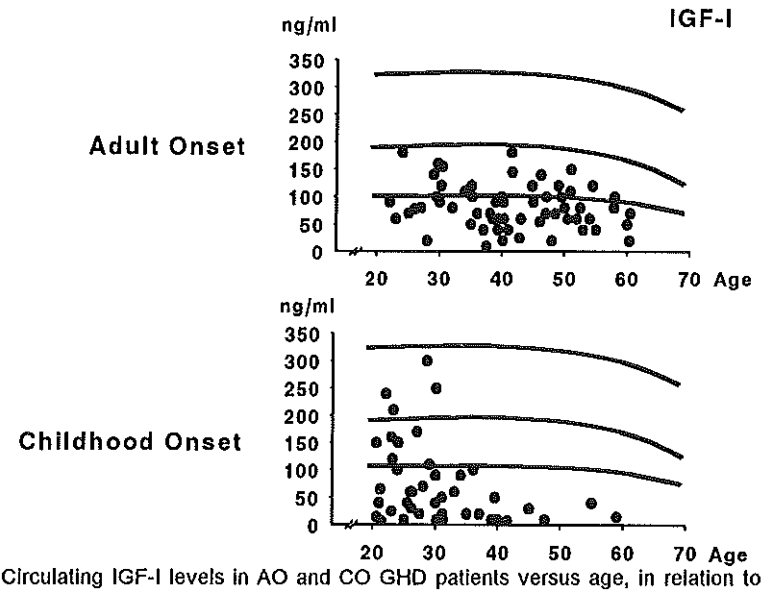

Fig.1 Circulating IGF-I levels in AO and CO GHD patients versus age, in relation to the 5% and 95% range of a normal population

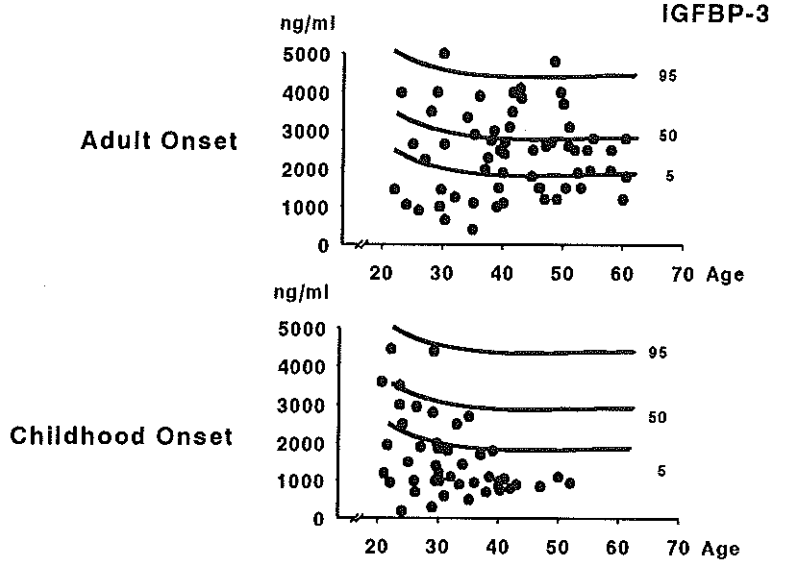

Fig.2 Circulating IGFBP-3 levels in AO and CO GHD patients versus age, in relation to the 5% and 95% range of a normal population

Mean IGFBP-3 levels were significantly higher in AO compared to CO (*P<0.001*) but there was no gender difference within each group. Individual IGFBP-3 values in AO and CO, plotted versus age, are presented in Figure 2 and were below the normal range in 76% of CO patients and in 35% of AO patients. Similar to IGF-I, IGFBP-3 levels were negatively correlated with duration of GHD in AO (*P=0.007*), and with age in CO (*P<0.001*).

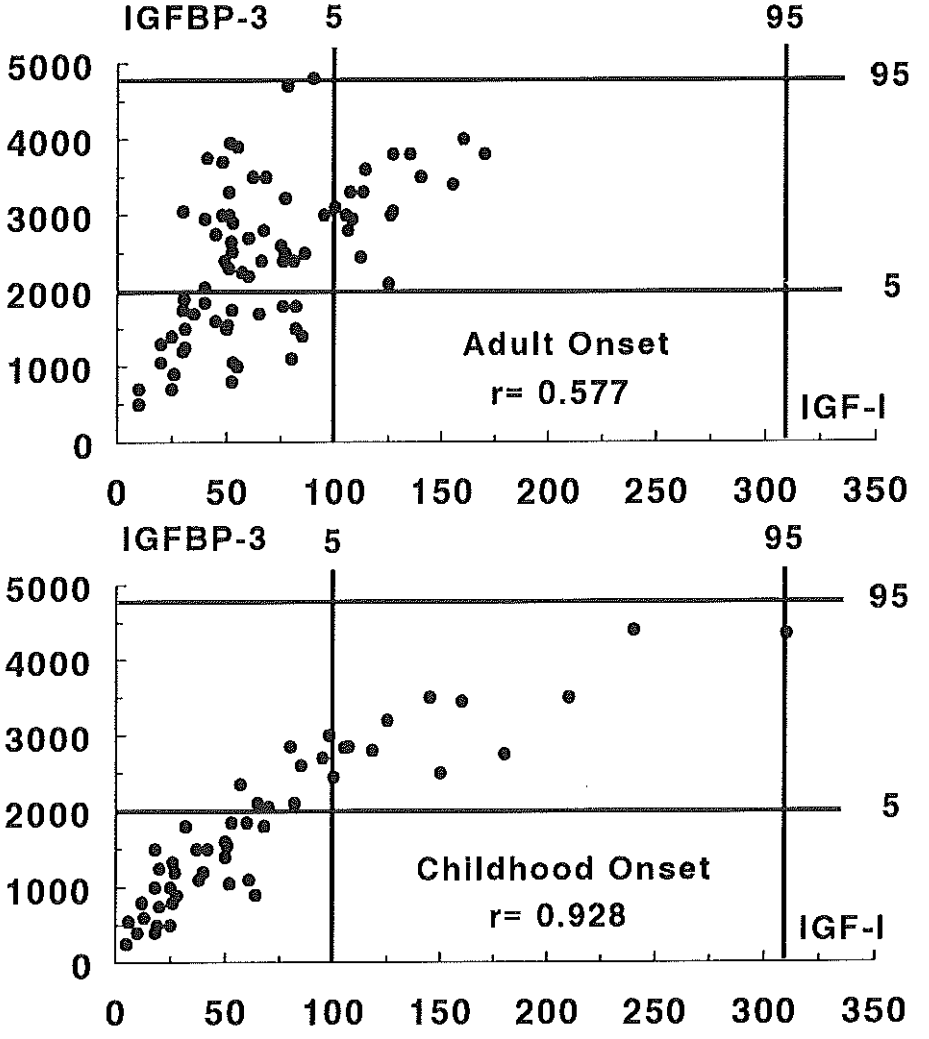

Fig.3 The relationship between IGF-I and IGFBP-3 levels in AO (r=0.58; *P < 0.001*) and CO GHD patients (r=0.93; *P < 0.001*).

In Figure 3 the relationship between IGF-I and IGFBP-3 levels is shown for CO and AO GHD patients, separately. In each group IGF-I and IGFBP-3 were significantly correlated, but the correlation was much stronger in CO (r=0.93; *P<0.001*) than in AO patients (r=0.58; *P<0.001*). Both IGF-I and IGFBP-3 values were positively correlated with height, weight and lean body mass in CO patients, but for weight this relationship was less clear in AO (Table V).

Osteocalcin

The mean serum osteocalcin level in CO GHD patients was 8.5 ± 3.3 ng/ml, which was above the normal range (Table IIC) and significantly (*P<0.001*) greater than the value in AO (5.6 ± 2.9 ng/ml) which was within the normal range. Individual analysis showed that elevated serum osteocalcin levels were present in 73% of CO and 30% of AO GHD patients.

Lipid values

Fasting serum cholesterol and other lipid concentrations are summarized in Table VIA. In comparison to CO subjects, the AO patients had significantly higher total cholesterol (*P<0.001*) levels and marginally lower HDL cholesterol values (*P=0.071*). Analysis of the total cholesterol concentrations in comparison with age- and sex-matched controls showed that 53% of the CO and 78% of the AO GHD adults had levels above the normal range. HDL-cholesterol levels were below normal in 54% of CO and 70% of AO individuals. In AO subjects HDL-cholesterol levels were negatively correlated to body weight (r=-0.32, *P=0.002*), BMI (r= -0.27, *P=0.01*) and waist/hip ratio (r=-0.25, *P=0.02*). Similar correlations were found for CO although they were less significant. There was no significant difference in mean serum apolipoprotein A_1 between the groups but apolipoprotein B was significantly (*P=0.022*) less in CO than in AO.

Table VI Lipid values and glucose homeostasis in GHD patients

	Total		Males		Females	
	CO	AO	CO	AO	CO	AO
A. Lipids						
Total cholesterol (mg/dl) (normal 150-200)	217 ± 54	240 ± 53[a]	212 ± 55	237 ± 52	227 ± 49	247 ± 55
HDL-cholesterol (mg/dl) (normal males: 35-55, females: 45-65)	35.9 ± 12	31.9 ± 11	35.0 ± 11	30.0 ± 10	38.2 ± 14	35.3 ± 11
Apolipoprotein A1 (mg/dl) (normal 115-190)	148 ± 25	148 ± 29	147 ± 24	141 ± 28	152 ± 26	161 ± 28
Apolipoprotein B (mg/dl) (normal 70-160)	124 ± 38	140 ± 43	123 ± 39	137 ± 37	126 ± 39	145 ± 54
Triglycerides (mg/dl) (normal 40-175)	130 ± 88	191 ± 171	140 ± 98	183 ± 101	106 ± 53	205 ± 252
B. Glucose Homeostasis						
Fasting glucose (mmol/l)	4.6 ± 0.9	4.4 ± 0.8	4.7 ± 0.8	4.4 ± 0.9	4.3 ± 1.1	4.3 ± 0.6
Fasting insulin (mU/ml)	13.9 ± 8.1	16.7 ± 11.6	14.5 ± 8.6	15.6 ± 8.4	12.9 ± 6.7	18.8 ± 15.4
HbA_1C (%)	5.02 ± 0.66	5.16 ± 0.54[a]	5.07 ± 0.71	5.18 ± 0.52	4.85 ± 0.44	5.13 ± 0.58

[a] *P< 0.001* vs CO

Glucose Homeostasis

Mean fasting glucose, insulin and glycosylated hemoglobin (HbA_1C) concentrations are shown in Table VIB. There were no significant differences between CO and AO patients in the measurements for fasting glucose and insulin. Fasting insulin concentrations in AO subjects were significantly correlated with weight (*P<0.001*), BMI (*P<0.001*), body fat mass (*P<0.001*) and waist/hip ratio (*P=0.016*) but in CO these correlations were absent or much weaker. HbA_1C levels were lower in CO than in AO patients (*P=0.031*), but were within the normal range. Glucose levels measured 120 min. after an oral glucose load were above 7.8 mmol/l in 3/61 (4.7%) of CO and in 11/85 (11.5%) of AO patients. This prevalence of impaired glucose tolerance is not different from the normal population.

Quality of life assessment

For the HAD measurements, 1/65 (1.5%) of the CO patients had a score between 8-10 and no patients higher than 10. In the AO group there were 10/97 (10.3%) patients with a score between 8 and 10, and 6/97 (6.2%) patients with a score higher than 10. The incidence of anxiety and depression in AO GHD patients was higher, than in CO individuals (*P<0.01* and *P<0.01* respectively).

Baseline mean NHP dimension scores are presented in Table VII, together with the 95% confidence intervals of these data. Also shown are the normal values of a reference population, derived from a large survey of a random population in central England (30-32) and carefully matched with regard to age and gender with each group of GHD patients in this study. Both the CO and AO onset patient groups score higher than the reference population on all six sections of the NHP. It is also evident that the CO patients generally had lower levels of distress than the AO patients. These differences were statistically significant for the physical mobility and energy level sections (Table VII).

In Figure 4 the mean baseline NHP scores for these AO and CO GHD patients are compared with mean scores for patient groups with other chronic illnesses: rheumatoid arthritis (33), breast cancer (34), ischemic heart disease (35), and diabetes (36).

Table VII. Baseline mean NPH scores and 95% confidence intervals (CI) in CO and AO patients, as well as the mean reference level of age- and sex-matched controls

	Childhood onset			Adult onset		
	Mean ± SD	95% CI	Reference level	Mean ± SD	95% CI	Reference level
Social isolation	5.9 ± 10.5	3.2 - 8.6	4.6	6.0 ± 13.0	4.7 - 10.1	5.1
Physical mobility	10.5 ± 14.0	4.7 - 12.9	1.4	18.4 ± 21.9[a]	12.8 - 21.7	3.4
Emotional reaction	17.5 ± 15.0	9.4 - 18.6	8.5	15.8 ± 14.6	11.3 - 18.1	9.6
Energy level	15.8 ± 25.7	8.6 - 20.9	6.4	24.2 ± 29.2[a]	22.0 - 34.7	12.0
Sleep	15.8 ± 18.4	9.4 - 20.1	8.5	18.5 ± 17.8	15.8 - 25.6	12.1
Pain	10.5 ± 15.7	4.5 - 11.9	2.8	11.6 ± 17.5	6.3 - 12.7	5.0

[a] *P<0.01* vs CO

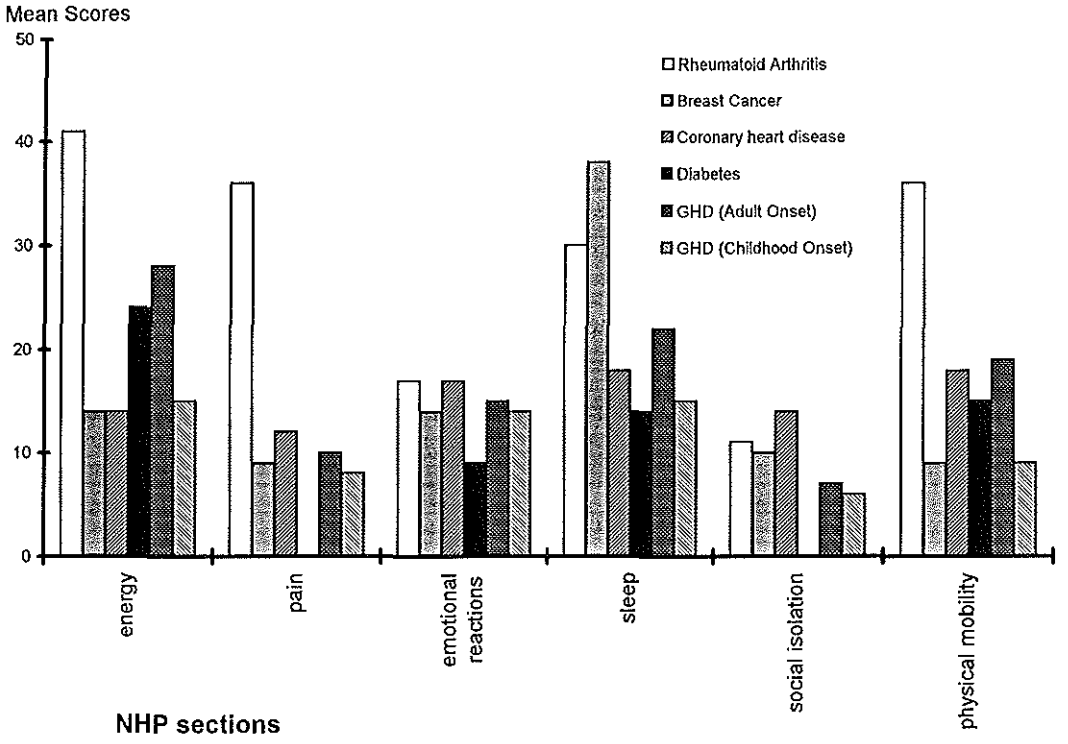

Fig. 4 A comparison of the mean scores of the sections of the Nottingham Health Profile questionnaire for Quality of Life in a number of chronic diseases and those in patients with adult onset and childhood onset Growth Hormone Deficiency (GHD).

DISCUSSION

The results described in the present study confirm the existence of "the clinical syndrome of GHD in adulthood", as defined by Sönksen's group (4). However, the pathogenesis and natural history of the GHD disease is different between AO and CO patients and although both groups exhibit symptoms of this syndrome, the magnitude and quality of the symptoms differ substantially between the two. Idiopathic growth hormone deficiency is the most common cause of GHD in childhood (2-4) and this is confirmed in our adult CO population in which only a

small percentage had acquired GHD. In AO patients, GHD was almost entirely due to pituitary organic disease and its pathogenesis and diagnosis are well defined by clinical criteria.

Decreased or low normal IGF-I levels have been reported previously in adult patients with GHD (37-39). Most of the circulating IGF-I is bound to specific binding proteins (IGFBPs), the predominant form in blood being IGFBP-3 (40,41). In childhood and adolescence, IGFBP-3 measurements are reliable indicators of GH secretory activity and a useful tool for GHD diagnosis (18). In the present patient population the IGF-I and IGFBP-3 levels clearly separate the two entities of CO and AO GHD. In CO patients, both levels were significantly lower than in AO patients and were strongly correlated, i.e. the relationship remained similar to that found in childhood (18). Also, their actual values were strongly correlated with weight, lean body mass, height and age of the patients. In AO patients, IGF-I and IGFBP-3 values did not correlate as strongly as in CO and their correlations with height, weight and lean body mass were less significant. Moreover, there was no correlation with age but only with GHD duration. It would seem that in AO patients the regulation of IGF-I and IGFBP-3 is different to that in CO. It follows that IGF-I, and certainly IGFBP-3, cannot be used in a straightforward manner for the diagnosis of adult onset GHD as established in pediatric patients.

Anthropometric and body composition measurements describe the clinical presentation of the adult GHD syndrome and of its deviation from normal, but also provide additional distinguishing features between the two types of onset. The major difference in the clinical presentation relates to height. About 50% of CO GHD adults had a final height which was 2 or more SD below the average of a normal adult population despite hGH treatment during childhood and adolescence.

One consequence of GHD is the accumulation of fat and obesity and in AO subjects this can be easily documented and quantified. Body weight, BMI, and waist/hip ratio compared to adult reference standards clearly demonstrated the high degree of obesity and its central (abdominal) accumulation. Although objective anthropometric evaluation is more difficult in CO subjects since adult reference standards do not correct for height, and pediatric reference standards do not correct for age and

maturation, it appears that these patients also were overweight. In fact the relative amount of body fat was virtually the same in both AO and CO, either measured by BIA or derived from skinfold thickness. However, AO patients had a significantly higher waist/hip ratio than CO which indicates that the distribution of body fat was not the same in the two groups, CO having less central fat accumulation than AO.

In absolute terms CO subjects had significantly less lean body mass than AO. Correction of lean body mass for height (g/cm) separates CO from AO patients even better. While in AO subjects the amount of lean body mass appeared to be appropriate for their height, this was not the case in CO. In spite of the fact that CO patients were younger than AO patients, their very low value for LBM/height indicates that they may not have attained adult muscle mass.

The smaller height, the decreased lean body mass and the amount and distribution of obesity of the CO GHD adult patients described indicate that the majority of them retained the full features of the clinical entity of hypopituitary dwarfism and in comparison to AO subjects they were somatically underdeveloped. It should be pointed out that the majority of our CO patients belong to a cohort of patients which had been treated with pituitary derived hGH, and hence could not benefit from the optimized therapy with biosynthetic hGH. Final height results in GHD children after hGH therapy have constantly improved since the advent of recombinant hGH, and a younger cohort of CO subjects may well have attained a better degree of somatic development than the patients of the present study (42). However, as a rule hGH therapy is being terminated with epiphyseal closure, and therefore the further steps to attain full somatic maturation, dependent upon the anabolic action of hGH, are not induced and completed.

The lipid values in our GHD adults confirm a considerable prevalence of a mild degree of hypercholesterolemia and lowered HDL-cholesterol levels. The mean baseline cholesterol levels for each group were slightly elevated above the mean normal values, while the difference in total cholesterol between CO and AO individuals could be attributed to group age differences. Lowered HDL cholesterol concentrations are considered to be an important risk-factor for atherosclerosis and

an increased incidence of premature atherosclerosis (43), as well as an increased mortality from cardiovascular causes have been reported in hypopituitary patients (8). This may be particularly important in idiopathic GHD where left ventricular mass has been shown to be reduced (44) although in patients who acquire GHD as adults ventricular mass was normal (45). The clustering of several metabolic risk factors for cardiovascular diseases in a single subject has been studied (46). These risk factors include lowered HDL-cholesterol, raised insulin, and raised triglyceride levels, as well as obesity, increased abdominal fat and hypertension. In the present study AO subjects exhibited clear correlations between HDL cholesterol levels and body weight, BMI, and waist/hip ratio, as well as between fasting serum insulin concentrations and weight, fat mass and waist/hip ratio. These correlations were much weaker or even absent in CO. The data do not support the concept that the same clustering of risk factors which has been referred to as syndrome X (46), the deadly quartet (47), or the insulin resistance syndrome (48) might exist in untreated GHD adults. Apart from the fact that no hypertensive patients were included in this study, very few of them had elevated fasting insulin levels or hypertriglyceridemia. We think that the abnormalities described are a genuine expression of the missing action of GH and are typically found in AO subjects in which GH withdrawal causes an acute imbalance of a previously normal metabolic control. In line with this, in CO subjects these abnormalities are much less pronounced, as these patients have adapted their metabolic control to GH deficiency during development.

Quality of life as assessed by the Nottingham Health Profile also demonstrated differences in the presentation of the adult GHD syndrome between CO and AO subjects. Like all generic measures the NHP contains items which might not be relevant to the specific health problems of adult GHD patients, and therefore may lack sensitivity (49,50). However, it was the only available questionnaire validated in the different countries participating into the study, it contained quality of life dimensions related to the biological effects of GH (e.g. physical mobility), and it has been used previously in the evaluation of GHD patients (49-52). The NHP scores in the present group of 173 GHD individuals demonstrated that these patients were under severe distress. This is documented in Figure 4 where the NHP scores of the

GHD patients are compared to the scores from patients with a number of other diseases.

The scores for the AO group were higher than those for CO in most dimensions, and the differences were statistically significant for physical mobility and energy level. This confirms the findings of Hunt et al (53) obtained from interviews with GHD patients, as well as other published data (51,52). It seems that most CO GHD patients have continuously adapted their lifestyle during growth and maturation to allow for their deficiency. In contrast, in AO patients the illness causing GHD has acutely affected a previously normal adult lifestyle. In line with the NHP findings, the HAD scale, which rates anxiety and depression, indicated a difference in these psychiatric variables between CO and AO GHD patients.

In conclusion, adult onset and childhood onset GHD are two separate entities. The CO GHD syndrome is a developmental disorder; the clinical and biochemical findings indicate that they could not achieve full somatic maturation and suggest that their lifespan might be shortened by this missing stage. Unlike the CO GHD syndrome, the AO GHD syndrome is a metabolic disorder. The patients, who previously have gone through a developmental lifetime of normal GH secretion and attained complete physical maturation are upset by the withdrawal of normal endogenous GH secretion which produces a metabolic imbalance and their QoL is affected.

When treating adult hypopituitary patients with hGH (and other necessary hormones) these differences between AO and CO should be kept in mind because the goal of therapy is different: induce and maintain full somatic development in CO patients, and restore normal QoL and prevent metabolic complications in AO patients.

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REFERENCES

1. **Wit JM.** 1986 Responses to growth hormone therapy. Thesis. Utrecht, The Netherlands.

2. **Salomon F, Cuneo RC, Hesp R, Sönksen PH.** 1989 The effects of treatment with recombinant human growth hormone on body composition and metabolism in adults with growth hormone deficiency. N Engl J Med. 321:1797-1803.

3. **Jørgensen JOL, Pedersen SA, Thuesen L, Jørgensen J, Skakkebæk NE, Christiansen JS.** 1989 Beneficial effects of growth hormone treatment in GH-deficient adults. Lancet. 1:1221-1225.

4. **Cuneo RC, Salomon F, McGauley GA, Sönksen PH.** 1992 The growth hormone deficiency syndrome in adults. Clin Endocrinol (Oxf). 37:387-397.

5. **Binnerts A, Swart GR, Wilson JH, et al.** 1992 The effect of growth hormone administration in growth hormone deficient adults on bone, protein, carbohydrate and lipid homeostasis, as well as on body composition. Clin Endocrinol (Oxf). 37:79-87.

6. **Cuneo RC, Salomon F, Wiles CM, Hesp R, Sönksen PH.** 1991 Growth hormone treatment in growth hormone-deficient adults. I. Effects on muscle mass and strength. J Appl Physiol. 70(2):688-694.

7. **Bengtsson B-Å, Edén S, Bosaeus I, et al.** 1993 Treatment of adults with growth hormone deficiency with recombinant human growth hormone. J Clin Endocrinol Metab. 76:309-317.

8. **Rosén T, Bengtsson B-Å.** 1990 Premature mortality due to cardiovascular disease in hypopituitarism. Lancet. 336:285-288.

9. **Markussis V, Beshyah SA, Fisher C, Sharp P, Nicolaides AN, Johnston DG.** 1992 Detection of premature atherosclerosis by high-resolution ultrasonography in symptom-free hypopituitary adults. Lancet. 340:1188-1192.

10. **de Boer H, Blok GJ, Voerman HJ, Phillips M, Schouten JA.** 1994 Serum lipid levels in growth hormone-deficient men. Metabolism. 43:199-203.

11. **Cuneo RC, Salomon F, Watts GF, Hesp R, Sönksen PH.** 1993 Growth hormone treatment improves serum lipids and lipoproteins in adults with growth hormone deficiency. Metabolism. 42:1519-1523.

12. **Edén S, Wiklund O, Oscarsson J, Rosén T, Bengtsson B-Å.** 1993 Growth hormone treatment of growth hormone-deficient adults results in a marked increase in Lp(a) and HDL cholesterol concentrations. Arterioscler Thromb. 13:296-301.

13. **Prader A, Largo RH, Molinari C, Issler C.** 1989 Physical growth of Swiss children from birth to 20 years of age. Helv Pae Acta. Suppl 52.

14. **Karlberg J, Mossberg H-O.** 1991 Weight-for-height standards in adulthood. J Intern Med. 229:303-308.

15. **Must A, Dallal GE, Dietz WH.** 1991 Reference data for obesity: 85th and 95th percentiles of body mass index (wt/ht^2) and triceps skinfold thickness. Am J Clin Nutr. 53:839-846.

16. **Lukaski HC, Bolonchuk WW, Hall CB, Siders WA.** 1986 Validation of tetrapolar bioelectrical impedance method to assess human body composition. J Appl Physiol. 60:1327-1332.

17. **Lukaski HC, Johnson PE, Bolonchuk WW, Lykken GI.** 1985 Assessment of fat-free mass using bioelectrical impedance measurements of the human body. Am J Clin Nutr. 41:810-817.

18. **Blum WF, Albertsson-Wikland K, Rosberg S, Ranke MB.** 1993 Serum levels of insulin-like growth factor I (IGF-I) and IGF binding protein 3 reflect spontaneous growth hormone secretion. J Clin Endocrinol Metab. 76:1610-1616.

19. **Strasburger CJ, Dressendörfer RA, Lee PDK.** 1994 Non-isotopic two site immunoassay for IGFBP-3. Growth Reg. 4 (Suppl 1):138.

20. **Snaith T.** 1985 Rating scales for depressionand anxiety: a current perspective. Brit J Clin Pharmacol. 19(Suppl 1):17-21.

21. **SAS Institute Inc, SAS/STAT.** 1989 User Guide, version 6. Cary NC: 891.

22. **Garrow J.S.** 1992 Obesity: definition assessment and dietary management. In: Grossman A. ed. Clinical Endocrinology, Blackwell Scientific Publications; 857-862.

23. **de Boer H, Blok GJ, Voerman HJ, de Vries PM, van der Veen EA.** 1992 Body composition in adult growth hormone-deficient man, assessed by anthropometry and bioimpedance analysis. J Clin Endocrinol Metab. 75:833-837.

24. **Binnerts A, Deurenberg P, Swart GR, Wilson JH, Lamberts SWJ.** 1992 Body composition in growth hormone-deficient adults. Am J Clin Nutr. 55:918-923.

25. **Deurenberg P, van der Kooij K, Evers P, Hulshof T.** 1990 Assessment of body composition by bioelectrical impedance in a population aged > 60 y. Am J Clin Nutr. 51:3-6.

26. **Deurenberg P, van der Kooij K, Leenen R, Weststrate JA, Seidell JC.** 1990 Sex and age specific prediction formulas for estimating body composition from bioelectrical impedance: a cross validation study. Int J Obesity. 15:17-25.

27. **Valk NK, van der Lely AJ, de Herder WW, Lindemans J, Lamberts SWJ.** 1994 The effects of human growth hormone (GH) administration in GH-deficient adults: a 20-day metabolic ward study. J Clin Endocrinol Metab. 79:1070-1076.

28. **Beshyah SA, Freemantle C, Thomas E, et al.** 1995 Abnormal body composition and reduced bone mass in growth hormone deficient hyopituitary adults. Clin Endocrinol (Oxf). 42:179-189.

29. **Barlett HL, Puhl SM, Hodgson JL, Buskirk ER.** 1991 Fat-free mass in relation to stature: ratios of fat-free mass to height in children, adults, and elderly subjects. Am J Clin Nutr. 53:1112-1116.

30. **Hunt SM, McEwen J, McKenna SP.** 1986 Measuring health status. London: Croom Helm.

31. **Hunt SM, McEwen J, McKenna SP, Backett EM, Pope C.** 1984 Subjective health assessment and the perceived outcome of minor surgery. J Psychosom Res. 28:105-114.

32. **Hunt SM, McKenna SP, McEwen J, Williams J, Papp E.** 1981 The Nottingham Health Profile: subjective health status and medical consultations. Soc Sci Med. 15A:221-229.

33. **Fitzpatrick R, Ziebland S, Jenkinson C, Mowat A.** 1992 A generic health status instrument in the assessment of rheumatoid arthritis. Brit J Rheumatology. 31:87-90.

34. **Hunt SM, Moriarty M.** 1984 Use of perceived health within a group of cancer patients. London: Croom Helm.

35. **Department of Health and Social Services.** 1985 Costs and benefits of the heart transplant programmes at Harefield and Papworth Hospitals. HMSO London.

36. **Dorman TL, Peck GM, Dow JDC, Tattersall RB.** 1992 A community survey of diabetes in the elderly. Diabetic Medicine. 9:860-865.

37. **Whitehead HM, Aiken B, Lewis S, Sheridan B, Hadden DR.** 1991 Physiological growth hormone secretion in adult growth hormone deficiency: comparison with normal controls. Clin Endocrinol (Oxf). 34:371-376.

38. **Jørgensen JOL.** 1991 Human growth hormone replacement therapy: pharmacological and clinical aspects. Endocrin Rev. 12:189-207.

39. **Hoffman DM, O'Sullivan AJ, Baxter RC, Ho KKY.** 1994 Diagnosis of growth-hormone deficiency in adults. Lancet. 343:1064-1068.

40. **Clemmons DR, Van Wyk JJ.** 1984 Factors controlling blood concentration of somatomedin C. Clin Endocrinol Metab. 13:113-143.

41. **Baxter RC.** 1993 Circulating binding proteins for the insulin-like growth factors. Trends Endocrinol Metab. 4:91-96.

42. **Brämswig JH, Schlosser H, Heise K.** 1995 Final height in children with growth hormone deficiency. Horm Res. 43:126-128.

43. **Kannel WB, Castelli WP, Gordon T, McNamara PM.** 1971 Serum cholesterol, lipoproteins and the risk of coronary artery disease. Ann Intern Med. 74:1-12.

44. **Merola B, Cittadini A, Colao A, et al.** 1993 Cardiac structural and functional abnormalities in adult patients with growth hormone deficiency. J Clin Endocrinol Metab. 77:1658-1661.

45. **Shahi M, Beshyah S, Hackett D, Sharp P, Johnston D, Foale R.** 1991 Cardiac structure and function in growth hormone deficiency. Br Heart J. 66:58.

46. **Reaven GM.** 1988 Role of insulin resistance in human disease. Banting lecture 1988. Diabetes. 37:1595-1607.

47. **Kaplan NM.** 1989 The deadly quartet: upper body obesity, glucose intolerance, hypertriglyceridemia and hypertension. Arch Intern Med. 149:1514-1520.

48. **Laakso M.** 1993 The possible pathophysiology of insulin resistance syndrome. Cardiovascular Risk Factors. 3:55-66.

49. **Björk S, Jönsson B, Westphal O, Levin J-E.** 1989 Quality of life of adults with growth hormone deficiency: a controlled study. Acta Paediatr Scand. 356:55-59.

50. **McGauley GA.** 1989 Quality of life assessment before and after growth hormone treatment in adults with growth hormone deficiency. Acta Paediatr Scand. 356:70-72.

51. **McGauley GA, Cuneo RC, Salomon F, Sönksen PH.** 1990 Psychological well-being before and after growth hormone treatment in adults with growth hormone deficiency. Horm Res. 33(Suppl 4):52-54.

52. **Rosén T, Wirén L, Wilhelmsen L, Wiklund I, Bengtsson B-Å.** 1994 Decreased psychological well-being in adult patients with growth hormone deficiency. Clin Endocrinol (Oxf). 40:111-116.

53. **Hunt SM, McKenna SP, Doward LC.** 1993 Preliminary report on the development of a disease specific instrument for assessing quality of life of adults with growth hormone deficiency. Acta Endocrinol (Copenh). 128:37-40.

3 The Effects of Human Growth Hormone (GH) Administration in GH-Deficient Adults: A 20-Day Metabolic Ward Study

ABSTRACT

The early effects of human GH administration in GH-deficient (GHD) adults on protein, electrolyte homeostasis and body composition were investigated in a metabolic ward study.

Four patients were studied. In addition to a constant caloric and nitrogen sufficient diet, the patients received GH for 15 days in dosages of 12.5-25 μg/kg.day with a maximum of 1.48 mg (4 IU)/day. GH replacement therapy was well tolerated by all patients. There was a slowly increasing effect on IGF-I levels, which reached a maximum after 8-12 days. The lowered IGFBP-3 levels normalized quicker, reaching maximum circulating concentrations 3 days after the start of GH treatment. Insulin concentrations maximally increased after 5 days, after which they leveled off. Insulin-like growth factor-binding protein-1 levels were maximally suppressed after 2 days of treatment.

Nitrogen balance became positive in all patients (mean +2.8 ± 0.2 g/ day). Maximal nitrogen retention occurred after 2-5 days of GH administration, after which adaptation occurred. This degree of nitrogen retention represents a formation of 20 g muscle/ day, which would mean an increase of 3.6 kg muscle over a period of 6 months of GH replacement therapy. A rapidly occurring positive sodium balance was observed within 24-72 h. Maximal sodium retention amounted to 61 mmol/ day. It slowly decreased spontaneously over the subsequent 12 days. In parallel rapid changes in bioelectrical impedance analysis (BIA) were observed. There was a close parallel between the net cumulative sodium retention and the decrease in BIA in these patients during the first 15 days of GH therapy. This suggests that the calculation of body composition compartments on the basis of BIA measurements during the initial phase of GH replacement does not represent actual changes in fat mass. This was substantiated with measurements of body composition using dual energy x-ray absorptiometry .

In conclusion, measurements of early metabolic changes in GHD adults during the first 15 days after the start of GH replacement indicate that IGF-I values reach maximal levels only after 8-12 days, that the measurements of changes in IGFBP-1 and IGFBP-3 levels probably do not contribute to a determination of the optimal GH replacement dose, that maximal nitrogen retaining effects occur within 2-5 days after which

adaptation occurs, that massive sodium retention occurs during this period which spontaneously levels off and that cumulative sodium retention closely correlates during this period with changes in BIA. This suggests that measurements of body composition in patients with GHD by BIA should be interpreted with caution.

INTRODUCTION

GH has been available for the treatment of children with short stature for more than 30 yr. (1). Lately it has also been used in clinical studies in GH-deficient (GHD) adults (2-5). Information concerning the early metabolic effects of replacement therapy with GH in GHD adults as well as on the course of the subsequent changes in body composition is limited. GHD adults have more body fat and less lean body mass than age-, sex-, height- and weight- matched controls (2-5). In hypopituitary dwarfs GH reduced the amount of subcutaneous (sc.) fat (6), whereas a reduction in both sc. and visceral adipose tissue was demonstrated during GH replacement in GHD adults (5,7). In a study of eight hypopituitary dwarfs an increase in muscle mass and a decrease in total body fat were recorded (8). These changes were also observed in GHD adults (4,5,7,9-12).

The most common side effects of growth hormone replacement therapy are transient fluid retention, arthralgia and discomfort in muscles and occasionally the occurrence of carpal tunnel syndrome (5,7). Some of these symptoms seem to be related to sodium retention caused by GH administration (13,14). Evidence has also been presented that body composition measurements using bioelectrical impedance analysis (BIA) may result in an overestimation of the lipolytic effects of GH replacement therapy (4,13-15).

GH exerts a positive effect on whole body nitrogen economy after operation (16). We found a decrease in nitrogen excretion after GH administration to catabolic elderly adults (17). Also during GH replacement therapy of GHD a net increase in protein synthesis using L-1-^{13}C-leucine and ^{15}N-glycine was found (4,18). The initial effects of GH replacement therapy on nitrogen balance in GHD adults have not been studied so far under metabolic ward conditions.

During GH replacement therapy of GHD adults, normalization of lowered insulin-like growth factor (IGF-I) levels was reported (2,3,5,7). Also the lowered IGF-binding protein-3 (IGFBP-3) concentrations normalize during GH administration in GHD patients (19,20). Long term GH replacement therapy causes a moderate deterioration of glucose tolerance, which is accompanied by an increase in insulin secretion (18), eventually followed by a decrease in circulating IGFBP-1 levels (21,22).

In the present study we investigated, under metabolic ward conditions the effects of GH replacement therapy in four GHD adults. During the first 15 days of GH administration the dynamics of the changes in circulating IGF-I, IGFBP-3, insulin and IGFBP-1 levels were monitored. Also the changes in nitrogen balance were measured. In addition the changes in sodium balance early after the start of GH replacement therapy are reported in relation to the rapid changes in bioelectrical impedance.

MATERIALS AND METHODS

Patients

Four patients with long-standing GHD were studied (Table 1). In all four patients GHD was confirmed by decreased circulating IGF-I concentrations and an insufficient rise of GH levels after iv GHRH administration. None of these patients suffered from renal or liver disease or diabetes mellitus, or had a history of cancer. One patient (no. 4) had osteogenesis imperfecta.

TABLE 1. Clinical and biochemical characteristics of the four patients included in the study

Case no.	Age (yr)	Ht (cm)	Wt (kg)	Sex	Cause of pituitary deficiency	Diagnosis[a]	Peak GH (mg/L)[b]	IGF-I (nmol/L)[c]
1	35	181	96	m	Craniopharyngeoma	TAGDI	2.3	2.7
2	49	138	69	f	Idiopathic	TAG	1.7	2.2
3	35	165	96	f	Craniopharyngeoma	TAG	1.1	6.2
4	37	162	52	m	Congenital	TAG	4.2	10.0

[a] T, thyroid deficiency; A, adrenal deficiency; G, gonadal deficiency; DI, diabetes insipidus.
[b] Peak GH, maximal GH level after iv injection of GHRH (1 μg/kg BW).
[c] Normal range, 12.0-49.8 nmol/L.

Protocol

The patients were admitted to the Clinical Research Unit for a period of 23 days. During the entire study they were given the same diet every day. The diets were prepared in accordance with the wishes of the patients, weighed and frozen before hospital admission. The patient's own account and visual confirmation by the nursing staff were used to check completeness of food intake. Two diets were diluted with distilled water and homogenized for measurements of the nitrogen and mineral contents.

The first 3 days after admission were used to achieve metabolic balance (these are not included in the figures). In the next 5 days, pretreatment measurements were made (-4,-3,-2,-1 and 0). From day 0 onward, the patients received recombinant derived human GH. The patients injected themselves daily sc. at bedtime. Patients 1, 3 and 4 received 25μg/kg.day with a maximum of 1.48 mg (4 IU)/day. Patient 2 received only half this dose (12.5 μg/kg.day). This lower dose was given because this patient already spontaneously complained of signs and symptoms of fluid retention. rhGH (Humatrope) was supplied by Eli Lilly Co.(Indianapolis, IN).

At 0800 h, weight, temperature, blood pressure, pulse rate and impedance were measured. Also fasting blood samples were taken for glucose, IGF-I, IGFBP-3, insulin and IGFBP-1 assessments. At regular intervals routine hematological and clinical chemical parameters were determined. Urine was collected in 24-h samples. In each sample, nitrogen, creatinine, sodium (Na), potassium, and glucose were measured quantitatively and ketone bodies were determined qualitatively.

Stools were collected in 4-day periods from day -4 onward. The 4-day periods were demarcated by the administration of 1200 mg polyethylene glycol every fourth day. Fecal nitrogen and Na were measured in each pooled sample after homogenization.

The aims and methods of the study were explained to the patients and written informed consent was obtained before the study, which was approved by the ethics committee of the University Hospital Dijkzigt (Rotterdam, The Netherlands).

Methods

Routine methods were used for the hematological and chemical measurements in blood. Urinary, fecal and dietary nitrogen levels were determined with the use of an automatic nitrogen analyzer (type 1400, Carlo Erba, Milan, Italy). The electrolytes were determined by flame photometry. Fecal polyethylene glycol was measured by a turbimetric method. Nitrogen balance was calculated by subtracting urinary, fecal and integumental nitrogen excretion from the dietary intake. Integumental nitrogen loss was estimated to be 0.5 g/day (23).

Total IGF-I levels were determined by RIA, using kits obtained from Medgenix Diagnostics [Brussels, Belgium; intra-assay coefficient of variation (CV), 4.1-6.1%; interassay CV 9.3-9.9%]. Insulin levels were determined by immunoradiometric assay (IRMA) using kits obtained from Medgenix Diagnostics (Brussels, Belgium, intraassay CV, 2.1-4.5%; interassay CV, 4.7-12.2%). IGFBP-1 levels were determined by IRMA (Diagnostic Systems Laboratories, Webster, TX; intraassay CV, 3.4-6.0%; interassay CV, 1.0-3.5%). IGFBP-3 levels were determined by IRMA (Diagnostic Systems Laboratories; intraassay CV, 0.56-3.90%; interassay CV, 0.49-1.9%). Normal values for these four parameters are shown in Tables 2, A and B.

Body composition

Body composition was measured by BIA and dual energy x-ray absorptiometry (DXA). Estimation of lean body mass, total body water, and fat mass was performed using a tetrapolar bioelectrical impedance analyzer (model BIA 101, RJL Systems, Detroit, MI). The principle of the BIA is that lean tissues, comprised largely of electrolyte containing water, readily conducts an applied current (of 800 μA at a signal frequency of 50 kHz) which is generated by a bioelectrical impedance analyzer, whereas fat conducts little current. After correct positioning of the patients and the electrodes and assuming that the two compartments act in parallel, the impedance of the body is controlled by the low impedance lean compartment and is a function of the specific resistance of lean tissue, the cross-sectional area and the length of the lean compartment. As water is the major component of the lean body compartment, total body water can be calculated from the

measured impedance (24-27). Fat free mass was calculated using the equation of Lukaski (25).

DXA is a noninvasive method of measuring bone and soft tissue body composition. DXA directly measures fat mass, lean tissue mass and total body bone mineral content. The reported CVs are 6.6%, 1.5% and 0.5% respectively (28). Body composition was determined by scanning each subject on a Lunar DPX Bone Densitometer (Lunar, Madison, WI). This equipment uses an x-ray source which produces a dual energy beam with energies of 38 and 70 kiloelectron volts by K-edge filtering using cerium. The DPX system performs rectilinear scans over the entire length of the patient's body, beginning at the top of the head and moving downward toward the feet. A total body scan takes approximately 15 min. and the subject receives less than 0.5 microSievert radiation exposure. The analysis software calculates fat mass, lean tissue mass and bone mineral content (29).

The statistical significance of the differences between mean values was determined using one-way analysis of variance. When significant overall effects were obtained by analysis of variance, multiple comparisons were made with the Newman-Keuls test. All data are shown as the mean ± SEM. $P < 0.05$ was considered significant.

RESULTS

The administration of GH was well tolerated by all four patients. None of them complained of signs or symptoms related to water retention.

The circulating IGF-I and IGFBP-3 levels increased in all patients during GH therapy (Table 2A). The course of this increase was variable: maximal levels were reached after 8-12 days of GH treatment in the three patients who were treated with 25 μg/kg.day of GH. In patient 2, who only received 12.5 μg GH/kg.day, IGF-I levels increased from 2.2 nmol/L to a maximum of 11.3 nmol/L (Fig. 1). IGFBP-3 levels were decreased in all four patients before the start of GH replacement and increased 2- to 5-fold in the subsequent period. The maximum increase in IGFBP-3 levels was reached after 3 days of GH replacement therapy when IGF-I levels were still rising (Fig. 2A). The relationship between IGF-I and IGFBP-3 levels in all four patients during the first 15 days of GH replacement therapy is shown in Fig. 3 (calculated linear regression: r=0.83; $P < 0.05$).

TABLE 2A. Overnight fasting levels of IGF-I and IGFBP-3

Patient no.	GH daily dose (μg)	IGF-I (nmol/L)		IGFBP-3 (mg/l)	
		pretreatment	day 15	pretreatment	day 15
1	1480	2.7	58.3	0.84	3.64
2	740	2.2	9.8	0.31	1.33
3	1480	6.2	35.1	1.79	3.42
4	1288	10.0	67.7	1.48	3.02
mean ± SEM		5.3 ± 1.8	42.7 ± 12.9[a]	1.10 ± 0.33	2.85 ± 0.52[a]
normal range		12.0-49.8		2.09-3.63	

[a] $P < 0.05$ vs. pretreatment.

TABLE 2B. Overnight fasting levels of insulin, IGFBP-1 and glucose

Patient no.	Insulin (mU/L)		IGFBP-1 (mg/L)		glucose (mmol/L)	
	pretr	day 15	pretr	day 15	pretr	day 15
1	9.1	31.7	3.7	0.3	3.5	3.9
2	10.7	17.7	12.0	9.1	3.4	4.1
3	11.6	12.0	4.9	0.7	5.6	5.4
4	4.4	7.5	72.6	5.8	3.4	4.7
mean ± SEM	8.9±1.6	17.2±5.3	23.3±16.5	4.0±2.1	4.0±0.5	4.5±0.3
normal range	2.0-25.0		10-150		3.3-6.0	

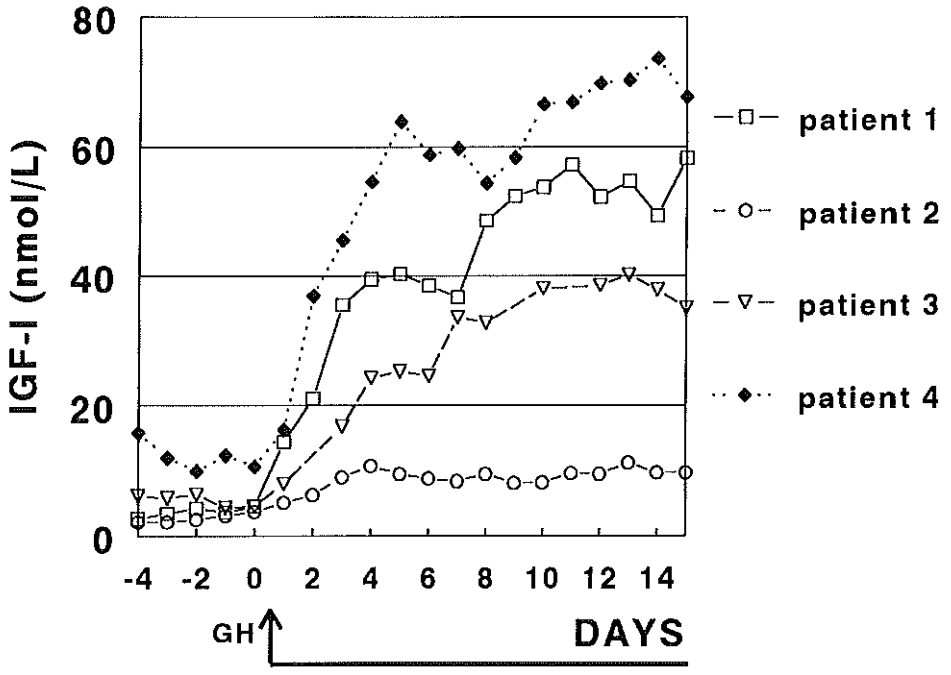

Fig.1 The course of the IGF-I levels during the study period in the four individual GHD patients. GH replacement therapy was initiated on the evening of day zero. Five pretreatment measurements were carried out.

Insulin levels increased during GH replacement therapy. The initial maximal increase in insulin levels 3-5 days after the start of GH replacement therapy tended to decrease in the subsequent 10 days (Fig. 2B). During this period, no glucosuria was found, whereas fasting and postprandial glucose levels did not change significantly (see also Table 2B). IGFBP-1 levels sharply decreased within 2 days after the start of GH replacement therapy and remained constant thereafter (Fig. 2B). During the first 5 days of GH administration there was a marginal negative correlation between the circulating insulin and IGFBP-1 levels ($r=-0.40$, $P = 0.056$).

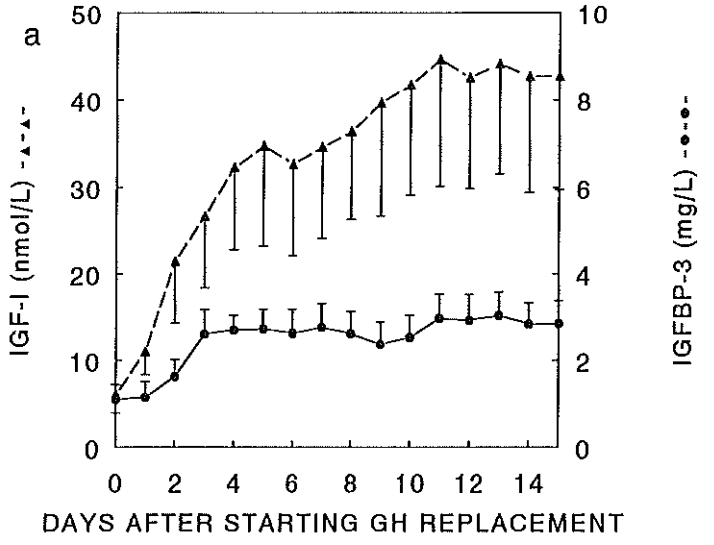

Fig. 2A IGF-I and IGFBP-3 levels (mean ± SEM) during GH replacement therapy in GHD patients. On day zero the mean of the five pretreatment values is presented

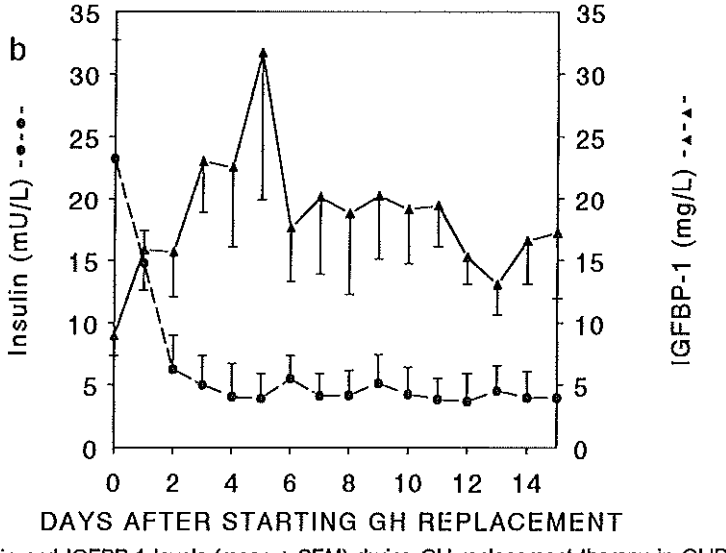

Fig. 2B Insulin and IGFBP-1 levels (mean ± SEM) during GH replacement therapy in GHD patients. On day zero the mean of the five pretreatment values is presented

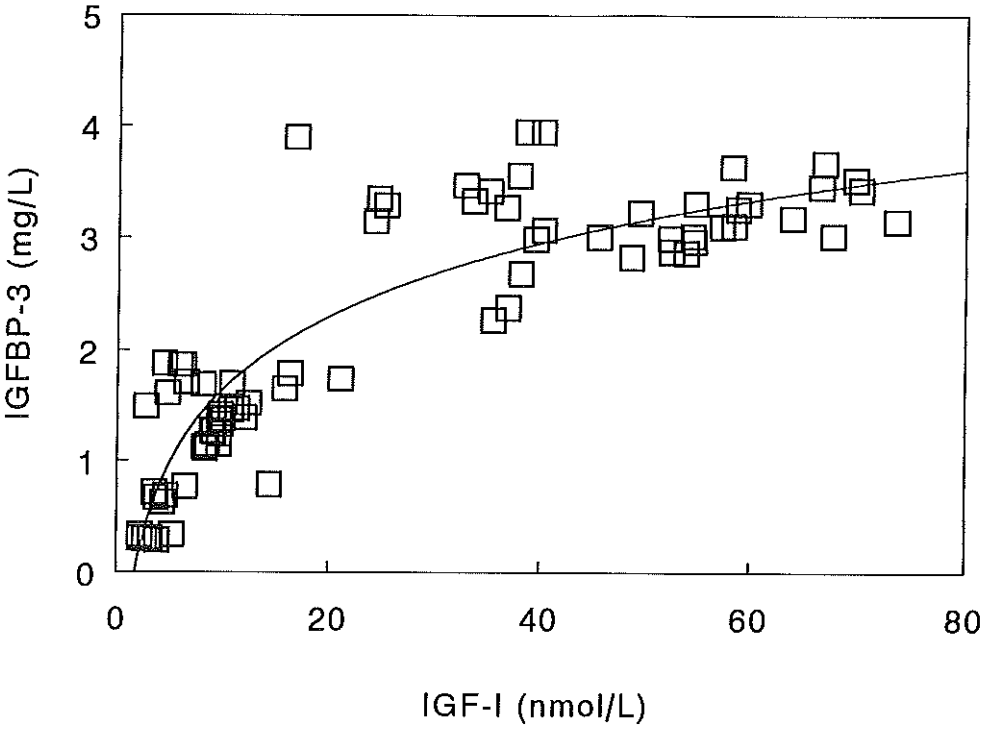

Fig. 3 The relationship between the changes in circulating IGF-I levels during GH replacement therapy. (calculated linear regression: r = 0.83; *P< 0.05*)

Nitrogen balance became positive in all four patients directly after the start of GH replacement therapy (Fig. 4A). Nitrogen retention was maximal from the second day onward, with a maximum of 3.8 g/day after two injections on day 2 (Fig. 4A). Statistical analysis showed that nitrogen balance was positive compared to pretreatment values only on days 2-5 after the start of GH administration (*P < 0.05* vs. control). Thereafter, no statistically significant changes occurred, which suggests an early adaptation of the nitrogen-retaining effects of GH administration from day 6 onward. The mean retention of nitrogen from days 1-15 after the start of GH administration amounted to 2.8 ± 0.2 g/day. Interestingly, in patient 2, who received 50% of the GH dose, which led to a subnormal increase in IGF-I levels, nitrogen retention was similar to that observed in the other three patients (2.7 ± 0.5 vs. 2.8 ± 0.5 g/day).

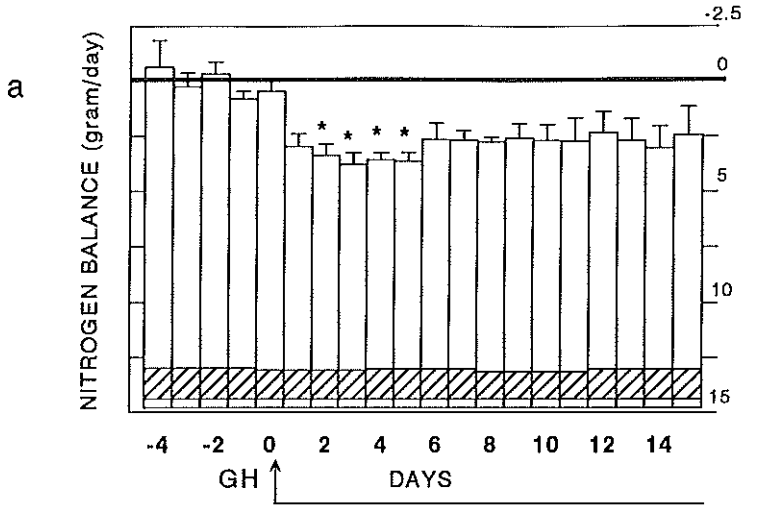

Fig. 4A Nitrogen balance (mean ± SEM) of four patients before and during GH replacement therapy of GHD patients. Positive balance is present when the bar is below the zero line (net balance).
* denotes significance vs. pretreatment values (*P<0.05*).

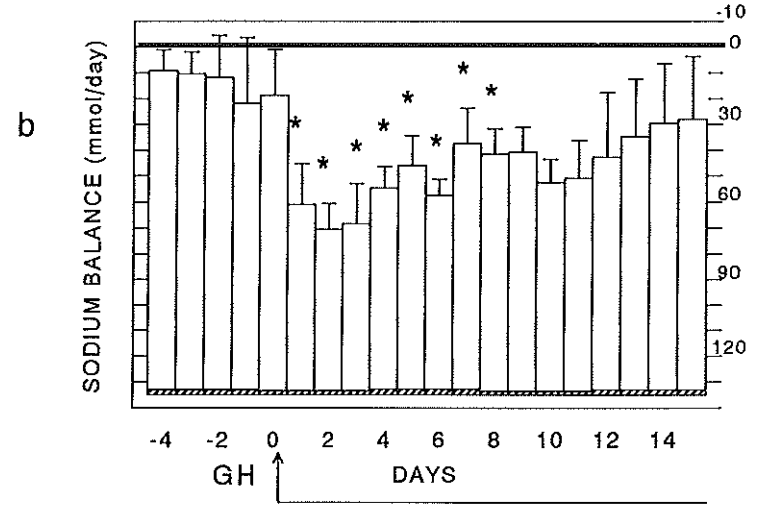

Fig. 4B Sodium balance (mean ± SEM) of four patients before and during GH replacement therapy of GHD patients. Positive balance is present when the bar is below the zero line (net balance).
* denotes significance vs. pretreatment values (*P<0.05*).

From the start of GH administration an early and considerable retention of sodium was observed in all patients (Fig. 4B). This was not accompanied by a change in body weight in three patients, whereas patient 4 showed an increase in body weight of 1.8 kg. During the course of the 15 days of GH administration, sodium balance tended to reverse spontaneously. Sodium retention on day 2 of GH administration was to 61 ± 16 mmol/day vs. the control value of 12 ± 16 mmol/day ($P < 0.05$). On day 15 of GH administration sodium retention was 28 ± 24 mmol/day ($P = NS$ vs. control). Potassium balance was positive in all four patients. There was a maximum retention of 22 ± 4 mmol on the third day of GH administration. This did not reach statistical significance because there was already modest potassium retention in the pretreatment period (8 ± 3 mmol/day). The pattern of the course of potassium balance was similar to that of sodium (data not shown).

No changes in kidney or liver function tests, electrolytes, cholesterol, triglycerides, total protein and albumin or leukocyte count were found.

Before GH administration, body composition was measured by BIA and DXA. In table 3, the course of BIA measurements before treatment and on days 3-6 and on 12-15 of treatment are shown together with the calculated fat mass and fat free mass on these days. The DXA measurements were performed before treatment and on day 15 of treatment (Table 3). According to the calculations by BIA, rapid changes were observed in the fat free mass as well as the absolute fat mass by 3-6 days after the start of GH administration. After 12-15 days of GH administration there was a decrease in fat percentage as measured by BIA, from a mean of 44.6% to 36.0%. The corresponding change measured by DXA amounted only to a decrease from 32.8% to 30.7%. In Fig. 5, the relationship between the course of cumulative sodium retention and the absolute values of bioelectrical impedance values during GH administration in all four patients is shown. Interestingly, there was a close correlation between cumulative sodium retention and the changes in bioelectrical impedance during GH administration ($r = -0.93$; $P < 0.0001$ in Fig. 5).

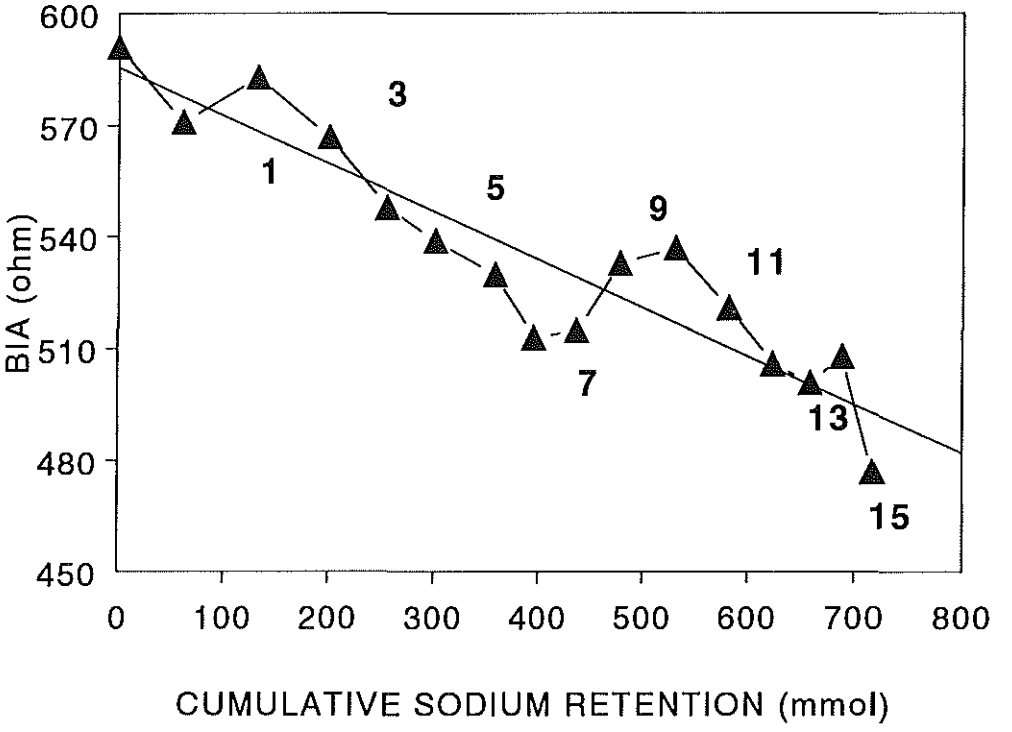

Fig.5 The relation between BIA values and cumulative sodium retention during GH replacement therapy in four patients with GHD. The numbers represent the days after the start of GH replacement therapy. There was a statistical significance between both parameters. ($r = -0.92$, $P<0.005$).

Table 3. Body composition measurements by BIA and DXA in the four patients.

Patient	Before treatment				Mean days 3-6			Mean days 12-15			
no.	R (ohm)	FFM (kg)	FM (kg)	FAT (%)	R (ohm)	FFM (kg)	FM (kg)	R (ohm)	FFM (kg)	FM (kg)	FAT (%)
BIA											
1	537±12	55.3±1.2	41.1±1.4	42.6	509±8	58.2±0.8	36.2±0.4	462±11	63.8±1.5	33.1±1.5	34.2
2	621±1	29.7±0.0	38.7±0.0	56.6	570±8	32.0±0.4	36.9±0.4	550±13	33.1±0.7	36.1±0.6	52.2
3	477±9	52.0±0.9	43.9±1.0	45.8	473±10	52.6±1.1	42.8±1.0	450±9	55.0±1.1	38.3±0.9	41.1
4	729±12	34.1±0.5	17.1±0.4	33.4	632±4	38.8±0.2	14.0±0.1	530±15	45.6±1.2	9.2±1.0	16.8
DXA											
1		67.7	25.8	27.6					72.0	22.5	23.8
2		34.4	33.1	49.1					35.1	32.7	48.2
3		55.6	37.3	40.2					56.3	34.0	37.7
4		43.9	7.3	14.3					47.7	7.2	13.1

The BIA measurements before as well as 3-6 and 12-15 days after GH administration are the mean ± SEM of four determinations.

DISCUSSION

In this study we investigated under metabolic ward conditions the course of the early effects of GH replacement therapy in GHD adults. Before the start of treatment IGF-I and IGFBP-3 levels were decreased in all patients. GH administration resulted in a rapid rise in IGFBP-3 levels, which seemed to be independent of the changes in circulating IGF-I concentrations. Maximal (normalized) IGFBP-3 levels were found after 3 days of GH therapy. This means that the measurement of IGFBP-3 levels, which has been suggested to be of value in the diagnosis of GH deficiency in adulthood (30-32), probably has limited value in determining the optimal dose of GH for replacement therapy in individual patients. Also the course of changes in IGF-I levels, as observed during the first 15 days of GH replacement therapy casts doubts on its value as a parameter to be used in determining the optimal dose of GH. This assumption is substantiated by the observation that nitrogen retention was already maximal from day 2 of GH administration, whereas circulating IGF-I levels had only just started to increase. In patient 2 who was treated with a low dose of 12.5 μg GH/kg.day, IGF-I levels did not normalize, whereas the degree of nitrogen retention, changes in sodium balance, as well as changes in body composition during the first 15 days of GH therapy were similar to those observed in the three patients who were treated with a double dose of GH, eventually resulting, after 8-15 days in a normalization of circulating IGF-I levels. Also the changes in IGFBP-1 levels were confirmed to be mainly dependent on the changes in insulin levels and the measurement of IGFBP-1 levels does not contribute to our understanding of the dynamic changes in IGF-I bioactivity, as maximal suppression of IGFBP-1 concentrations was reached within 2 days after the start of GH replacement therapy.

Our studies on the effects of GH replacement therapy on nitrogen balance confirm previous observations in GHD children (33). A remarkably rapid nitrogen-retaining effect, with a maximum reached after 2 days, was observed. Also, interestingly, this positive effect of GH seemed, under these metabolic ward conditions, in which nitrogen intake was standardized, to level off after 5 days. Whole body studies on nitrogen retention reflect total protein synthesis which represents the net effect of potentially antagonizing changes involving both protein synthesis as well as breakdown (34).

Earlier studies in postoperative patients receiving various nutritional regimens showed that short term (7 days) GH administration increased nitrogen turnover and synthesis, whereas degradation either increased or remained unchanged (35). In obese patients receiving hypocaloric nutrition, the N-retaining effect of GH appeared to wear off after 3-5 weeks (36). Our own previous studies on the effect of GH replacement in GHD adults with ^{15}N-glycine incorporation measurements also demonstrated an increased (initial) rise of protein synthesis, followed by a higher rate of protein degradation (4).

During GH treatment for 6 months significant increases were noted in the cross-sectional area of thigh and quadriceps muscle which correlated with the changes in lean body mass measured with ^{40}K (34). We observed in our patients a mean nitrogen retention of 2.8 g/day during the first 15 days of GH replacement therapy. This accounts for approximately 17 g of whole body protein synthesis, which represents 20 g of muscle (34,37,38). In 2 weeks, an estimated mean increase in muscle mass of 300 g is to be expected, which over a 6-month period would lead to an increase in total muscle mass of 3.6 kg. This indeed is in the range of the changes that have been reported during GH replacement therapy in GHD adults in several studies (3,5,9).

BIA determines the percentage of fat from total body water measurement (24). The accuracy of the percentage of body fat derived from this method depends on the hydration status of the body. Exercise-induced dehydration causes a decrease in the percentage of fat measured by BIA because of a decrease in total body weight, without a change in the fat free mass (39). Acute changes in the circulating volume in patients receiving hemodialysis cause a rapid change in resistance measured by BIA. Although no estimates of body composition were reported in these patients, these acute changes may also affect estimates of body composition (40). In GHD patients there is an increased resistance that cannot only be explained by their lower lean body mass. Resistance is determined by both the hydration state and the electrolyte content of the lean body mass (14). In our patients, body composition measurements by BIA showed rapid initial changes in fat mass and fat free mass shortly after the initiation of GH replacement therapy. These changes in BIA were demonstrated to be largely due to sodium and water retention, taking place soon after the start of therapy. The observed rapid decrease in total fat mass was overestimated by BIA. In a comparative study in

elderly subjects an accurate estimation of the amount of total body fat could be made by BIA and anthropometry depending on the regression equations used (41). This study also showed a considerable lack of agreement between body fat measured by DXA and body fat measured by BIA in several equations. There was an overestimation of body fat by most of the equations. The DXA method measures fat, bone mineral and lean tissue separately. In this it seems to have a greater precision than most of the other currently used methods (42). In our patients there was a decrease in fat percentage measured by BIA from 44.6% to 36.0%. With DXA there was a decrease only from 32.8% to 30.7%. This difference was shown to be mainly caused by overestimation of the decrease in fat mass by BIA. In conclusion, the changes in resistance reflected mainly the changes in the extracellular water composition. Therefore the results of this technique should be interpreted with great reserve in patients with GHD (14).

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REFERENCES

1. **Raben MS.** 1958 Treatment of a pituitary dwarf with human growth hormone. J Clin Endocrinol Metab.18:901-903.

2. **Salomon F, Cuneo RC, Hesp R, Sönksen PH.** 1989 The effects of treatment with recombinant human growth hormone on body composition and metabolism in adults with growth hormone deficiency. N Engl J Med.321:1797-1803.

3. **Jørgensen JOL, Pedersen SA, Thuesen L, Jørgensen J, Skakkebæk NE, Christiansen JS.** 1989 Beneficial effects of growth hormone treatment in GH-deficient adults. Lancet.1:1221-1225.

4. **Binnerts A, Swart GR, Wilson JH, et al.** 1992 The effect of growth hormone administration in growth hormone deficient adults on bone, protein, carbohydrate and lipid homeostasis, as well as on body composition. Clin Endocrinol (Oxf).37:79-87.

5. **Bengtsson B-Å, Edén S, Bosaeus I, et al.** 1993 Treatment of adults with growth hormone deficiency with recombinant human growth hormone. J Clin Endocrinol Metab.76:309-317.

6. **Tanner JM, Whitehouse RH.** 1967 The effect of human growth hormone on subcutaneous fat thickness in hyposomatotrophic and panhypopituitary dwarfs. J Endocrinol.39:263-268.

7. **Sönksen PH, Cuneo RC, Salomon F, et al.** 1991 Growth hormone therapy in adults with growth hormone deficiency. Acta Paediatr Scand.379:139-146.

8. **Parra AP, Argote RM, García G, Cervantes C, Alatorre S, Pérez-Pasten E.** 1979 Body composition in hypopituitary dwarfs before and during human growth hormone therapy. Metabolism.28:851-857.

9. **Cuneo RC, Salomon F, Wiles CM, Hesp R, Sönksen PH.** 1991 Growth hormone treatment in growth hormone-deficient adults. I. Effects on muscle mass and strength. J Appl Physiol.70(2):688-694.

10. **Whitehead HM, Boreham C, McIlrath EM, et al.** 1992 Growth hormone treatment of adults with growth hormone deficiency: results of a 13-month placebo controlled cross-over study. Clin Endocrinol (Oxf).36:45-52.

11. **Christiansen JS, Jørgensen JO, Møller J, Jørgensen J, Skakkebæk NE.** 1990 Effects of growth hormone on body composition in adults. Horm Res.33(Suppl 4):61-64.

12. **Degerblad M, Elgindy N, Hall K, Sjöberg H-E, Thorén M.** 1992 Potent effect of recombinant growth hormone on bone mineral density and body composition in adults with hypopituitarism. Acta Endocrinol (Copenh).126:387-393.

13. **Binnerts A, Deurenberg P, Swart GR, Wilson JH, Lamberts SWJ.** 1992 Body composition in growth hormone-deficient adults. Am J Clin Nutr.55:918-923.

14. **de Boer H, Blok GJ, Voerman HJ, de Vries PM, van der Veen EA.** 1992 Body composition in adult growth hormone-deficient man, assessed by anthropometry and bioimpedance analysis. J Clin Endocrinol Metab.75:833-837.

15. **Henneman PH, Forbes AP, Moldawer M, Dempsey EF, Carroll EL.** 1960 Effects of human growth hormone in man. J Clin Invest.39:1223-1238.

16. **Hammarqvist F, Strömberg C, von der Decken A, Vinnars E, Wernerman J.** 1992 Biosynthetic human growth hormone preserves both muscle protein synthesis and the

decrease in muscle-free glutamine, and improves whole-body nitrogen economy after operation. Ann Surg.216:184-191.

17. **Binnerts A, Wilson JH, Lamberts SWJ.** 1988 The effects of human growth hormone administration in elderly adults with recent weight loss. J Clin Endocrinol Metab.67:1312-1316.

18. **Russell-Jones DL, Weissberger AJ, Bowes SB, et al.** 1993 The effects of growth hormone on protein metabolism in adult growth hormone deficient patients. Clin Endocrinol (Oxf).38:427-431.

19. **Jørgensen JOL, Blum WF, Møller N, Ranke MB, Christiansen JS.** 1991 Short-term changes in serum insulin-like growth factors (IGF) and IGF binding protein 3 after different modes of intravenous growth hormone (GH) exposure in GH-deficient patients. J Clin Endocrinol Metab.72:582-587.

20. **Møller J, Jørgensen JOL, Lauersen T, et al.** 1993 Growth hormone dose regimens in adult GH deficiency: effects on biochemical growth markers and metabolic parameters. Clin Endocrinol (Oxf).39:403-408.

21. **Suikkari A-M, Koivisto VA, Koistinen R, Seppälä M, Yki-Järvinen H.** 1989 Dose-response characteristics for suppression of low molecular weight plasma insulin-like growth factor-binding protein by insulin. J Clin Endocrinol Metab.68:135-140.

22. **Lee PDK, Jensen MD, Divertie GD, Heiling VJ, Katz HH, Conover CA.** 1993 Insulin-like growth factor-binding protein-1 response to insulin during suppression of endogenous insulin secretion. Metabolism.42:409-414.

23. **Calloway DH, Odell ACF, Margen S.** 1971 Sweat and miscellaneous nitrogen losses in human balance studies. J Nutr.101:775-786.

24. **Lukaski HC, Johnson PE, Bolonchuk WW, Lykken GI.** 1985 Assessment of fat-free mass using bioelectrical impedance measurements of the human body. Am J Clin Nutr.41:810-817.

25. **Lukaski HC, Bolonchuk WW, Hall CB, Siders WA.** 1986 Validation of tetrapolar bioelectrical impedance method to assess human body composition. J Appl Physiol.60:1327-1332.

26. **Kushner RF, Schoeller DA.** 1986 Estimation of total body water by bioelectrical impedance analysis. Am J Clin Nutr.44:417-424.

27. **Segal KR, Van Loan M, Fitzgerald PI, Hodgdon JA, Van Itallie TB.** 1988 Lean body mass estimation by bioelectrical impedance analysis: a four-site cross-validation study. Am J Clin Nutr.47:7-14.

28. **Mazess RB, Barden HS, Bisek JP, Hanson J.** 1990 Dual-energy x-ray absorptiometry for total-body and regional bone-mineral and soft-tissue composition. Am J Clin Nutr.51:1106-1112.

29. **Stewart SP, Bramley PN, Heighton R, et al.** 1993 Estimation of body-composition from bioelectrical impedance of body segments: comparison with dual-energy x-ray absorptiometry. Br J Nutr.69:645-655.

30. **Blum WF, Ranke MB, Kietzmann K, Gauggel E, Zeisel HJ, Bierich JR.** 1990 A specific radioimmunoasssay for the growth hormone (GH)-dependent somatomedin-binding protein: its use for diagnosis of GH deficiency. J Clin Endocrinol Metab.70:1292-1298.

31. **Jørgensen JOL, Blum WF, Møller N, Ranke MB, Christiansen JS.** 1990 Circadian patterns of serum insulin-like growth factor (IGF) II and IGF binding protein 3 in growth hormone-deficient patients and age- and sex-matched normal subjects. Acta Endocrinol (Copenh).123:257-262.

32. **Hasegawa Y, Hasegawa T, Aso T, et al.** 1993 Comparison between insulin-like growth factor-I (IGF-I) and IGF binding protein-3 (IGFBP-3) measurement in the diagnosis of growth hormone deficiency. Endocr J.40:185-190.

33. **Dahms WT, Owens RP, Kalhan SC, Kerr DS, Danish RK.** 1989 Urea synthesis, nitrogen balance, and glucose turnover in growth-hormone deficient children before and after growth hormone administration. Metabolism.38:197-203.

34. **Welle S, Thornton C, Jozefowicz RF, Statt M.** 1993 Myofibrillar protein synthesis in young and old man. Am J Physiol.264:E693-E698.

35. **Ziegler TR, Young LS, Manson JM.** 1988 Metabolic effects of recombinant human growth hormone in patients receiving parenteral nutrition. Ann Surg.208:6-16.

36. **Snyder DK, Clemmons DR, Underwood LE.** 1988 Treatment of obese , diet restricted subjects with growth hormone for 11 weeks: effects on anabolism, lipolysis, and body composition. J Clin Endocrinol Metab.67:54-61.

37. **Young VR.** 1990 Protein and amino acid metabolism with reference to aging and the elderly. Prog Clin Biol Res.326:279-300.

38. 1977 Energetics and coupled systems. In: Biochemistry, Montgomery R., Dryer R.L., Conway T.W.Spector A.A. eds. St Louis: Mosby; 232.

39. **Runge VM, Eisenmann PA, Johnson SC.** 1987 Effects of exercise induced dehydration on BIA to determine body composition. Med Sci Sports Exerc.19:S38.

40. **Zabetakis GW, Gleim GW, Vitting KE, Gardenswartz MH.** 1987 Volume changes effect electrical impedance measurements of body composition. Med Sci Sports Exerc.19:S40.

41. **Svendsen OL, Haarbo J, Heitman HL, Gotfredsen A, Christiansen C.** 1991 Measurement of body fat in elderly subjects by dual-energy x-ray absorptiometry, bioelectrical impedance and anthropometry. Am J Clin Nutr.53:1117-1123.

42. **Pritchard JE, Nowson CA, Strauss BJ, Carlson JS, Kaymakci C, Wark JD.** 1993 Evaluation of dual-energy x-ray absorption as a method of measurement of body fat. Eur J Clin Nutr.47:216-228.

4 Combined Treatment of Growth Hormone (GH) and the Bisphosphonate Pamidronate vs. Treatment with GH Alone in GH-Deficient Adults: The Effects on Renal Phosphate Handling, Bone Turnover and Bone Mineral Mass

ABSTRACT

A potential drawback of GH replacement therapy in GH deficient (GHD) patients is the initial decrease in bone mass. The present study investigates the effects of the addition of pamidronate to GH replacement therapy in adult GHD subjects, on serum levels of parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$), renal phosphate handling, bone turnover and bone mineral content (BMC).

Six GHD adult patients were studied for two periods of six months with a wash out period of three years. In the first period they were treated with conventional replacement therapy and GH. In the second study period GH treatment was identical, while after two weeks 150 mg pamidronate per day was added.

In the first study period (GH only) there was a significant increase of phosphate reabsorption, without a change in serum PTH and 1,25-$(OH)_2D_3$ levels. This suggests a specific effect of GH or IGF-I on renal phosphate handling. This, indeed was supported by the close correlation between serum IGF-I levels and TmP/GFR ($r=0.75$, *$P<0.0001$*). When GH was administered together with pamidronate, this correlation was less, but remained significant ($r=0.44$, *$P<0.001$*).

The increase in bone turnover and decrease in BMC, as initially observed during GH replacement therapy alone, were attenuated by simultaneous pamidronate administration. The percentual decline of lumbar spine BMC (measured with dual-photon absorptiometry) at six months was -3.1 ± 1.5% during GH replacement therapy alone, versus +3.8 ±2.0% during the administration of the combination of GH and pamidronate (measured with dual-energy x-ray absorptiometry). At the distal and proximal forearm the changes amounted -0.5 ± 3.4% versus +4.5 ± 1.8% and -1 ± 1.2% versus +1.2 ± 1.1% respectively.

In conclusion this study shows that the addition of a bisphosphonate to GH replacement therapy in GHD adults counteracts the GH (or IGF-I) induced increase in renal phosphate reabsorption. Furthermore, it reduces GH-induced bone turnover and prevents the initial decrease in bone mineral content as seen during GH treatment alone, resulting in a beneficial effect on bone mineral mass. Pamidronate might therefore be an important adjunct to GH replacement therapy in adults with

GHD and severe osteopenia during the early phase of GH-induced stimulation of bone turnover.

INTRODUCTION

Several studies indicate that GH increases renal phosphate reabsorption and stimulates the renal production of the active vitamin D metabolite 1,25-dihydroxyvitamin D (1,25-$(OH)_2D_3$ (1,2). In other words at the level of renal phosphate handling GH appears to act as a parathyroid hormone (PTH) antagonist, while at the level of the renal 1α-hydroxylase an opposite more PTH-like action appears to take place (3). This apparent dichotomy in the interaction between GH and PTH prompted us to compare two periods of GH replacement therapy in the same GH deficient (GHD) individuals; one period with GH treatment alone and a second period whereby GH treatment was combined with the bisphosphonate pamidronate. It is known from other studies that pamidronate treatment results in an increase of PTH levels (4-6).

Recent reports also indicate a high prevalence of osteopenia in subjects with growth hormone deficiency (GHD) of both childhood (7,8) and adult onset (9,10). Other investigators showed an increased susceptibility for fractures in this group, which is compatible with the inverse relationship between bone mineral density (BMD) and fracture risk (11-13). Also a positive correlation between circulating insulin-like growth factor (IGF-I) and BMD in individuals with acquired GHD has been reported (14,15). Based on these observations clinical studies were initiated to establish the potential effects of GH replacement therapy on bone turnover and bone mass (16-18). Several reports indicate that GH replacement results in a considerable increase of bone turnover as indicated by an increase of markers of bone resorption and bone formation (18-23). This increase in bone remodeling might result in an increase of remodeling space with a subsequent loss of bone mineral mass. This indeed was observed in a group of GHD adults we have followed on GH replacement therapy for six months (24). However, long-term treatment with GH up to 30 months leads, after an initial downwards trend, to a sustained increase of bone

mineral content (BMC) as measured in the axial and appendicular skeleton (25-28). In other words long-term GH replacement therapy appears to result in a favourable shift in the balance between bone resorption and bone formation. In the present study we investigated the effects of GH replacement therapy in a group of GHD patients in combination with an inhibitor of bone resorption, the bisphosphonate pamidronate. Given the fact that bone turnover is initiated with bone resorption, we added in the present study pamidronate two weeks after the initiation of GH replacement therapy.

Taken together, the present study performed in GHD subjects compares the effects of GH alone and GH with pamidronate on parameters of renal phosphate handling, bone turnover and bone mineral mass.

MATERIALS AND METHODS

Patients

Eight patients with long-standing GHD were treated with GH alone for six months (24). The baseline characteristics of the six patients who agreed to enter the second part of the study three years later are shown in Tables 1 and 2.

TABLE 1. Clinical and biochemical characteristics of the six patients included in the study

Case no.	GH daily dose(μg)	Sex	Cause of pituitary deficiency	Diagnosis[a]	Peak GH (mg/L)[b]
1	1480	m	Craniopharyngeoma	TAGDI	2.3
2	740	f	Idiopathic	TAG	1.7
3	1480	f	Craniopharyngeoma	TAG	1.1
4	1288	m	Congenital	TAG	4.2
5	1110	f	Craniopharyngeoma	G	2.9
6	1480	m	Craniopharyngeoma		3.2

[a] T, thyroid deficiency; A, adrenal deficiency; G, gonadal deficiency; DI, diabetes insipidus.
[b] Peak GH, maximal GH level after iv injection of GHRH (1 μg/kg BW).

Age of onset of GHD was between 13 and 19 years of age. Two patients received GH treatment during childhood (no. 4 and 6) more than 10 years earlier. In the first study period patients 1, 3 ,4 and 6 received 25 μg/kg.day with a maximum of 1.48

TABLE 2. Clinical and biochemical characteristics of the patients

Characteristic	GH (period I)		GH + APD (period II)		
	mean	range	mean	range	*P*
Age (yr)	33.7	25-46	36.7	28-49	
Height (cm)	162.3	137-182	163.2	138-181	NS
Weight (kg)	73.3	47-95	80.8	52-102	0.004
BMI (kg/m^2)	27.6	18.1-33.8	30.3	19.8-36.2	0.007
Serum calcium (mmol/L)*	2.21	2.16-2.23	2.17	2.08-2.42	NS
Serum phosphate (mmol/L)	1.19	1.11-1.28	1.10	0.98-1.22	NS
$1,25\text{-}(OH)_2D_3$ (pmol/L)	80	57-103	77	54-114	NS
PTH (pg/mL)	20	13-32	26	15-32	0.017
TmP/GFR (mmol/L)	1.0	0.8-1.2	1.1	0.9-1.2	NS
Apase (U/L)	71	39-150	58	37-97	NS
Osteocalcin (μg/L)	4.1	2.6-7.5	3.2	1.-8.0	NS
Procollagen I (μg/L)	85.7	56-116	72	21-139	NS
Urinary OH-proline (mmol/mol creat)	20.8	9.6-39.4	23.5	10.2-60.0	NS
BMC SPA_{DIST} (U/cm)	30.9	18.4-42.6	32.9	25.2-42.2	NS
BMC SPA_{PROX}	33.8	22.3-44.5	34.7	25.5-44.9	NS

NS: no significance between groups

* serum calcium corrected for serum albumin.

mg (4 U) per day. Patient 2 received only half this dose (12.5 μg/kg.day). This lower dose was given because this patient already spontaneously complained of signs and symptoms of fluid retention. Patient 5 received 18μg/kg.day. She complained of fluid retention in the initial part of the study at which time the dose was reduced. Patients received GH replacement therapy for six months at the same dose in the two study periods. rhGH (Humatrope) was generously supplied by Eli Lilly Co., Indianapolis, IN.. In the second study period, from day 16 onward, the patients were cotreated with pamidronate 150 mg per day orally. Measurements were performed

at baseline, 1 month, 3 months and six months in the first study period and again at baseline, two weeks, 3 months and six months in the second study period. During and in between both treatment periods conventional pituitary replacement therapy remained unaltered.

The aims and methods of the study were explained to the patients, and written informed consent was obtained before the study, which was approved by the ethics committee of the University Hospital Dijkzigt (Rotterdam, The Netherlands).

Bone mass measurements

Lumbar bone mass (lumbar vertebrae, L2-L4) was measured, during the first study period using Dual-Photon Absorptiometry (DPA, Novo BMC-Lab 22a scanner). In the second study period a Lunar DPX Bone Densitometer (Lunar Corp., Madison, WI) was used. The short-term coefficients of variation (CV) in normal subjects for DPA and DXA were 2.3% and 1.1% respectively (29,30); *in vivo* correlation between both methods, as measured on the same day, was high (based on 252 lumbar spine measurements, $r=0.984$, $\beta=1.29 \pm 0.015$, $P < 0.001$). The results are expressed as BMC (g hydroxyapatite (Ha)). Quality assurance including calibration, was performed routinely every morning. No drift was observed during both study periods. Single-Photon Absorptiometry (SPA) of the right forearm was performed at the distal and proximal sites using a Nuclear Data 1100a scanner (31). The (fat-corrected) results are expressed as BMC (arbitrary units (U) per cm). The CV in our institution is 1.9% for the distal site and 1.0% for the proximal site (29).

Measurements and laboratory tests

Routine methods were used for the measurements of total alkaline phosphatase (APase) and inorganic phosphate (Pi). The renal tubular reabsorption maximum of phosphate corrected for glomerular filtration rate (TmP/GFR) was calculated according to Bijvoet (32). Serum osteocalcin and the carboxyterminal propeptide of type I collagen (PICP) were determined by commercially available kits (INCSTAR Corp., Stillwater, MN and Orion Diagnostica, Espoo, Finland respectively).

24-hour Urinary excretion of hydroxyproline (Organon Technika, Oss, The Netherlands) expressed as mmol OH-proline per mol creatinine was assessed at each visit. Prior to urine collections patients were on a gelatin free diet for three days.

25-hydroxyvitamin D_3 (25-(OH)D_3), 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) and intact PTH (1-84) were determined using commercially available kits (INCSTAR Corp., Stillwater, MN).

Statistical analysis

Paired T-tests were performed to analyze the effects on renal phosphate handling, bone turnover parameters and BMC between the two periods as well as within the two study periods. Results are expressed as mean ± SEM. $P < 0.05$ was considered significant.

RESULTS

All patients gained weight during the three year interval. Since height did not change, this also resulted in a significant increase of BMI (Table 1). PTH levels were significantly different at the beginning of the two study periods. Although none of the other parameters differed significantly, there was a tendency to a somewhat lower bone turnover and higher BMC at the beginning of the second study period (Table 2).

GH replacement therapy was well tolerated. In the second study period, one patient was treated with furosemide 20 mg for two weeks in the third and fourth week of GH therapy because of tense feeling in the fingers due to mild edema. Also in the second study period two of the six patients complained of gastrointestinal discomfort after the initiation of pamidronate. This disappeared spontaneously within two weeks.

The rise of IGF-I levels in the second study period was similar to that reported for the first study period (24).

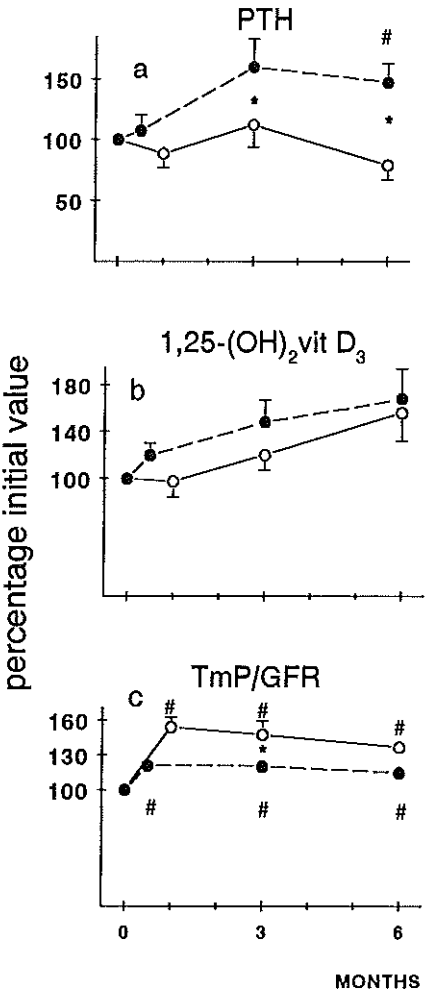

Fig.1 Time course of serum PTH, 1,25-dihydroxyvitamin-D_3--levels and TmP/GFR. Results are presented as mean ± SEM.

-o -; GH.-• -; GH + pamidronate.

# $P < 0.05$ vs. baseline.

* $P < 0.05$ difference between periods.

Biochemical measurements

Despite the higher baseline serum PTH levels during combined treatment, the rise of PTH levels was more prominent than during treatment with GH alone (Fig. 1A). The increase of PTH levels was significantly higher on combined treatment ($P < 0.05$). A similar trend was observed for 1,25-$(OH)_2D_3$ levels, although no significant difference between treatment periods could be observed (Fig. 1B).

25-$(OH)D_3$ levels did not change in either treatment period (results not shown). Serum phosphate increased from 1.19 ± 0.03 to 1.48 ± 0.03 mmol/L in the first study period and from 1.10 ± 0.03 to 1.35 ± 0.03 mmol/L in the second study period ($P < 0.05$ baseline vs. six months). TmP/GFR increased in both treatment periods, with a less prominent rise during combined treatment, reaching significance at three months (Fig. 1C). Although the correlation between serum IGF-I and TmP/GFR was highly significant ($P < 0.001$) during both treatment periods, addition of pamidronate weakened this relationship (r=0.75 vs. r=0.44) (Fig. 2).

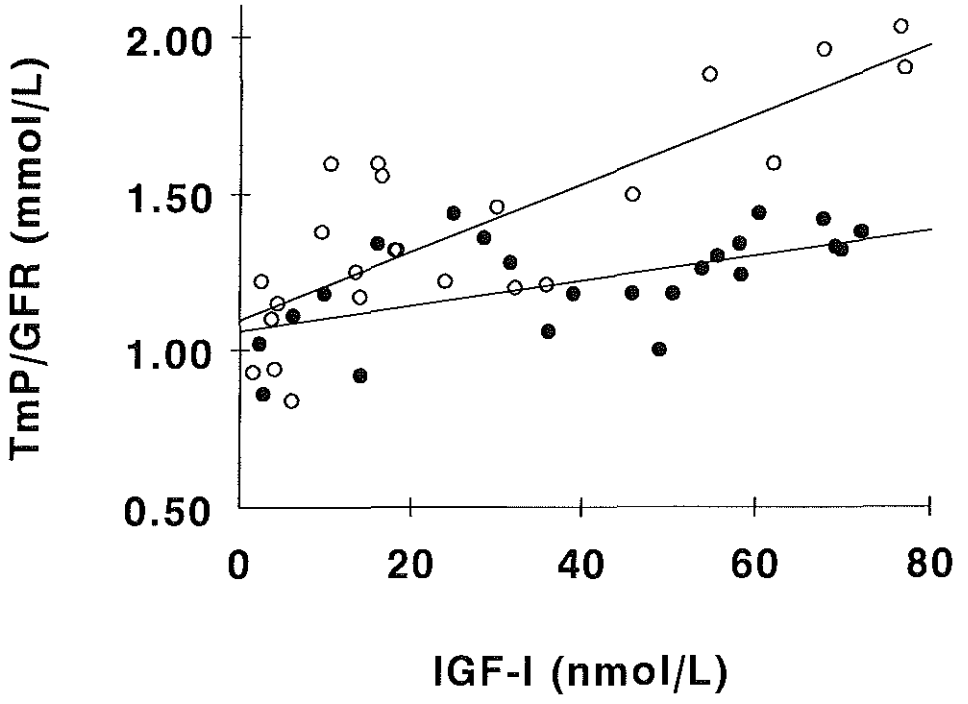

Fig. 2 Correlation between TmP/GFR and IGF-I. -o -; GH ($r = 0.75$). -• -; GH + pamidronate ($r = 0.44$). $P < 0.001$ both periods.

In both treatment periods an increase in markers of bone formation (ic. osteocalcin, APase and PICP) was observed. However, versus baseline the increase of the biochemical markers of bone turnover was markedly blunted in the second study period from the moment that GH replacement therapy was combined with pamidronate (Fig. 3A-C).

Urinary hydroxyproline as a measure of bone resorption rose significantly in both treatment periods. It reached a maximum after 1 month during treatment with GH alone, whereas an attenuated response was observed after addition of pamidronate (Fig. 3D).

24-hour Urinary calcium excretion increased significantly, but was not significantly different between the two periods (data not shown).

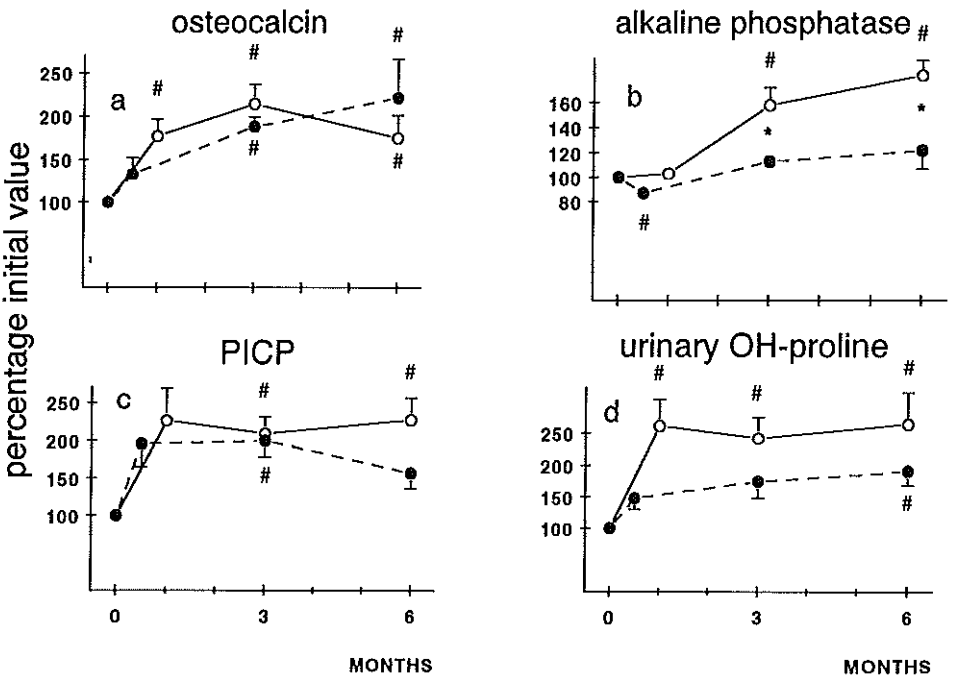

Fig. 3 Time course of bone turnover parameters. See also legend to Fig. 1.

Bone mineral content

The baseline date for bone mineral mass are shown in Table 1. The changes of the bone mass measurements during the two treatment periods are shown in Fig. 4. Only BMC data are shown, because bone mineral density data showed similar changes.

Lumbar spine. In the first treatment period there was a significant decrease in BMC after three and six months of GH replacement therapy (-1.5 ± 1.3% and -3.1 ± 1.5%, respectively). In the second treatment period there was an increase in BMC, which already reached significance versus baseline at six months (3.8 ± 2.0%). The difference between the periods was already nearly significant at three months and reached significance at six months (*P=0.052* and *P=0.04* respectively)

Distal forearm. In the first treatment period there was a significant decrease in BMC as measured in the distal forearm at three months. At six months BMC was still

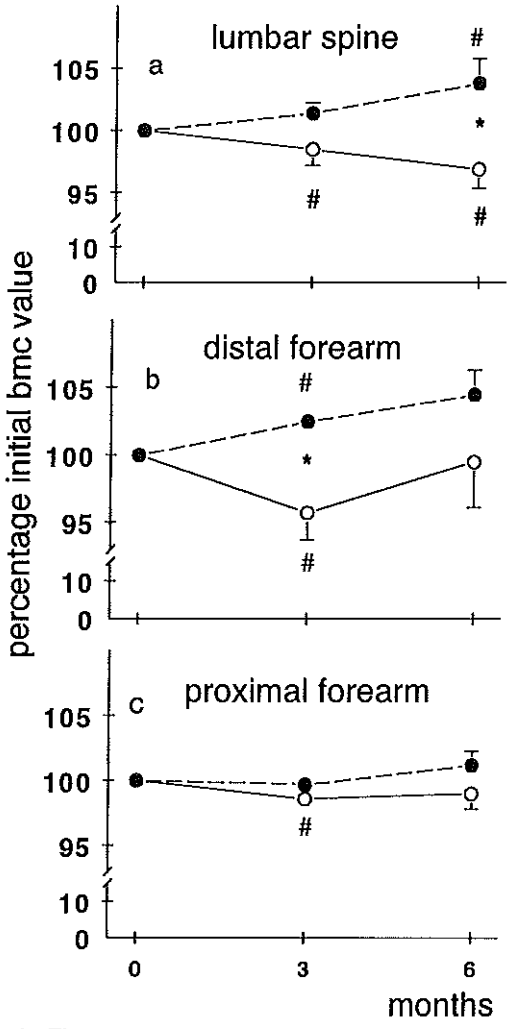

Fig. 4 Time course of bone mass measurements (BMC). See also legend to Fig. 1.

lower vs. baseline, however not significantly (-4.8 ± 2.0% and -0.5 ± 3.4%). In the second treatment period there was an increase at three and six months (2.5 ± 0.7% and 4.5 ±1.8%). The difference between the periods was significantly different at three months, but not at six months of therapy (*P=0.036* and *P=0.239* respectively).

Proximal forearm. The initial decline in BMC in the first treatment period was significantly greater than in the second treatment period. After six months in the second treatment period BMC levels were above baseline (-1.4 ± 0.6% and -1 ± 1.2% in the first period versus -0.3 ± 0.4% and 1.2 ± 1.1% at three and six months, respectively). Changes were not significantly different between periods.

DISCUSSION

Our study indicates that addition of pamidronate to GH replacement therapy in GHD adults attenuates the GH-induced increase of the renal phosphate threshold and prevents the initial bone loss as observed during treatment with rhGH alone.

As observed during the first study period GH induced a pronounced increase in renal phosphate reabsorption. This increase was not accompanied by changes of the phosphate regulating hormones PTH and 1,25-$(OH)_2D_3$. Therefore, a direct effect of GH or IGF-I on renal phosphate handling is more likely, which is also supported by the high correlation observed between IGF-I and TmP/GFR. Also recent in vivo and in vitro studies (33,34) suggest a similar mode of action. That GH does not affect the PTH-induced phosphaturic response is provided by the observation that the rise of PTH levels, as observed after the addition of pamidronate, is accompanied by a lowering of the renal phosphate threshold. In other words it appears that the GH-induced increase in phosphate reabsorption is counteracted by a PTH-induced decrease. Based on these observations it is unlikely that GH at the level of renal phosphate handling acts as a PTH-antagonist (3).

Recent studies also indicate that GH or IGF-I stimulates renal 1α-hydroxylase activity (3). In the first study period we did not observe a significant rise of 1,25-$(OH)_2D_3$ levels, which might be explained by the prominent increase of serum phosphate levels during GH treatment. It is known that hyperphosphatemia is a potent inhibitor of 1α-hydroxylase activity (35). The lower phosphatemic response after addition of pamidronate might, therefore, explain the trend towards higher 1,25-$(OH)_2D_3$ levels during the second study period. Of course, it is also possible that this represents a direct effect of the raised PTH levels on the renal 1α-hydroxylase.

When we compare the pretreatment values of biochemical markers of bone turnover and bone mass between both study periods, there appears to be a tendency to a somewhat lower bone turnover and a somewhat higher BMC at the start of the second study period. Nevertheless, the differences in these markers did not reach significance, which exclude an important "carry-over" effect of GH treatment from the first to the second study period. The somewhat higher bone mass at the start of the second study period could be related to the increase in body weight between both study period. Several studies have shown a positive relationship between body weight and bone mineral measurements.

As shown by the results obtained during the first study period GH leads to an increase in bone turnover. During an initial phase the GH-induced increase in the number of activated bone remodeling units will result in a larger bone surface undergoing bone resorption and subsequently will result in bone loss. An initial decrease in BMC was indeed found during treatment with GH alone in our patients, as well as by others (26). During a second phase the coupling between bone resorption and formation will result in a new balance, which will limit bone loss (32). However, Vandeweghe et al observed that long-term treatment with rhGH, beyond six months, even resulted in an increase of bone mass. Therefore, this suggests that besides an increase in activation frequency of bone remodeling units, long-term rhGH treatment may result in a loss of synchronization between resorption and formation with an apparent shift towards bone formation.

Several studies in postmenopausal women have shown that a reduction of bone turnover, as can be induced by pamidronate, is accompanied by a transient increase of bone mass (for review see Heaney (32)). However, in the present study treatment with pamidronate only attenuated the GH-induced increase in bone turnover, but did not result in a reduction below pretreatment level. Therefore, it is unlikely that the observed gain in BMC during additional pamidronate treatment can simply be explained by the so-called "transient". A shift of the bone balance towards formation is more likely. As stated above this shift towards formation may be related to GH treatment. It is known that IGF-I can stimulate osteoblast-like cells (36). Furthermore the effects of GH treatment can be indirect via an increase in muscle mass and physical activity. However, an anabolic effect on bone of the raised PTH levels during pamidronate treatment can not be excluded. Although the mechanism for the anabolic action of PTH is poorly understood, GH is required for its effect in animals (37). As the volume to surface ratio of trabecular bone is higher than that of cortical bone, changes in bone turnover are more reflected in trabecular bone. This also explains the more pronounced change of BMC at the distal radius and lumbar spine, compared to the proximal forearm.

This study shows that the effects of GH-replacement therapy and bisphosphonates on renal phosphate handling are partly antagonistic and that these effects are probably not mediated through PTH. GH therapy results in an increased bone turnover and consequently in bone loss. Inhibition of bone turnover with the bisphosphonate pamidronate, prevents this initial loss and even results in an increase of bone mass. Additional studies are needed to see whether pamidronate interacts with long-term effects of GH on bone mass in GHD patients.

Acknowledgements

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REFERENCES

1. **Harbison MD, Gertner JM.** 1990 Permissive action of growth hormone on the renal response to dietary phosphorus deprivation. J Clin Endocrinol Metab. 70:1035-1040.

2. **Bouillon R.** 1991 Growth hormone and bone. Horm Res. 36(Suppl 1):49-55.

3. **Lieberman SA, Holloway L, Marcus R, Hoffman AR.** 1994 Interactions of growth hormone and parathyroid hormone in renal phosphate, calcium, and calcitriol metabolism and bone remodeling in postmenopausal women. J Bone Miner Res. 9:1723-1728.

4. **Adami S, Frijlink WB, Bijvoet OLM, O'Riordan JLH, Clemens TL, Papapoulos SE.** 1982 Regulation of calcium absorption by 1,25,dihydroxy-vitamin D-studies of the effects of a bisphosphonate treatment. Calc Tissue Int. 34:317-320.

5. **Bonjour JP, Trechsel U, Taylor CM, Fleisch H.** 1988 Parathyroid hormone-independent regulation of $1,25(OH)_2D$ in response to inhibition of bone resorption. Am J Physiol. 254:E260-E264.

6. **Netelenbos JC, van Ginkel FC, Lips P, et al.** 1991 Effect of a single infusion of aminohydroxypropylidene on calcium and bone metabolism in healthy volunteers monitored during 2 months. J Clin Endocrinol Metab. 72:223-228.

7. **Kaufman J-M, Taelman P, Vermeulen A, Vandeweghe M.** 1992 Bone mineral status in growth hormone-deficient males with isolated and multiple pituitary deficiencies of childhood onset. J Clin Endocrinol Metab. 74:118-123.

8. **Hyer SL, Rodin DA, Tobias JH, Leiper A, Nussey SS.** 1992 Growth hormone deficiency during puberty reduces adult bone mineral density. Arch Dis Child. 67:1472-1474.

9. **Elgindy N, Grunditz R, Thorén M, Degerblad M, Sjöberg HE, Ringertz H.** 1991 Long term follow-up of metacarpal cortical thickness and bone mineral density in panhypopituitarism. Radiol Diagn. 32:326-330.

10. **Holmes SJ, Economou G, Whitehouse RW, Adams JE, Shalet SM.** 1994 Reduced bone mineral density in patients with adult onset growth hormone deficiency. J Clin Endocrinol Metab. 78:669-674.

11. **Wüster C, Slencka E, Ziegler R.** 1991 Erhöhte prävalenz von osteoporose und arteriosklerose bei konventionell substituierter hypophysenvorderlappeninsuffizienz: bedarf einer zusätzlichen wachstumshormonsubstitution? Klin Wochenschr. 69:769-773.

12. **Black DM, Cummings SR, Genant HK, Nevitt MC, Palermo L, Browner W.** 1992 Axial and appendicular bone density predicts fractures in older women. J Bone Miner Res. 7:633-638.

13. **Cummings SR, Black DM, Nevitt MC, et al.** 1993 Bone density at various sites for prediction of hip fractures. Lancet. 341:72-75.

14. **Johansson AG, Burman P, Westermark K, Ljunghall S.** 1992 The bone mineral density in acquired growth hormone deficiency correlates with circulating levels of insulin-like growth factor I. J Intern Med. 232:447-452.

15. **Bing-You RG, Denis M-C, Rosen CJ.** 1993 Low bone mineral density in adults with previous hypothalamic-pituitary tumors: Correlations with serum growth hormone responses to GH-releasing hormone, insulin-like growth factor I, and IGF binding protein 3. Calc Tissue Int. 52:183-187.

16. **Salomon F, Cuneo RC, Hesp R, Sönksen PH.** 1989 The effects of treatment with recombinant human growth hormone on body composition and metabolism in adults with growth hormone deficiency. N Engl J Med. 321:1797-1803.

17. **Jørgensen JOL, Pedersen SA, Thuesen L, Jørgensen J, Skakkebæk NE, Christiansen JS.** 1989 Beneficial effects of growth hormone treatment in GH-deficient adults. Lancet. 1:1221-1225.

18. **Bengtsson B-Å, Edén S, Bosaeus I, et al.** 1993 Treatment of adults with growth hormone deficiency with recombinant human growth hormone. J Clin Endocrinol Metab. 76:309-317.

19. **Brixen K, Nielsen HK, Mosekilde L, Flyvbjerg A.** 1990 A short course of recombinant human growth hormone treatment stimulates osteoblasts and activates bone remodeling in normal human volunteers. J Bone Miner Res. 5:609-618.

20. **Johansen JS, Pedersen SA, Jørgensen JOL, et al.** 1990 Effects of growth hormone (GH) on plasma bone gla protein in GH-deficient adults. J Clin Endocrinol Metab. 70:916-919.

21. **Nielsen HK, Jørgensen JOL, Brixen K, Christiansen JS.** 1991 Serum osteocalcin and bone isoenzyme alkaline phosphatase in growth hormone-deficient patients: dose-response studies with biosynthetic human GH. Calc Tissue Int. 48:82-87.

22. **Schlemmer A, Johansen JS, Pedersen SA, Jørgensen JOL, Hassager C, Christiansen C.** 1991 The effect of growth hormone (GH) therapy on urinary pyridinoline cross-links in GH-deficient adults. Clin Endocrinol (Oxf). 35:471-476.

23. **Whitehead HM, Boreham C, McIlrath EM, et al.** 1992 Growth hormone treatment of adults with growth hormone deficiency: results of a 13-month placebo controlled cross-over study. Clin Endocrinol (Oxf). 36:45-52.

24. **Binnerts A, Swart GR, Wilson JH, et al.** 1992 The effect of growth hormone administration in growth hormone deficient adults on bone, protein, carbohydrate and lipid homeostasis, as well as on body composition. Clin Endocrinol (Oxf). 37:79-87.

25. **Degerblad M, Elgindy N, Hall K, Sjöberg H-E, Thorén M.** 1992 Potent effect of recombinant growth hormone on bone mineral density and body composition in adults with hypopituitarism. Acta Endocrinol (Copenh). 126:387-393.

26. **Vandeweghe M, Taelman P, Kaufman J-M.** 1993 Short and long-term effects of growth hormone treatment on bone turnover and bone mineral content in adult growth hormone-deficient males. Clin Endocrinol (Oxf). 39:409-415.

27. **O'Halloran DJ, Tsatsoulis A, Whitehouse RW, Holmes SJ, Adams JE, Shalet SM.** 1993 Increased bone density after recombinant human growth hormone (GH) therapy in adults with isolated GH deficiency. J Clin Endocrinol Metab. 76:1344-1348.

28. **Rosén T, Johansson G, Hallgren P, Caidahl K, Bosaeus I, Bengtsson B-Å.** 1994 Beneficial effects of 12 months replacement therapy with recombinant human growth hormone to growth hormone deficient adults. Endocrinol Metab. 1:55-66.

29. **van Berkum FNR, Birkenhäger JC, van Veen LCP, et al.** 1989 Non-invasive axial and peripheral assessment of bone mineral content: A comparison between osteoporotic women and normal subjects. J Bone Miner Res. 4:679-685.

30. **Erdtsieck RJ, Pols HAP, Algra D, Kooy PPM, Birkenhäger JC.** 1994 Bone mineral density in healthy Dutch women; spine and hip measurements using dual-energy x-ray absorptiometry. Neth J Med. 45:198-205.

31. **Nilas L, Borg J, Gotfredsen A, Christiansen C.** 1985 Comparison of single- and dual-photon absorptiometry in postmenopausal bone mineral loss. J Nucl Med. 26:1257-1262.

32. **Heaney RP.** 1994 The bone-remodelling transient: implications for the interpretation of clinical studies of bone mass change. J Bone Miner Res. 9:1515-1523.

33. **Caverzasio J, Bonjour JP.** 1988 Influence of recombinant IGF-I (somatomedin-C) on sodium-dependent phosphate transport in cultured renal epithelium. Prog Clin Biol Res. 252:385-386.

34. **Caverzasio J, Montessuit C, Bonjour JP.** 1990 Stimulatory effect of insulin-like growth factor-1 on renal Pi transport and plasma 1,25-dihydroxyvitamin D_3. Endocrinology. 127:453-459.

35. **Holick MF, Adams JS.** 1990 Vitamin D metabolism and biological function. In: Avioli LV, Krane SM eds. Metabolic bone disease and clinically related disorders, Philadelphia: W.B. Saunders; 155-195.

36. **Kassem M, Blum WF, Ristelli J, Mosekilde L, Eriksen EF.** 1993 Growth hormone stimulates proliferation and differentiation of normal human osteoblast-like cells in vitro. Calc Tissue Int. 52:222-226.

37. **Hock JM, Fonseca J.** 1990 Anabolic effect of human synthetic parathyroid-hormone-(1-34) depends on growth hormone. Endocrinology. 127:1804-1810.

5 Treatment of Postmenopausal Osteoporosis with a Combination of Growth Hormone and Pamidronate. A Placebo-Controlled Trial

ABSTRACT

It is known that growth hormone (GH) can induce accelerated bone turnover in GH deficient people as well as healthy elderly people. In this study we examined the effect of recombinant human GH (rhGH) on bone mineral mass and bone turnover in the presence of the bone resorption inhibiting agent, pamidronate. Also effects on body composition were studied.

21 postmenopausal osteoporotic women were treated with the bisphosphonate pamidronate during twelve months. During the initial 6 months rhGH was administered (0.0675 IU/kg, 3 times/week) in a placebo-controlled fashion (10 vs. 11 patients).

Bone mineral content (BMC) of the lumbar spine and femoral neck was measured with dual energy X-ray absorptiometry and BMC of the distal and proximal forearm with single photon absorptiometry. Body composition was measured with bioelectrical impedance and total body dual energy X-ray absorptiometry. Furthermore serum IGF-I and biochemical indices of bone turnover were measured.

The group treated with rhGH showed a two- to three-fold increase in serum IGF-I levels. No effects on bone mineral mass were observed in the group treated with rhGH, while in women treated with pamidronate only a consistent increase of about 5 % was found at the lumbar spine. In both groups no change in BMC at the femoral neck and forearm was observed.

Compared to baseline the biochemical measurements of bone turnover showed a decrease of about 50 % in the pamidronate-treated group, whereas in the group additionally treated with rhGH this effect was blunted.

The body composition measurements showed clear effects of rhGH administration, i.e. a decrease of fat mass of about 5 % and an increase of lean body mass of about 3 %. However, these effects disappeared after the treatment with rhGH was stopped; fat mass as well as lean body mass returned to initial values.

The present study suggests that treatment with rhGH blunted both the pamidronate-induced accumulation of bone mineral mass and the reduction of biochemical markers of bone turnover. Furthermore the positive effect of rhGH on body composition disappears completely after cessation of treatment with rhGH.

INTRODUCTION

Several approaches for the medical treatment of postmenopausal osteoporosis are available at present. Among these are estrogens, bisphosphonates, anabolic steroids, calcitonin, fluoride and vitamin D preparations (1-7). Most of these treatment schemes are effective by diminishing bone resorption, whereas only fluoride is able to increase osteoblastic activity (8,9). Recently Heaney argued that much of the apparent gain in bone mass produced by several agents currently employed to treat osteoporosis can be explained by the so called "bone-remodelling transient" (10). In other words the gain of bone induced by an inhibitor of bone resorption will only be transient because the associated rate of change in bone mass persists for only one remodelling period. A more fundamental change in bone mineral mass can only be obtained if a given drug can induce a change in the balance between formation and resorption at each individual remodelling locus.

Recent evidence suggests a role for growth hormone (GH) as an osteotrophic factor. It is known that GH is responsible for longitudinal bone growth through a direct stimulation of chondrocytes and osteoblasts (11,12). In adults GH also activates bone remodelling (13,14). Furthermore, several studies indicate that in GH deficient (GHD) adults treatment with recombinant human GH (rhGH) results in an increase of bone turnover parameters and after long-term treatment even a rise of bone mineral mass has been observed (15-18). In addition studies of patients with endogenous high levels of GH (acromegaly) have shown a stimulated bone turnover and an increased bone mineral mass (19,20).

Normal ageing and GHD show some striking common features. Both GHD as well as normal ageing are associated with decreases in protein synthesis, decreases in percentages of lean body and bone mass as well as with increases in percentage body fat (21-26). It is possible that reduced GH and IGF-I secretion may account, at least in part, for one or more of the above effects of ageing (27,28) and that elderly individuals might benefit from treatment with rhGH. In several studies administration of rhGH for periods varying from a few weeks to twelve months, resulted in improvement of nitrogen balance, an increase in lean body mass, and a decrease in percentage body fat (14,29-31).

Very little is known about the effects of rhGH in the treatment of postmenopausal osteoporosis. In a small study in which GH was administered in sequence with calcitonin in women with postmenopausal osteoporosis a small favourable effect was observed (32). In the present placebo-controlled study we tested whether rhGH might have an additional anabolic effect on bone formation in postmenopausal osteoporotic women treated with the bone resorption inhibitor pamidronate. Furthermore, the effects of rhGH on body composition were evaluated.

MATERIALS AND METHODS

Patients and treatment

After review and approval of the protocol by the Ethical Committee of our institution, written informed consent was obtained from the patients. All 23 patients were Caucasian postmenopausal osteoporotic women with at least one non-traumatic vertebral fracture. Patients with any disorder known to affect bone mass were excluded from this study, as were women with a history of malignancy. All patients were treated with pamidronate. The patients were randomly assigned to receive either rhGH or placebo.

All patients received during 12 months pamidronate as an enteric-coated tablet, 150 mg per day, orally on an empty stomach. During the initial six months rhGH or placebo was self administered by means of subcutaneous injection of 0.0625 IU/kg with a maximum of 4 IU, 3 times per week (monday, wednesday, friday). In a pilot-study this dose resulted in high normal IGF-I levels. Treatment was double blinded with regard to rhGH or placebo. rhGH (Humatrope) and placebo was supplied by Eli Lilly Co. Indianapolis, IN., USA. Placebo vials were indistinguishable from rhGH vials.

In patients with a calcium intake below 1000 mg (according to dietary history), a Ca-Sandoz effervescent tablet containing 0.5 g elemental calcium was given. This applied to 5 patients in the GH-group and 6 patients in the placebo group. Compliance was measured by means of tablet and vial counting. The baseline characteristics of the patients are shown in Table 1.

TABLE 1. Clinical and biochemichal characteristics of the patients

Characteristic	rhGH (n=10)		placebo (n=11)		
	mean	range	mean	range	*P*
Age (yr)	63.5	57-74	62.8	55-72	NS
YSM (yr)	20.2	7-30	14.4	5-27	NS
BMI (kg/m^2)	25.0	19.6-27.4	25.6	19.0-32.5	NS
Height (cm)	162.5	149-179	160.5	153-178	NS
Weight (kg)	65.3	56.0-77.5	66.7	45.0-89.1	NS
BMC_{SPINE} (gHa)	32.7	23.4-39.7	31.8	21.2-44.6	NS
$BMC_{FEM\ NECK}$ (gHa)	3.5	3.1-4.4	3.6	1.9-6.0	NS
BMC_{WARD} (gHa)	1.6	1.2-2.4	1.7	1.0-3.5	NS
BMC_{TROCH} (gHa)	8.2	4.6-14.3	7.3	3.2-11.6	NS
BMC SPA_{DIST} (U/cm)	29.4	22.2-38.2	27.8	14.0-40.3	*0.05*
BMC SPA_{PROX} (U/cm)	30.8	19.5-38.4	30.3	16.3-42.9	NS
Serum calcium (mmol/L)	2.28	2.10-2.40	2.29	2.15-2.44	NS
Serum phosphate (mmol/l)	1.13	0.99-1.27	1.19	0.81-1.41	NS
Creatinine (μmol/l)	67	48-91	78	49-113	NS
APase (U/l)	57.2	44-82	79.5	37-124	*<0.05*
IGF-I (nmol/l)	20.0	9.6-33.0	24.2	13.2-48.0	NS
Osteocalcin (μg/l)	3.0	1.0- 6.5	4.7	0.6- 8.5	NS
Procollagen I (μg/l)	104.2	44-286	108.1	74-178	NS
2 hr fasting:					
Urinary OH-proline (mmol/mol creat)	20.8	9.6-39.4	23.5	10.2-60.0	NS
Urinary pyrilinks (nmol/mol creat)	80.3	36.7-171.0	51.3	14.6-88.1	NS

NS: no significance between groups.

Bone mineral mass measurements

Single photon absorptiometry (SPA) of the right forearm was performed at the distal and proximal sites with an interval of 3 months using a Nuclear Data 1100a scanner (33). The (fat-corrected) results are expressed as bone mineral content (BMC; arbitrary units, U/cm). The coefficient of variation in our hands is 1.9 % for the distal site and 1.0 % for the proximal site.

Dual energy X-ray absorptiometry (DXA; Lunar DPX-L, Lunar Radiation, Madison, WI, USA) was performed at the lumbar vertebrae 2-4 (L2-4) and in the left proximal femur: the femoral neck, Ward's triangle and trochanteric region according to the instructions of the manufacturer (34). BMC is expressed as grams of bone mineral (g/cm). For osteoporotic women the coefficient of variation is 1.5% for the lumbar spine and 1.8, 2.4 and 2.8% in the femoral neck, Ward's triangle and trochanteric region, respectively.

Quality assurance, including calibration with the standard of the machines (SPA and DXA), was performed routinely every working day. No drift was observed during the whole study period.

Body composition

Body composition was measured after an overnight fast by the bioelectrical impedance method (BIA) with a body composition analyser (RJL-systems BIA-101 Detroit, MI, USA) as described previously (35,36). Through regression equation, fat free mass (FFM) was calculated. Fat mass was calculated by subtracting FFM from body weight (36).

Body composition was also measured by using the DPX-L densitometer. The instrument measures fat mass, lean mass and bone mineral content. The method is well validated (37).

Biochemistry

At baseline and after 1, 3, 6, 9, and 12 months the following fasting serum parameters were determined: Calcium, phosphate, alkaline phosphatase, creatinine, albumin, glucose and glycosylated haemoglobin (HbA_1C) with standard methods.

Insulin-like growth factor-I (IGF-I), osteocalcin and procollagen type 1 (PICP) with radioimmuno-assay (RIA) (Medgenix Diagnostics, Brussels, Belgium; Incstar Corporation, Stillwater, USA and Orion Diagnostica, Espoo, Finland respectively).
At baseline and after 3, 6, 9 and 12 months the following urinary parameters were determined: Fasting (2h) hydroxyproline by Hypronosticon (Organon Technika, Oss, The Netherlands) and excretion of free deoxypyridinoline by enzyme linked immuno sorbent assay (Pyrilinks D, Metra biosystems, Palo Alto, USA).
All samples were collected after an overnight fast on the day after administration of rhGH or placebo.

Statistics

Values are expressed as means ± SEM. At baseline differences between groups were tested by Student's t-test for unpaired data, with a P value of < 0.05 for significance.
Statistics within and between treatment groups were performed by means of repeated measures analysis of variance (rmANOVA). The dependent variable is assumed to have a linear trend relationship with time; in the model the slope is different between the two treatment groups. In the GH group the model includes additional effects after stopping rhGH in month 9 and 12.
If the rmANOVA resulted in a significant main effect between treatment groups, we performed a Student's t-test posthoc analysis for paired data within groups and for unpaired data between groups, with a P value of < 0.05 for significance.

RESULTS

Side effects, baseline values

Initially 23 patients entered the study. 12 patients were treated with pamidronate only (placebo-group). One of these patients decided to stop at 4 months, because of gastric complaints, related to the use of the bisphosphonate. The 11 patients of the other group were treated with APD and rhGH (rhGH-group). In this group one patient was taken out at 3 months because of cardiac complaints related to fluid retention (minor cardiac failure, which was treated with diuretics). The remaining 21 patients (placebo-group; n=11, rhGH-group; n=10) completed the 12 months study period and were eligible for evaluation. Except for 1 patient complaining temporarily of gastric complaints no side effects were observed in these patients. rhGH injections were tolerated well, no dose adjustments were necessary.

The baseline values are presented in Table 1. No significant differences as to age, or years since menopause (YSM) were found between both groups. Also anthropometric data were comparable. The initial bone mass measurements showed no significant differences except for the distal forearm BMC, measured with SPA (Table 1). Body composition did not show any differences at baseline when calculated with BIA or measured with DXA. The baseline biochemical measurements showed a higher serum alkaline phosphatase ($P < 0.05$) in the placebo-treated group, otherwise there were no differences (Table 1).

Longitudinal bone mass measurements

The time course of the different bone mineral mass measurements are shown in Fig. 1A-D. For the lumbar spine a rapid increase in the placebo-group was observed after three months with a much slower increase during the subsequent nine months. For the rhGH-group no significant changes were observed during (0-6 months) and after cessation (6-12 months) of treatment. In the femoral neck (as well as the Ward's triangle and trochanteric region) no significant changes during the whole treatment period (12 months) for either group were observed. For the distal forearm a similar pattern as in the lumbar spine was found, however, no significance was reached between both groups. The proximal forearm showed no clear changes in either group.

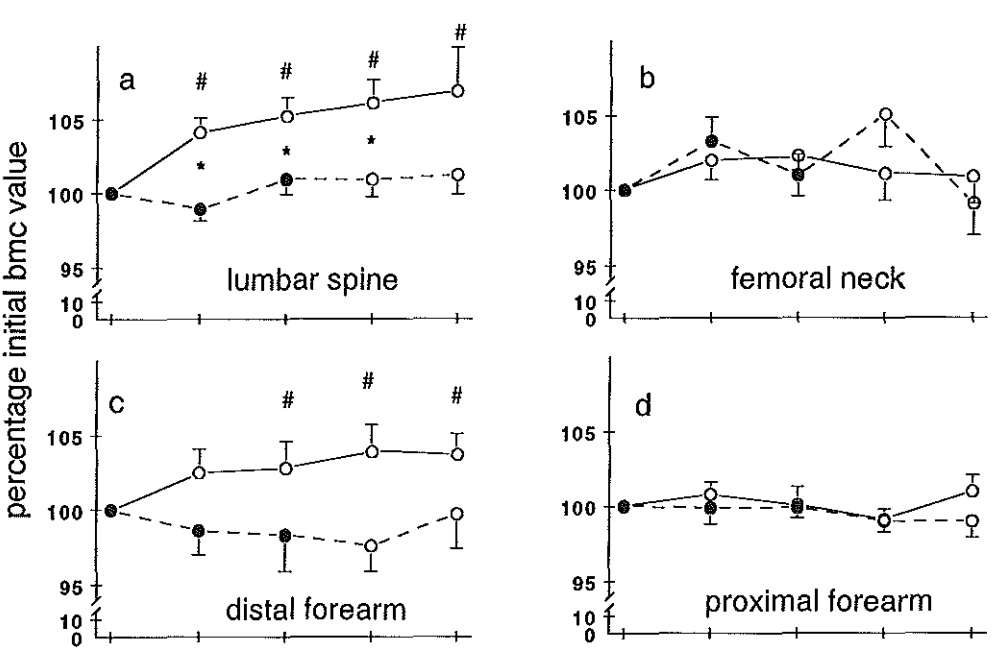

Fig. 1 Course of the BMC. Treatment with Pamidronate during twelve months. Treatment with rhGH or placebo during the first six months. Measurement at 0, 3, 6, 9 and 12 months. Results are presented as mean ± SEM and as percentage of initial value. For statistical analyses see material and methods section. - o - ; pamidronate. - ● - ; GH + pamidronate

Significance within groups vs. baseline: *# P < 0.05, # # P < 0.01.*

Significance between groups :* *P < 0.05, ** P < 0.01.*

Body composition

Baseline measurements for body composition showed a correlation between fat mass assessment by BIA and DXA (r = 0.74, *P <0.001*). Also the changes in time measured by the two methods were well correlated (r = 0.85, *P <0.001*).

In the placebo-group no changes in body composition were observed during the year of treatment. In the rhGH-group, irrespective of the method used, a significant decline of the percentage fat of about 5-7 % compared to initial values occurred. After the treatment with rhGH was withdrawn an increment of fat mass occurred, reaching pre-treatment levels within 3 months, when measured by BIA (Fig. 2A).

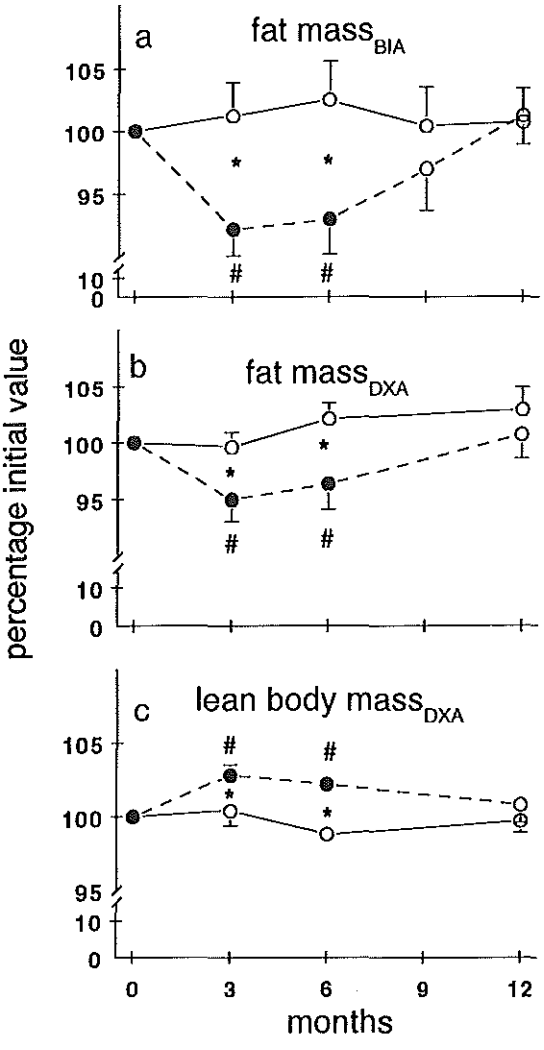

Fig. 2 Course of the fat mass: measured by impedance (BIA) (a) and DXA (b). Course of lean body mass by DXA (c). See also the legend to Fig. 1.

Total body DXA measurements were not performed at 9 months (Fig. 2B). When lean body mass was analysed by DXA, again no changes in the placebo-group were found whereas a significant increase in lean mass of about 2.5 % after 3 months was found in the rhGH-group (Fig. 2C). Again, these changes disappeared after treatment with rhGH was stopped at six months and returned to pre-treatment values.

Biochemical parameters

Serum levels of IGF-I increased rapidly after 1 month in the rhGH-group and stabilised during rhGH-treatment. After cessation of rhGH-treatment a rapid return to pre-treatment values was observed (Fig. 3).

Serum Ca, P and creatinine did not change during the treatment period between or within groups. During the treatment period no changes in glucose metabolism (glucose, glycosylated Hb) were observed in both groups.

The relative changes of serum alkaline phosphatase, osteocalcin, procollagen type 1 over time within and between both treatment groups are depicted in Fig. 4 A-C.

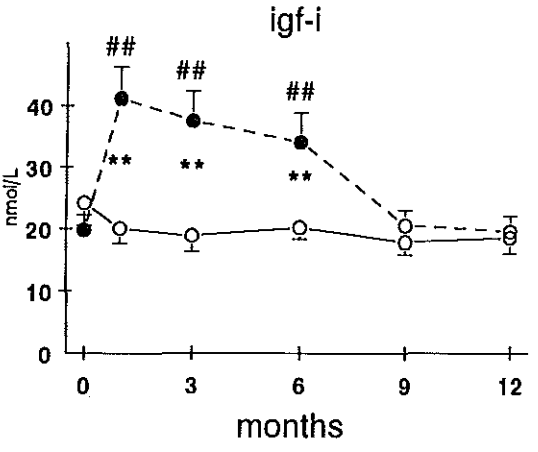

Fig. 3 Course of the serum IGF-I levels. Treatment with Pamidronate during twelve months. Placebo-controlled treatment with GH during first six months. Measurements at 0, 1, 3, 6, 9 and 12 months. Results are expressed as mean ± SEM in nmol/L. See also the legend to Fig. 1.

In the placebo-group as well as in the rhGH-group a significant increase in alkaline phosphatase was found after 1 month. From 6 months onwards in the placebo-group and from 9 months onwards in the GH-group a significant decline of serum alkaline phosphatase was found. For osteocalcin a rapid decrease in the placebo-group was observed, whereas in the rhGH-group a non-significant increase was observed. From 3 months onwards a significant difference between both treatment groups existed.

Procollagen type 1 showed a small increase after 1 month in the rhGH-group, followed by a non-significant decline (compared to initial values). In the placebo-group an instant decline was observed from initiation of treatment onwards.

In the placebo-group, urinary OH-proline (corrected for urinary creatinine) showed a rapid although not significant decline, whereas in the rhGH-group no changes at all were observed (Fig 4D). Also urinary free deoxypyridinoline, which is a more specific marker of bone resorption, showed only in the placebo-group a temporarily significant decline.

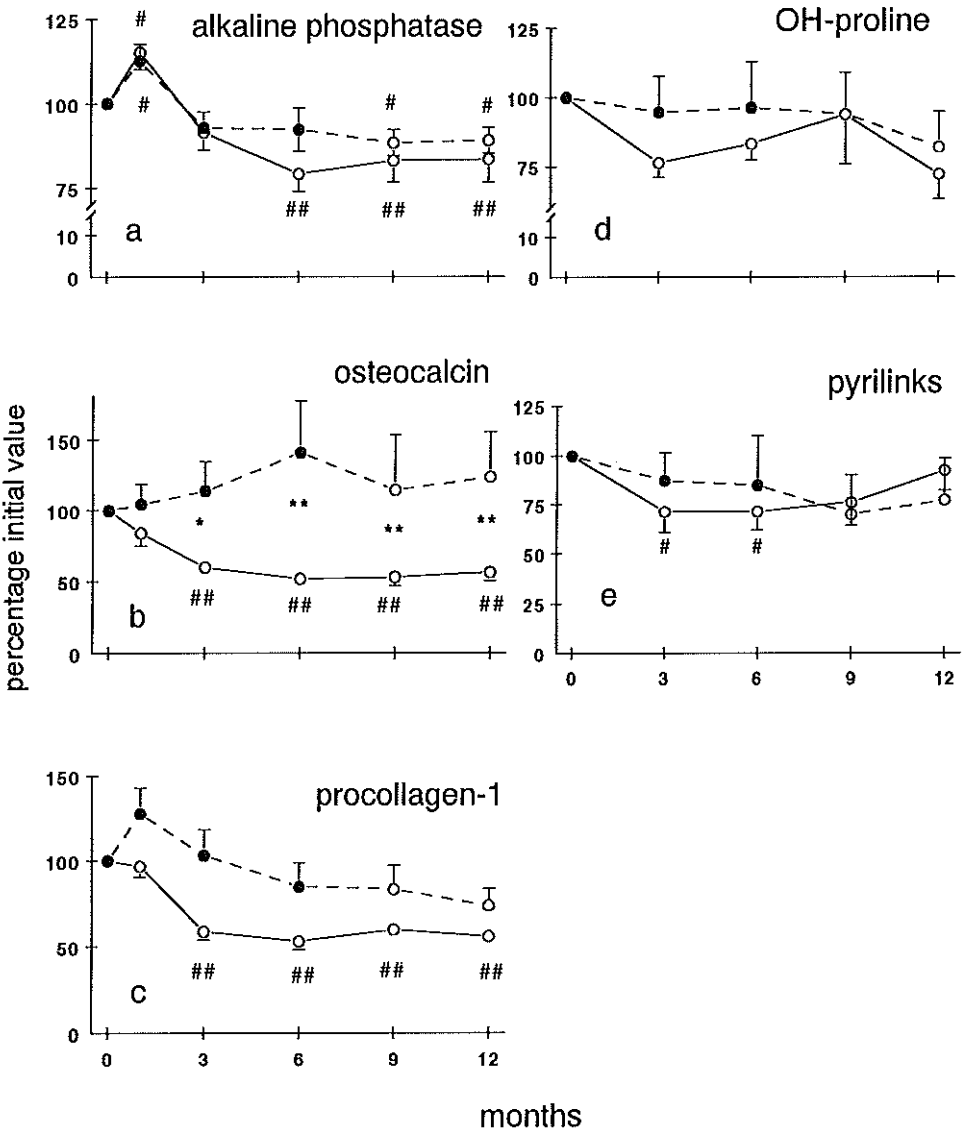

Fig. 4 Course of serum alkaline phosphatase (a), osteocalcin (b), procollagen type 1 (c), fasting 2h urinary hydroxyproline (d), and pyrilinks (e) during the treatment period. Urinary measurements are corrected for creatinine excretion. Measurements at 0, 1, 3, 6, 9 and 12 months. (Urinary measurements not performed at 1 month). Results are expressed as mean ± SEM and as percentage of initial value. See also the legend to Fig. 1.

DISCUSSION

In this study, in which we treated postmenopausal osteoporotic women for one year with pamidronate, half of the women received during the initial six months also rhGH in a placebo-controlled manner. No additional effect of rhGH treatment was found on bone mineral mass. On the contrary, the beneficial effect of pamidronate on bone mass was blunted by the addition of rhGH.

In the placebo-group, treated with pamidronate only an increase of bone mineral mass at sites representing cancellous bone (lumbar spine and distal forearm) was observed, whereas no significant changes were observed at sites merely containing cortical bone (femoral neck and proximal forearm). A similar gain of bone mineral mass, explained by the so-called "transient" (10), has been reported by other investigators using bisphosphonates (4,38).

In the rhGH-group a rapid and consistent increase of circulating IGF-I levels was observed, indicating that the dose of rhGH used (0.0625 IU/kg, 3 times/wk) was sufficient to reach a level which is supraphysiological for this age group. After withdrawal of rhGH administration, IGF-I values returned to baseline values. Other investigators recently showed that in postmenopausal women a comparable dosage of rhGH as used in our study induces a prompt and sustained increase in bone remodelling (14). However, in our osteoporotic women the simultaneous addition of pamidronate appears to attenuate this rhGH-induced increase in bone turnover. Therefore, the absence of a clear change in the activation of bone remodelling units, as reflected by the markers of bone turnover, might explain that in the rhGH-group no change of bone mineral mass was observed. It became only apparent after the study that the baseline bone turnover rate appeared somewhat lower in the rhGH treated patients. However, this does not detract from our main conclusion that combined treatment of rhGH and pamidronate does not result in a positive effect on bone mineral mass.

The present combination of therapeutic modalities in the treatment of postmenopausal osteoporosis was based on the assumption that it might be possible to activate bone formation by rhGH in the presence of an anti-resorptive therapy (pamidronate). However, this turned out not to occur. Several possible explanations can be given. First,

we have to take into account that the postmenopausal women we have treated are not growth hormone deficient and may thereby be less sensitive to supraphysiological dosages of growth hormone. However, several studies have indicated that both short- (30) and long-term (14) treatment with comparable dosages rhGH resulted in an increased bone turnover. In the present study we also observed in the rhGH treated patients at least initially a trend towards an increase of all bone formation markers, which was not accompanied by an increase of bone resorption. However, this apparent shift of the balance towards formation did not result in a clear rise of bone mass.

Secondly, the treatment period might have been too short to observe an osteotropic effect of rhGH. In GHD patients only long-term treatment with rhGH beyond six months resulted in a sustained increase of bone mineral mass (18).

Thirdly, the present scheme of continuous rhGH and pamidronate treatment might have inhibited potential beneficial effects of rhGH on osteoblastic function. Whether it may be necessary to administer rhGH cyclically in tandem with pamidronate remains to be seen. Previous results (32) using GH in sequence with the bone resorption inhibitor calcitonin do not support the idea that such an approach indeed results in a more favourable response than could be expected from treatment with an inhibitor of bone resorption alone.

Finally, one can argue that the number of patients in this study is too small. However, based on the present results it is highly unlikely that inclusion of more patients will change the results towards a more favourable effect of combined treatment versus treatment with pamidronate alone.

rhGH treatment affects body composition with a decrease of fat mass and increase of lean body mass (24,29,39). Body composition in this group of elderly patients also changed accordingly during rhGH treatment with a decrease of fat mass and a rise of lean body mass. The changes observed were comparable to those obtained in GHD adult individuals during rhGH replacement therapy (29,40).

Still, the methodology used in this study to measure body composition merits some further comments. The validation of the BIA method has not been carried out for different patient groups. The hydration status significantly influences the outcome, in the sense that a slight dehydration, as might be the case in the elderly, overestimates the

percentage body fat (41,42). The DXA method is better validated and measures fat directly (43). The DXA technique does not distinguish between intra- and extracellular water. The increase in free fat mass as observed in our patients during GH treatment indicates an accumulation of body water. The decrease in fat mass, as measured by DXA, however, is a true phenomenon. As the changes in body composition, measured by BIA and DXA are closely in parallel, we consider these changes as clinically significant.

Taken together our results clearly show that simultaneously started combined treatment with rhGH and pamidronate during six months offers no new approach in the treatment of postmenopausal osteoporosis. Even if long-term treatment with rhGH results in a favourable shift of the balance between bone resorption and formation than it is still questionable whether such an effect justifies long-term treatment given the side-effects of GH-treatment and its high costs. Nevertheless, further studies are needed to elucidate whether in selected cases with a low bone turnover administration of rhGH in sequence with an inhibitor of bone resorption is a useful option.

Acknowledgements

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1. **Mamelle N, Dusan R, Martin JL, et al.** 1988 Risk-benefit ratio of sodiumfluoride treatment in primary vertebral osteoporosis. Lancet. 2:361-365.

2. **Need AG, Horowitz M, Bridges A, Rad ADipD, Morris GA, Nordin BEC.** 1989 Effects of nandrolone decanoate and antiresorptive therapy on vertebral density in osteoporotic postmenopausal women. Arch Intern Med. 149:57-60.

3. **Overgaard K, Riis BJ, Christiansen C, Hansen MA.** 1989 Effect of salcatonin given intranasally on early postmenopausal bone loss. Br Med J. 299:477-479.

4. **Storm T, Thamsborg G, Steiniche T, Genant HK, Sørensen OH.** 1990 Effect of intermittent cyclical etidronate therapy on bone mass and fracture rate in women with postmenopausal osteoporosis. N Engl J Med. 322:1265-1271.

5. **Erdtsieck RJ, Pols HAP, van Kuijk C, et al.** 1994 Course of bone mass during and after hormonal replacement therapy with and without addition of nandrolone decanoate. J Bone Miner Res. 9:277-283.

6. **Ettinger B, Genant HK, Cann CE.** 1994 Long-term estrogen replacement therapy prevents bone loss and fractures. Ann Intern Med. 102:319-324.

7. **Tilyard MW, Spears GFS, Thomson J, Dovey S.** 1992 Treatment of postmenopausal osteoporosis with calcitriol and calcium. N Engl J Med. 326:357-362.

8. **Riggs BL.** 1985 Treatment of osteoporosis with sodium fluoride: an appraisal. In: Peck WA. ed. Bone and Mineral Research 2, Amsterdam: Elsevier; 366-393.

9. **Riggs BL, Hodgson SF, O'Fallon WM, et al.** 1990 Effect of fluoride treatment on the fracture rate in postmenopausal women with osteoporosis. N Engl J Med. 322:802-809.

10. **Heaney RP.** 1994 The bone-remodelling transient: implications for the interpretation of clinical studies of bone mass change. J Bone Miner Res. 9:1515-1523.

11. **Isaksson OGP, Lindahl A, Nilsson A, Isgaard J.** 1987 Mechanism of the stimulatory effect of growth hormone on longitudinal bone growth. Endocrin Rev. 8:426-438.

12. **Jørgensen PH, Bak B, Andreassen TT.** 1991 Mechanical properties and biochemical composition of rat cortical femur and tibia after long-term treatment with biosynthetic human growth hormone. Bone. 12:353-359.

13. **Kassem M, Blum WF, Ristelli J, Mosekilde L, Eriksen EF.** 1993 Growth hormone stimulates proliferation and differentiation of normal human osteoblast-like cells in vitro. Calc Tissue Int. 52:222-226.

14. **Holloway L, Butterfield G, Hintz RL, Gesundheit N, Marcus R.** 1994 Effects of recombinant human growth hormone on metabolic indices, body composition, and bone turnover in healthy elderly women. J Clin Endocrinol Metab. 79:470-479.

15. **Degerblad M, Elgindy N, Hall K, Sjöberg H-E, Thorén M.** 1992 Potent effect of recombinant growth hormone on bone mineral density and body composition in adults with hypopituitarism. Acta Endocrinol (Copenh). 126:387-393.

16. **Bengtsson B-Å, Edén S, Bosaeus I, et al.** 1993 Treatment of adults with growth hormone deficiency with recombinant human growth hormone. J Clin Endocrinol Metab. 76:309-317.

17. **Clemmesen B, Overgaard K, Riis BJ, Christiansen C.** 1993 Human growth hormone and growth hormone releasing hormone: a double masked, placebo-controlled study of their effects on bone metabolism in elderly women. Osteoporosis Int. 3:330-336.

18. **Vandeweghe M, Taelman P, Kaufman J-M.** 1993 Short and long-term effects of growth hormone treatment on bone turnover and bone mineral content in adult growth hormone-deficient males. Clin Endocrinol (Oxf). 39:409-415.

19. **Diamond T, Nery L, Posen S.** 1989 Spinal and peripheral bone mineral densities in acromegaly: the effects of excess growth hormone and hypogonadism. Ann Intern Med. 111:567-573.

20. **Ezzat S, Melmed S, Endres D, Eyre DR, Singer FR.** 1993 Biochemical assessment of bone formation and resorption in acromegaly. J Clin Endocrinol Metab. 76:1452-1457.

21. **Forbes GB, Reina JC.** 1970 Adult lean body mass declines with age: some longitudinal observations. Metabolism. 19:653-663.

22. **Young VR, Steffee WP, Pencharz PB, Winterer JC, Scrimshaw NS.** 1975 Total human body protein synthesis in relation to protein requirements at various ages. Nature. 253:192-194.

23. **Forbes GB.** 1976 The adult decline in lean body mass. Hum Biol. 48:161-173.

24. **Jørgensen JOL, Pedersen SA, Thuesen L, Jørgensen J, Skakkebæk NE, Christiansen JS.** 1989 Beneficial effects of growth hormone treatment in GH-deficient adults. Lancet. 1:1221-1225.

25. **Cuneo RC, Salomon F, McGauley GA, Sönksen PH.** 1992 The growth hormone deficiency syndrome in adults. Clin Endocrinol (Oxf). 37:387-397.

26. **Corpas E, Harman SM, Piñeyro MA, Roberson R, Blackman MR.** 1993 Continuous subcutaneous infusions of growth hormone (GH) releasing hormone 1-44 for 14 days increase GH and insulin-like growth factor-I levels in old men. J Clin Endocrinol Metab. 76:134-138.

27. **Rudman D, Kutner MH, Rogers CM, Lubin MF, Fleming GA, Bain RP.** 1981 Impaired growth hormone secretion in the adult population. Relation to age and adiposity. J Clin Invest. 67:1361-1369.

28. **Zadik Z, Chalew SA, McCarter RJ, Meistas M, Kowarski AA.** 1985 The influence of age on the 24-hour integrated concentration of growth hormone in normal individuals. J Clin Endocrinol Metab. 60:513-516.

29. **Binnerts A, Swart GR, Wilson JH, et al.** 1992 The effect of growth hormone administration in growth hormone deficient adults on bone, protein, carbohydrate and lipid homeostasis, as well as on body composition. Clin Endocrinol (Oxf). 37:79-87.

30. **Marcus R, Butterfield G, Holloway L, et al.** 1990 Effects of short term administration of recombinant human growth hormone to elderly people. J Clin Endocrinol Metab. 70:519-527.

31. **Rudman D, Feller AG, Nagraj HS, et al.** 1990 Effects of human growth hormone in man over 60 years old. N Engl J Med. 323:1-6.

32. **Aloia JF, Vaswani A, Meunier PJ, et al.** 1987 Coherence treatment of postmenopausal osteoporosis with growth hormone and calcitonin. Calc Tissue Int. 40:253-259.

33. **Nilas L, Borg J, Gotfredsen A, Christiansen C.** 1985 Comparison of single- and dual-photon absorptiometry in postmenopausal bone mineral loss. J Nucl Med. 26:1257-1262.

34. **Mazess RB, Barden HS.** 1988 Measurement of bone by dual-photon absorptiometry (DPA) and dual-energy x-ray absorptiometry (DEXA). Ann Chir Gynaecol. 77:197-203.

35. **Lukaski HC, Johnson PE, Bolonchuk WW, Lykken GI.** 1985 Assessment of fat-free mass using bioelectrical impedance measurements of the human body. Am J Clin Nutr. 41:810-817.

36. **Lukaski HC, Bolonchuk WW, Hall CB, Siders WA.** 1986 Validation of tetrapolar bioelectrical impedance method to assess human body composition. J Appl Physiol. 60:1327-1332.

37. **Svendsen OL, Haarbo J, Hassager C, Christiansen C.** 1993 Accuracy of measurements of body composition by dual-energy x-ray absorptiometry in vivo. Am J Clin Nutr. 57:605-608.

38. **Valkema R, Vismans FJFE, Papapoulos SE, Pauwels EKJ, Bijvoet OLM.** 1989 Maintained improvement in calcium balance and bone mineral content in patients with osteoporosis treated with the bisphosphonate APD. Bone Miner. 5:183-192.

39. **Salomon F, Cuneo RC, Hesp R, Sönksen PH.** 1989 The effects of treatment with recombinant human growth hormone on body composition and metabolism in adults with growth hormone deficiency. N Engl J Med. 321:1797-1803.

40. **Martha PM, Reiter EO, Davila N, Shaw MA, Holcombe JH, Baumann G.** 1992 The role of body mass in the response to growth hormone therapy. J Clin Endocrinol Metab. 75:1470-1473.

41. **Deurenberg P, van der Kooij K, Leenen R, Weststrate JA, Seidell JC.** 1990 Sex and age specific prediction formulas for estimating body composition from bioelectrical impedance: a cross validation study. Int J Obesity. 15:17-25.

42. **Deurenberg P, van der Kooij K, Evers P, Hulshof T.** 1990 Assessment of body composition by bioelectrical impedance in a population aged > 60 y. Am J Clin Nutr. 51:3-6.

43. **Mazess RB, Barden HS, Bisek JP, Hanson J.** 1990 Dual-energy x-ray absorptiometry for total-body and regional bone-mineral and soft-tissue composition. Am J Clin Nutr. 51:1106-1112.

6 The Effects of Long-Term Growth Hormone (GH) Replacement Therapy in Patients with *Adult Onset* GH-deficiency.

ABSTRACT

GH-deficiency in adults (GHDA) has become recognized as a specific clinical syndrome. Its main features are changes in body composition, decreased psychological well-being, reduced bone mineral content and disorders in lipids metabolism. We report here the results of the effects of long-term rhGH replacement therapy in twenty patients with adult onset growth hormone deficiency. There was an initial placebo-controlled phase of six months, followed by a period of open treatment for both patient groups of 12 months. We measured body composition with several techniques in order to compare these techniques. Body composition was measured by bioelectrical impedance analysis (BIA), anthropometry, skinfolds (SF) and total body dual energy x-ray absorptiometry (DXA). Also measurements were performed of bone turnover parameters and bone mass, quality of life, carbohydrate and lipid metabolism. There was a significant difference in the age between the groups ($P = 0.034$). Eighteen patients completed the eighteen month study period. Fifteen patients reached normal IGF-I levels. Three patients needed dose reduction. Glucose metabolism was slightly affected temporarily. Body composition changed significantly, with a decrease in fat mass (FM) and an increase of fat free mass (FFM). These changes were detected well by SF but not by BIA. Quality of life measurements showed modest improvement. Lipid profile improved significantly by an elevation of HDL-cholesterol levels. Bone turnover was activated, in favor of bone formation. However, this did not result in an increase in bone mineral content measurements.

We conclude that long-term GH replacement therapy in patients with adult onset GHD results in favorable effects on body composition, quality of life, HDL-cholesterol levels, and bone turnover. Changes in body composition are easily and accurately detected by skinfold anthropometry and not by bioelectrical impedance analysis. As skinfold anthropometry is an easy, patient-friendly and cheap procedure, this method should be used in the evaluation of the efficacy of GH replacement therapy in GHDA patients.

INTRODUCTION

GH-deficiency in adults (GHDA) has become recognized as a specific clinical syndrome (1). Its main features are changes in body composition (2-5), reduced bone mineral content (6-8) and a decreased psychological well-being (9-12). Other reported findings are alterations in renal function (13), reduced cardiac function and premature atherosclerosis with increased cardiovascular morbidity and mortality (14-17), disorders in lipid and carbohydrate metabolism (18), and impaired thermoregulation (19).

An important aspect of GHD is an increase in body fat and a decrease in lean body mass compared to age-, sex-, height- and weight- matched controls (2,20-22). In childhood onset GHD GH treatment reduced the amount of subcutaneous (sc.) fat (23), whereas a reduction mainly in sc. and visceral adipose tissue was demonstrated during GH replacement in GHD adults (22,24). In another study in children with GHD an increase in muscle mass and a decrease in total body fat were recorded (25). These changes were also observed in GHD adults by using several techniques of body composition assessment (22,24,26-30). The validity of measuring changes of body composition by bioelectrical impedance analysis (BIA) in GHD is debated and might be not suitable in evaluating effects of GH replacement therapy (3,31). In this study we investigated and compared the long-term effects of GH on body composition by BIA, total body dual energy x-ray absorptiometry (DXA) and skinfold anthropometry.

We report here the results of the effects of rhGH replacement therapy in twenty patients with adult onset growth hormone deficiency. There was an initial placebo-controlled phase of six months, followed by a period of open treatment for both patient groups of 12 months. We measured body composition with several techniques in order to compare these techniques. Also measurements were performed of bone turnover parameters and bone mass, quality of life, carbohydrate and lipid metabolism, and physical performance.

MATERIALS AND METHODS

Patients

GHD was considered to be present, when there was a history of pituitary disease, in combination with maximum GH levels in at least one provocation test below 5 mg/L. Twenty patients (13 males and 7 females, mean age 47 yr., range 30-59) were included in the study (Table 1). In the twenty patients that entered the study, thirteen patients had undergone transsphenoidal hypophysectomy: all but three of these patients, who had a craniopharyngeoma, received additional radiation therapy. Two patients had undergone transfrontal hypophysectomy and additional radiotherapy. Two patients received primary radiotherapy (one with Cushing's disease and one with prolactinoma). Two received primary medical treatment of prolactinoma. One patient presented with an enlarged pituitary fossa, liquorrhoea and partial hypopituitarism. Patients, who previously received GH replacement therapy, were excluded. All patients received conventional replacement therapy, of which the doses, apart from temporary increases (less than one week) in corticosteroids at the event of intercurrent illnesses, were not altered during the entire study period. The six patients with a prolactinoma received dopamine agonist therapy.

Protocol

The trial had a run-in period of four weeks, after which the patients were randomized into two groups to receive (double blind) treatment with recombinant human GH (rhGH) or placebo for six months. The GH dose was 6.25 µg/kg.day in the first month and 12.5 µg/kg.day (0.236 IU/kg.week) for the following five months. Maximum dose was 1000 µg/day (2.7 IU/day). After six months all patients continued on rhGH therapy, at which time the dose was again reduced to 6.25 µg/kg.day for one month. From the seventh month onward all patients were treated with 12.5 µg GH/kg.day. The aims and methods of the study were explained to the patients and written informed consent was obtained before the study, which was approved by the ethics committee of the University Hospital Dijkzigt (Rotterdam, The Netherlands).

TABLE 1. Clinical and biochemical characteristics of the patients included in the study.

Characteristic	rhGH Group (n=9)	Placebo Group (n=9)
Mean age (yr)	42 ± 2.8	52 ± 2 *
Range	30-53	39-59
Sex (M/F)	(7/2)	(4/5)
Known duration of GHD (yr)	10	10
Range	2-16	2-17
Diagnosis		
Cushing's disease	2	1
Prolactinoma	3	2
Chromophobe adenoma	3	3
Craniopharyngeoma	1	3
Conventional replacement[a]		
T,A	1	
T,G	1	2
A,G	1	1
T,A,G	6	5
T,A,G,DI		1
IGF-I baseline (ng/mL) (± SEM) Normal range (91-356)	132 ± 23	103 ± 16
Range	52-263	41-204
Ht (cm)	177	172
Range	162-188	161-186.6
Wt (kg)	86	87
Range	68.9-103.4	63-104.7
Body Mass Index (kg/m^2)	27.5	29.5
Range	22.6-36.8	24.3-35.2

[a] T, thyroid deficiency; A, adrenal deficiency; G, gonadal deficiency; DI, diabetes insipidus.
* $P < 0.05$

Methods

Routine methods were used for the hematological and chemical measurements and measurement of cholesterol, HDL-cholesterol (HDL-c), LDL-cholesterol (LDL-c) and VLDL-cholesterol (VLDL-c).

Glucose metabolism

An oral glucose tolerance test (OGTT, 75g glucose) was performed at several visits during the study. Plasma blood glucose was measured at 0, 30, 60, 90 and 120 min. At the same points serum samples were collected for insulin determinations. Insulin was determined by RIA using a commercially available kit (INCSTAR Corp., Stillwater, MN; intraassay CV, 6.4-10.9%; interassay CV, 8.5-10.0%). Impaired glucose tolerance was defined as a 2-h value between 7.8 and 11.1 mmol/L with another value during the 2-h test period equal to or greater than 11.1 mmol/L (32). The total areas under the glucose (AUCG, mmol.h/L) and insulin (AUCI, mU.h/L) curve were calculated trapezoidally. Also calculations were made to measure peripheral insulin resistance according to methods described earlier (33). Glycosylated hemoglobin (HbA_1C) was assessed using high-performance liquid chromatography using routine methods (intraassay CV, 0.41%-1.99%; interassay CV, 1.1-1.62%). HbA_1C values exceeding 6.3% of the total hemoglobin were regarded as abnormal.

Insulin-like Growth Factor I (IGF-I) determinations were performed by a RIA kit (Mediagnost, Tübingen, Germany) (34). Normal range in adults 20-30 yr.: 115-340 ng/mL; 50-60 yr.: 97-295 ng/mL. IGF binding protein-3 (IGFBP-3) was determined by IRMA using a non-isotopic sandwich assay (35). Normal range for adults 1800-4800 ng/mL.

Bone turnover parameters

Osteocalcin was determined using a RIA kit (Diagnostic Systems Laboratories, Webster, TX; intraassay CV, 5.4-8.4%; interassay CV, 5.5-14.7%). Normal range 1.8-6.6 ng/mL.

Deoxypyridinoline cross-links (DPD, pyrilinks-D) were quantified by using a high-performance liquid chromatography technique described earlier (36), (intraassay CV, 3.4%; interassay CV, 8-12%). Concentrations of urinary DPD were expressed relative to urinary creatinine (nmol/mmol creatinine).

Body composition

Body composition was measured by bioelectrical impedance analysis (BIA), anthropometry, skinfolds (SF) and total body dual energy x-ray absorptiometry (DXA).

Estimation of fat free mass and fat mass was performed using a Holtain Body Composition Analyzer (Holtain Ltd., Croswell, Dyfed, England) as described earlier (4,31).

DXA analysis was performed with a Lunar DPX Densitometer (Lunar Corp., Madison, WI) (37). DXA directly measures fat mass, lean tissue mass and total body bone mineral content (38).

Anthropometric assessment of body composition was performed by measuring the sum of skinfold thickness with calipers (Holtain Ltd., England) at biceps, triceps, subscapular and suprailiac sites at the left side of the body according to standardized guidelines. Through equations developed by Durnin and Womersley, body density, body fat and fat mass were calculated (39).

Quality of life

For assessment of quality of life part I of the Nottingham Health Profile (NHP) was used (40). This is a self-rating questionnaire by which perceived health problems and their impact on task performance in the physical, mental and social spheres are measured. Part I of the NHP consists of 38 statements covering six sections, each of which refers to a separate area of functioning. The sections comprise physical mobility, pain, sleep, energy, social isolation, and emotional reactions. Respondents are required to read each statement and to tick either 'yes' or 'no', depending on whether that problem applies to them in general at the time.

The sections contain between 3 and 9 items, each of which has received a scoring weight in order to make the maximum score per section 100. Thus a respondent affirming all the statements in one section would obtain a score of 100.
Several studies have established the validity and reliability of the NHP (41,42). Validity and reliability of the Dutch adaptation were found good, with exception of the section social isolation (43).

Bone mass measurements

Lumbar bone mass (lumbar vertebrae, L2-L4) was measured with the Lunar DPX Bone Densitometer (Lunar Corp., Madison, WI) (44). The coefficient of variation (CV) in normal subjects for DXA was 1.1% (37). The results are expressed as BMC (g hydroxyapatite (Ha)). Quality assurance including calibration with the standard of the machine was performed routinely every morning.
Single-Photon Absorptiometry (SPA) of the right forearm was performed at the distal and proximal sites using a Nuclear Data 1100a scanner (45). The (fat-corrected) results are expressed as BMC (arbitrary units (U) per cm). The CV in our institution is 1.9% for the distal site and 1.0% for the proximal site (46).

RESULTS

There was a significant difference in the age between the groups (*P =0.034*). Other baseline characteristics were not significantly different. Eighteen patients completed the eighteen month study period. One patient, who was in the 5th month of the placebo period, decided to stop due to marital problems. Another patient stopped after 7 months, in the open phase of the study, because of complaints of arthralgia and swelling mainly localized in the hands. There were no complaints in the placebo period. Reported complaints during active treatment were arthralgia in 12, muscle aches in 10, ankle edema in 6, occasional headache in 6, paresthesias in 4, palpitations in 3, and acne in 2 patients. Slight hypertension developed in one patient. Almost all complaints resolved spontaneously. One patient needed short-term (two weeks) diuretics because of edema. In two patients dose reduction was performed, one because of persisting invalidating arthralgia, the other one because of ankle edema persisting after diuretic therapy.
There were no significant changes in leukocyte count, hemoglobin concentration, serum creatinin, sodium, potassium, calcium and liver enzymes. Mean blood pressure, heart rate, and electrocardiograms did not change in both groups during the entire study period. No GH antibodies were found.

IGF- I and IGFBP-3

Mean IGF-I levels were lowered and not significantly different between groups at baseline. Only two patients in the GH group had IGF-I levels that were within the normal range when adjusted for sex and age compared to normals (one male 30 y, IGF-I 141 ng/mL, and another male 42 y IGF-I 180 ng/mL). After the initiation of GH replacement therapy, there were significant differences versus baseline and between groups (Fig. 1A). IGFBP-3 levels were also not significantly different between groups at baseline. Mean levels were in the normal range for adults. After the initiation of GH replacement therapy, there were significant differences versus baseline and between groups (Fig.1B). Fifteen patients reached normal IGF-I levels during GH replacement therapy, while in three patients IGF-I levels higher than 340 ng/mL were recorded at eighteen months.

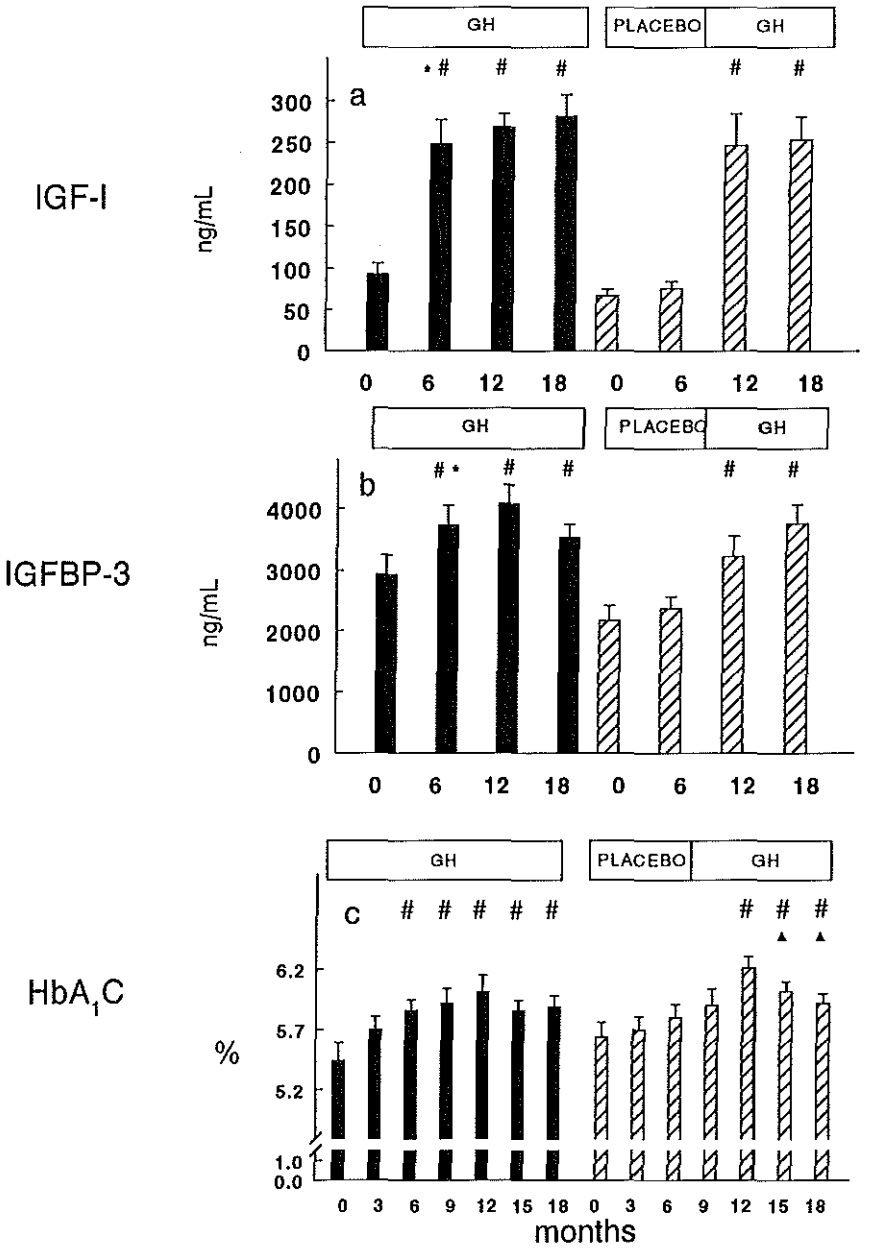

Fig. 1 A-C. Insulin-like growth factor I (IGF-I), IGF binding protein-3 levels (IGFBP-3) and percentage of glycosylated haemoglobin (HbA_1C) during the entire study period in both groups of patients. Results are presented as mean ± SEM. # $P < 0.05$ within groups vs. baseline, * $P < 0.05$ between groups. ▲ $P < 0.05$ within groups vs. 12 months.

Glucose metabolism

Overnight glucose had elevated significantly after twelve weeks of GH treatment in the group that first received placebo treatment (4.2 ± 0.1 *vs.* 4.6 ± 0.1 mmol/L, $P < 0.001$). During continued treatment overnight glucose decreased and eventually were not significantly different anymore from baseline. Apart from this, there were no significant between, nor within group differences in glucose or insulin levels measured overnight and during OGTT, nor in AUCG or AUCI or measurements of peripheral insulin resistance. HbA_1C levels increased significantly during GH treatment within groups. No statistically significant difference between groups was reached (Fig. 1C). In the group that started GH therapy at six months a significantly elevated HbA_1C was found at six months of active treatment (i.e. 12 months of the trial period) which was maintained during the remaining six months. At nine and twelve months (i.e. 15 and 18 months of the trial period), however, HbA_1C levels had again decreased significantly vs. the maximum at six (12) months. In the other treatment group the same pattern was observed. However, the decrease of HbA_1C levels at 15 and 18 months did not reach significance (Fig. 1C).

Body composition

Body composition measurements before the start of therapy demonstrated, by all techniques, differences between groups in fat mass and fat free mass. This was probably due to the significant difference in age between the groups. These differences in fat mass and fat free mass, however, were not statistically significant. Body composition changed significantly, with a decrease in fat mass (FM) and an increase of fat free mass (FFM), which were comparable but not identical among the three techniques that were used. When compared to placebo there was a significant difference in FM between groups at six months when assessed by SF (FM_{SF}, $P < 0.05$), but not by DXA (FM_{DXA}, $P = 0.056$).

Within groups FM_{DXA} had decreased after 6 months of GH therapy and after 12 months, respectively. FM_{DXA}: 20.2 ± 2.8 kg (baseline) *vs.* 17.8 ± 3.6 kg (six months), $P < 0.05$ and *vs.* 19.1 ± 3.8 kg (18 months), $P = n.s.$, in the group that started with GH. FM_{DXA}: 25.9 ± 2.6 kg (baseline) *vs.* 23.0 ± 2.8 kg (18 months), $P = 0.015$, in the group that started with

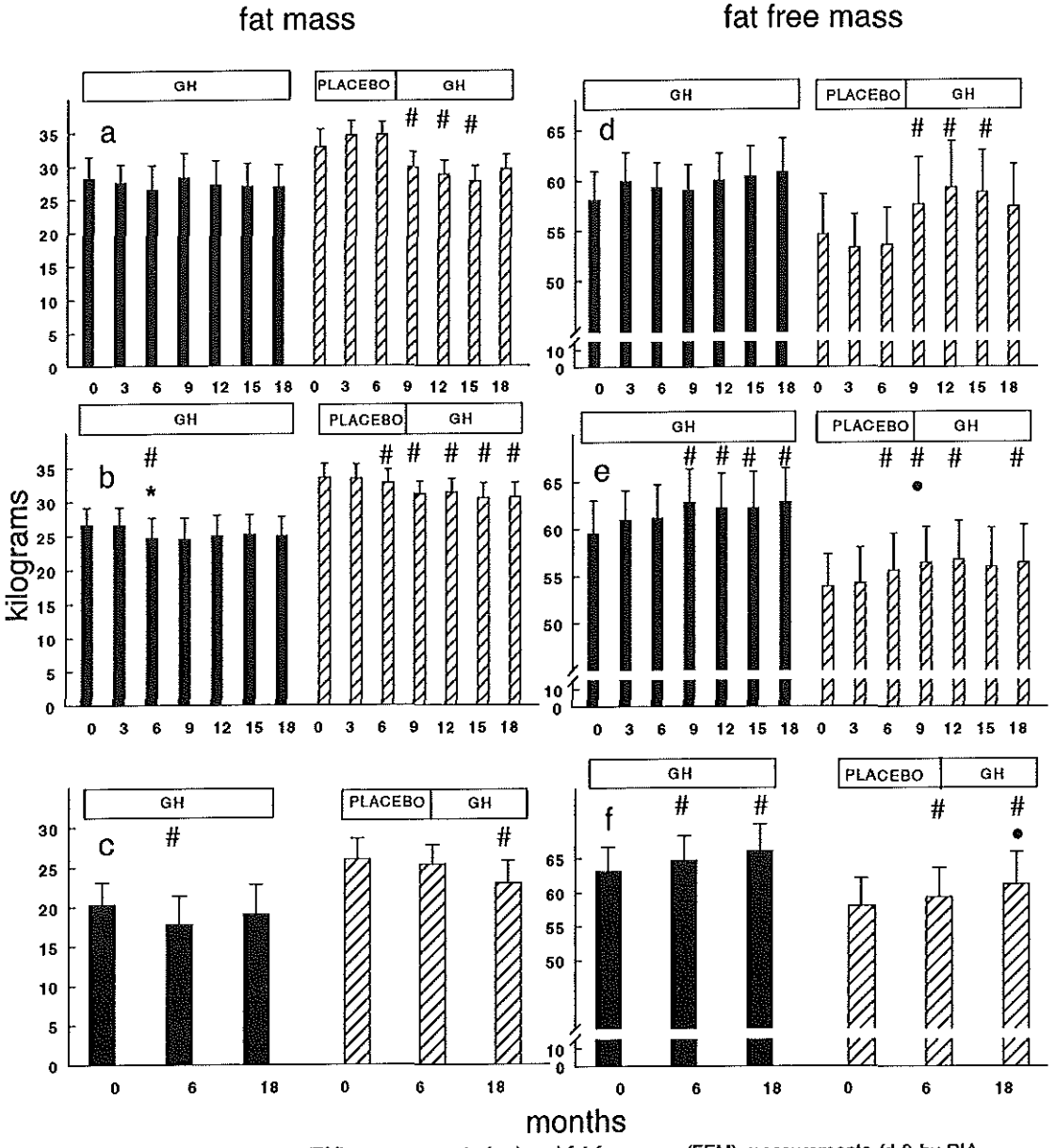

Fig. 2 A-F Fat mass (FM) measurements (a-c) and fat free mass (FFM) measurements (d-f) by BIA (a,d), SF (b,e) and DXA (c,f). • $P < 0.05$ within group vs. six months. See also legend to Fig. 1.

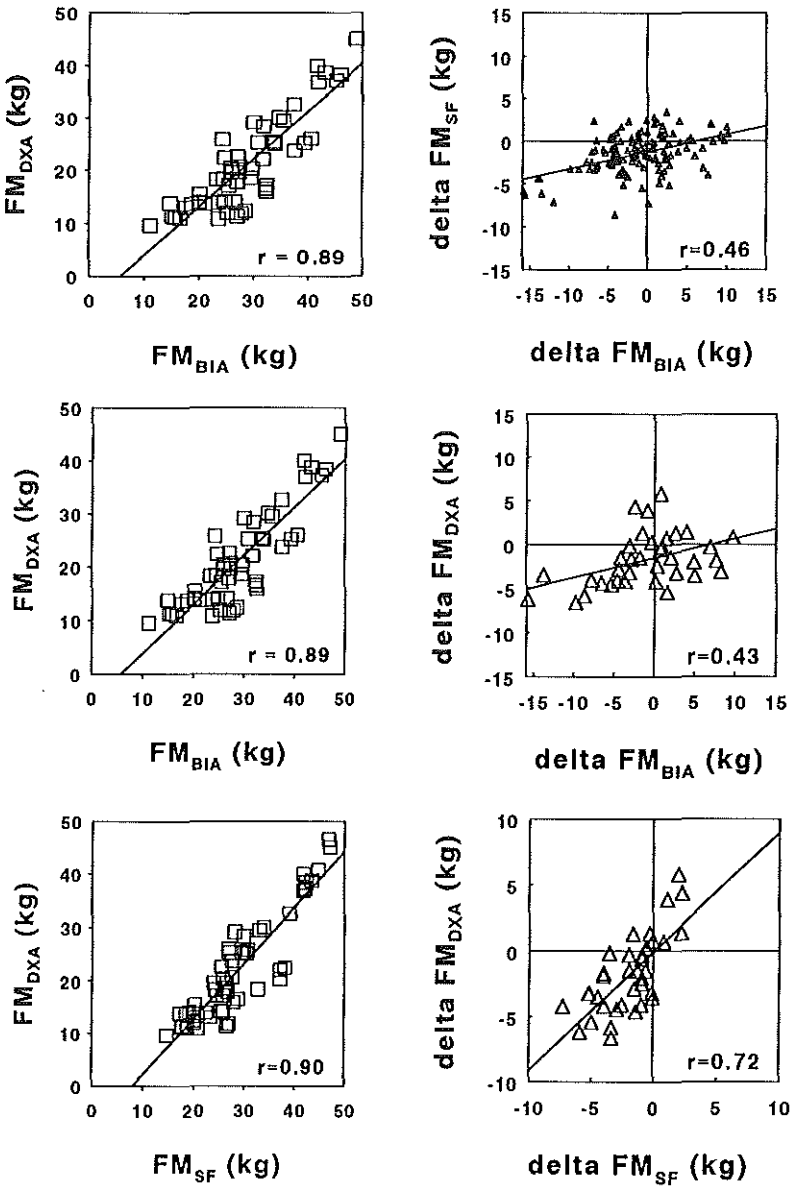

Fig. 3 Correlations between absolute values and delta changes of fat mass as measured by BIA, SF and DXA

placebo. In this group, there was a slight reduction in fat mass in the placebo period, which did not reach significance, however (Fig. 2A-C).

Increases in FFM_{BIA} were not significant in the GH group. FFM_{SF} and lean mass by DXA (LM_{DXA}) had significantly increased. (LM_{DXA}: 63.1 ± 3.4 kg (baseline) *vs.* 66.0 ± 3.9 kg (18 months), $P = 0.008$). In the group that started GH replacement at six months there was a significant increase in FFM by SF and DXA in the placebo period. (LM_{DXA}: 58.1 ± 4.0 kg (baseline) *vs.* 59.3 ± 4.3 kg (6 months), $P = 0.03$). Increases versus six months were significant at 9 months when assessed by SF ($P = 0.04$) and at eighteen months when assessed by DXA ($P = 0.03$), (Fig. 2D-F).

Overall measurements of FM showed good correlations; BIA with SF, r = 0.84, BIA with DXA, r = 0.89, SF with DXA, r = 0.90, $P < 0.001$ in all cases (Fig. 3). The changes in FM as measured by DXA correlated better with skinfold measurements than with BIA; BIA with SF, r = 0.46, ($P < 0.001$), BIA with DXA, r = 0.43, ($P = 0.008$), SF with DXA, r = 0.72, ($P < 0.001$).

Quality of life

Quality of life assessment showed no significant differences between groups in perceived health status at baseline. There was a considerable intra-individual variation in several subsections of the NHP. At six months there was a significant difference in the section of physical mobility. This is partly due however to an increase of perceived problems in the placebo group (Table 2). At eighteen months the two groups were significantly different for this section. The increase in energy in the GH group did not reach significance. In the group that started with placebo there was an increase in energy after six months of active treatment. However this difference was not maintained (Table 2). On an individual basis there was substantial improvement in the patients that scored high at baseline.

TABLE 2. Nottingham Health Profile

	GH				Placebo	GH		
months	0	6	12	18	0	6	12	18
Section								
Physical Mobility	3.7 ± 1.7	2.4 ±1.5*	3.7 ± 2.5	0 ± 0*	9.5 ± 3.6	15.2 ± 3.0	11.0 ± 4.4	10.7 ± 3.8
Pain	1.11 ± 1.0	4.0 ± 2.9	3.3 ± 2.1	1.4 ± 1.4	3.1 ± 1.5	7.5 ± 4.1	14.1 ± 6.2	8.6 ± 5.4
Sleep	7.8 ± 4.2	9.2 ± 4.1	11.7 ± 4.9	1.4 ± 1.3	22.5 ± 9.4	11.4 ± 8.0	12.4 ± 8.5	13.8 ± 8.3
Energy	32.2 ± 11.5	12.4 ± 6.8	9.7 ± 6.8	9.7 ± 4.8	32.2 ± 11.5	21.1 ± 8.7	11.4 ± 7.3#	15.5 ± 10.8
Social Isolation	8.5 ± 4.6	6.7 ± 4.5	2.2 ± 2.1	2.2 ± 2.0	6.4 ± 6.0	13.3 ± 8.7	13.3 ± 8.1	13.3 ± 8.1
Emotional Reactions	16.6 ± 6.1	6.5 ± 2.5	3.0 ± 1.4	0.8 ± 0.8#	21.3 ± 11.1	17.6 ± 8.1	8.8 ± 5.4	10.6 ± 8.1
All	11.6 ± 3.8	6.9 ± 2.0	5.6 ± 2.7	2.6 ± 0.8#	15.8 ± 5.6	14.3 ± 4.4	11.8 ± 4.9	12.1 ± 5.5

Results are presented as mean ± SEM.

# $P < 0.05$ within group.

* $P < 0.05$ between groups.

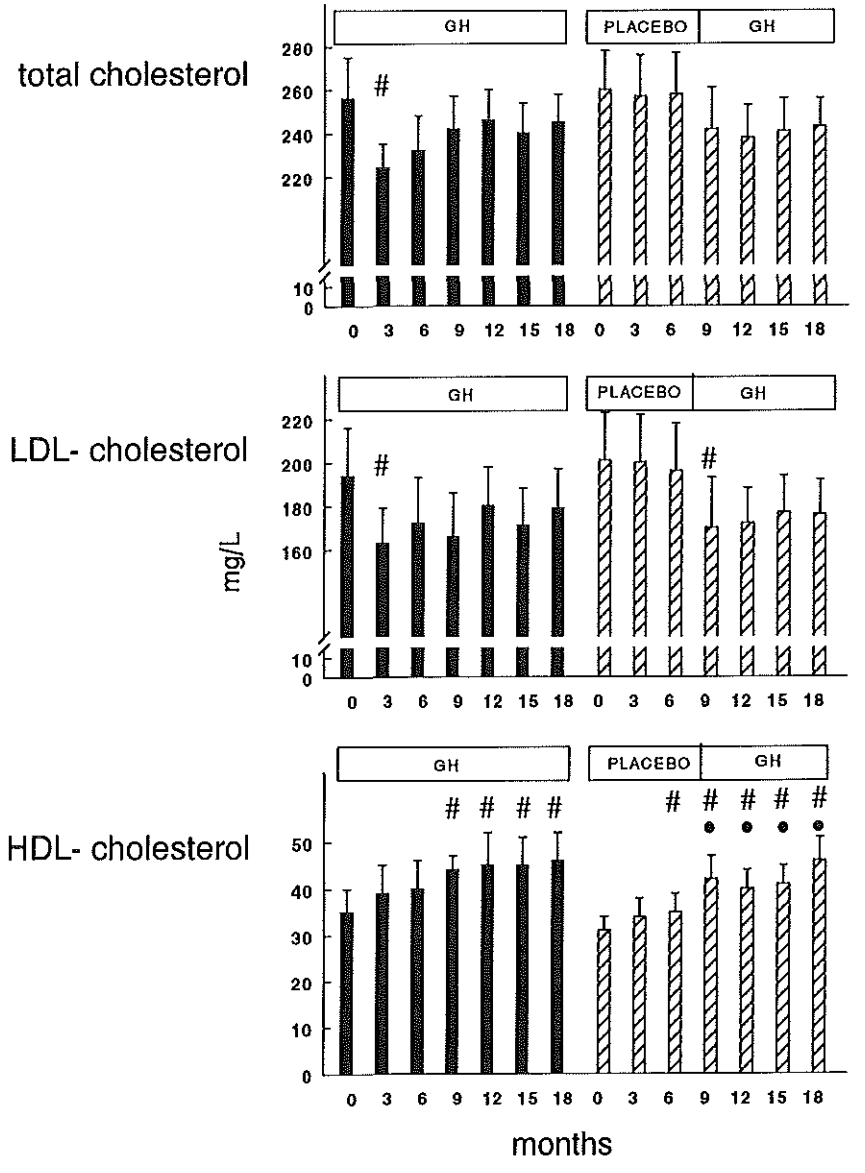

Fig. 4 Lipid parameters. See also legends to Fig. 1 and 2.

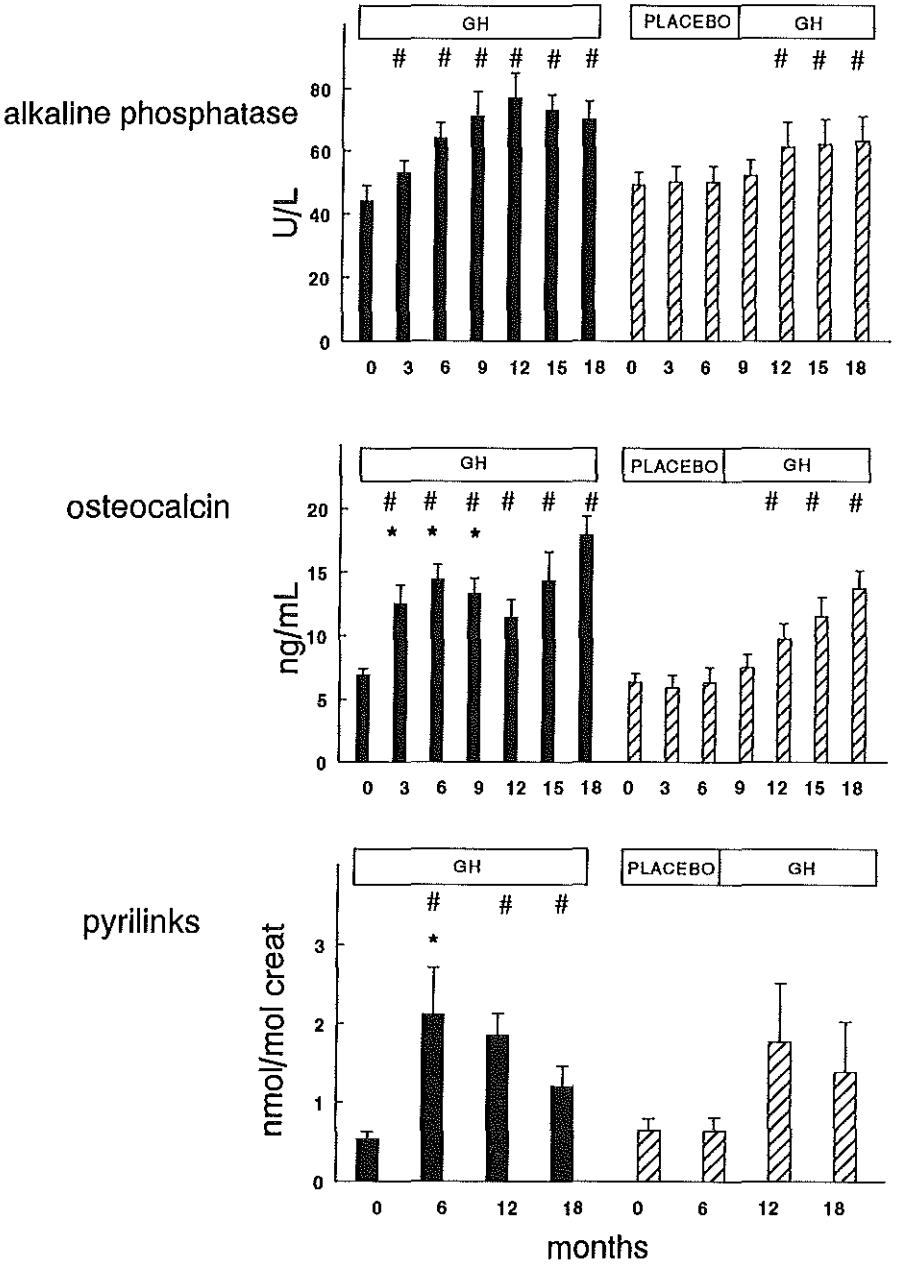

Fig. 5 Markers of bone formation (alkaline phosphatase and osteocalcin) and bone resorption (deoxypyridinoline). See also the legend to Fig. 1.

TABLE 3. Bone mineral content of lumbar spine and proximal and distal forearm

	GH			Placebo	GH	
months	0	6	18	0	6	18
DXA_{SPINE}	55.5 ± 4.4	54.0 ± 4.3	55.0 ± 4.6	59.4 ± 3.6	59.5 ± 3.5	59.8 ± 3.6
SPA_{PROX}	50.6 ± 3.4	49.8 ± 3.6	48.8 ± 3.6#	46.6 ± 3.9	46.7 ± 3.8	45.7 ± 3.7
SPA_{DIST}	53.0 ± 4.2	51.9 ± 4.7	50.6 ± 4.3	47.6 ± 3.8	47.5 ± 3.8	46.4 ± 3.5

Results are presented as mean ± SEM.

 # $P < 0.05$

Lipid profile

here was an improvement of the lipid profile with a temporary decrease of total and LDL-cholesterol and a sustained increase in HDL-cholesterol. There were no between group differences. In the group that started with placebo there was a significant increase of HDL-cholesterol after six months. After starting active therapy there was also a significant increase versus six months (Fig. 4).

Bone turnover and bone mineral content

There was a significant increase in bone turnover reflected in markers of bone formation as well as resorption (Fig. 5). The bone formation markers alkaline phosphatase and osteocalcin were significantly increased versus placebo. After starting GH therapy in the placebo group these differences were no longer significant between the groups. However significance versus baseline remained. The marker of bone degradation (DPD) showed a significant difference between the two treatment groups at six months. After six months DPD crosslinks started to decrease, which almost reached significance at 18 months (*P =0.051*). Bone mass measurements showed a significant reduction at the proximal forearm after eighteen months of GH treatment (Table 3).

DISCUSSION

In this study we report the beneficial effects of long-term GH replacement therapy in patients with adult onset GHD. Effects are reported on body composition, quality of life, lipid metabolism, bone turnover and bone mineral mass.

Since there were significant changes in the placebo period in body composition and lipids, we considered the patients groups separately during the entire eighteen month study period. In contrast to others we did not pool the data into one group of GH replacement therapy (47).

In this study we used lower GH doses, than in previously reported studies (11.3 µg/kg.day; 0.210 IU/kg.week) which explains the fewer incidence of side effects and the fact that dose reduction was necessary in only three patients (including the one that stopped treatment) (20-22,47).

When studying the effects of GH therapy there were significant increases in IGF-I and IGFBP-3 levels. Fifteen patients had normalized IGF-I levels at eighteen months. Three patients had levels that could be considered too high. However, individual GH dose adjustment, with careful monitoring of side effects, until normal high IGF-I levels are reached, might prove an interesting strategy to obtain maximum biological effects of GH therapy in GHD patients. IGFBP-3 levels were in the normal range at baseline for the two groups. Mean levels were in the normal range for adults. After starting GH therapy there was an initial increase with a non significant tendency to decrease thereafter. Since this decrease is not found in the IGF-I levels this further corroborates our earlier observation that IGFBP-3 levels are not helpful in defining the optimal GH dose (31).

Glucose metabolism was only slightly affected in our patients. Although a significant increase in HbA_1C levels implicates that mean glucose levels must have increased, no support for this was found in extensive studies of OGTT data on glucose and insulin. The fact that HbA_1C levels decrease, while mean GH dose was only slightly reduced, suggests that there is a mild, transient glucose intolerance. Evaluation with euglycemic clamp studies might further elucidate this point.

Overall correlations between the three methods of body composition assessment (BIA, SF, DXA) are good. The reduction of FM, however, was best demonstrated by SF and DXA, and not by BIA.

The validation of the BIA method under different circumstances (hydration status), in different patient groups (obese, growth hormone deficient, elderly), using different body impedance analyzers, differences in electrode placement and using different prediction formulas is still a matter of debate (48-53). Also the validity of the basic equation is discussed in that it cannot detect changes in body composition unless they are considerable (54). The BIA method assesses TBW or FFM depending on the regression equation used. From TBW, FFM and FM can be calculated with the formula's FFM = TBW/0.73 and FM = BW - FFM. This formula uses the Pace constant for the hydration of the lean body of 0.732 (55). In other words water constitutes 73.2% of FFM in adult man. However, this assumption is based upon animal studies, in guinea pigs and several other small animals and has certainly not been validated in humans (55,56). From an extensive review of the literature, it has been concluded that in normal humans

the water content of the FFM ranges from 70 to 78%, with a mean value of 73% (56). This, however, requires a normal hydration status and the absence of electrolyte disturbances, which do not change during GH therapy. This is obviously not the case in GHD patients (3,31). Therefore the BIA technique cannot be used in longitudinal studies on GH therapy to assess changes in FM.

Since SF represents measurements of FM and not FFM it might be more accurate in detecting changes in FM. The only adjustments that have to be made are for age and sex. The DXA method is well validated and measures fat directly (37,57,58). The measurement of fat by DXA is derived from the ratio of the mass attenuation coefficients for the two energies of a constant x-ray source of 40 and 70 keV (R). This R-value has a linear relation to fat percentage. The %fat times the measured soft tissue mass gives the fat mass; the remainder is the lean tissue mass. This also implicates that DXA does not distinguish between intra- and extracellular water. The DXA method therefore is different from the other methods of body composition assessment, because it measures body composition independently from age, water, or any other factor of the FFM.

In the group as a whole, there were only minor changes in perceived health status. In the NHP, patients score higher when they perceive more problems. Some patients, do not score high at baseline, maybe because they have become adjusted to their impaired capacities. On an individual basis there was substantial improvement in the patients that score high at baseline, especially in the section energy. The intra-individual variation was considerable. This means that larger patient groups are needed to prove a beneficial effect of GH therapy on quality of life.

The rise of HDL-cholesterol in the placebo group is unexplained. Changes in lipid profile during GH treatment were favorable and persisted throughout the study period as elevated HDL-cholesterol level. Since HDL-cholesterol is an important independent risk factor in cardiovascular disease, this increase will probably improve long-term prognosis in GHD patients.

The increases in bone turnover parameters were sustained throughout the study period. Interestingly the markers of bone resorption (deoxypyridinoline) start to decline, while the markers of bone formation are still rising (osteocalcin) or maintain elevated (alkaline phosphatase). This could implicate uncoupling of bone formation and resorption, in

favor of bone formation. In this study with 12 months and 18 months of GH therapy only minor changes in BMC were found. A longer observation period turned out to be necessary to demonstrate long-term positive effects on bone mineral mass in GHD adult patients (59).

We conclude that long-term GH replacement therapy in patients with adult onset GHD results in favorable effects on body composition, HDL-cholesterol levels and bone turnover. Side effects of long-term GH replacement therapy are fewer when smaller doses are used. The changes in body composition are easily and accurately detected by skinfold anthropometry and not by bioelectrical impedance analysis. As skinfold anthropometry is an easy, patient-friendly and cheap procedure, this method should be used in the evaluation of the efficacy of GH replacement therapy in GHDA patients.

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REFERENCES

1. **Cuneo RC, Salomon F, McGauley GA, Sönksen PH.** 1992 The growth hormone deficiency syndrome in adults. Clin Endocrinol (Oxf). 37:387-397.

2. **Binnerts A, Deurenberg P, Swart GR, Wilson JH, Lamberts SWJ.** 1992 Body composition in growth hormone-deficient adults. Am J Clin Nutr. 55:918-923.

3. **de Boer H, Blok GJ, Voerman HJ, de Vries PM, van der Veen EA.** 1992 Body composition in adult growth hormone-deficient man, assessed by anthropometry and bioimpedance analysis. J Clin Endocrinol Metab. 75:833-837.

4. **Orme SM, Sebastian JP, Oldroyd B, et al.** 1992 Comparison of measures of body composition in a trial of low dose growth hormone replacement therapy. Clin Endocrinol (Oxf). 37:453-459.

5. **Rosén T, Bosaeus I, Tölli J, Lindstedt G, Bengtsson B-Å.** 1993 Increased body fat mass and decreased extracellular fluid volume in adults with growth hormone deficiency. Clin Endocrinol (Oxf). 38:63-71.

6. **Elgindy N, Grunditz R, Thorén M, Degerblad M, Sjöberg HE, Ringertz H.** 1991 Long term follow-up of metacarpal cortical thickness and bone mineral density in panhypopituitarism. Radiol Diagn. 32:326-330.

7. **Bing-You RG, Denis M-C, Rosen CJ.** 1993 Low bone mineral density in adults with previous hypothalamic-pituitary tumors: Correlations with serum growth hormone responses to GH-releasing hormone, insulin-like growth factor I, and IGF binding protein 3. Calc Tissue Int. 52:183-187.

8. **Holmes SJ, Economou G, Whitehouse RW, Adams JE, Shalet SM.** 1994 Reduced bone mineral density in patients with adult onset growth hormone deficiency. J Clin Endocrinol Metab. 78:669-674.

9. **Björk S, Jönsson B, Westphal O, Levin J-E.** 1989 Quality of life of adults with growth hormone deficiency: a controlled study. Acta Paediatr Scand. 356:55-59.

10. **McGauley GA.** 1989 Quality of life assessment before and after growth hormone treatment in adults with growth hormone deficiency. Acta Paediatr Scand. 356:70-72.

11. **McGauley GA, Cuneo RC, Salomon F, Sönksen PH.** 1990 Psychological well-being before and after growth hormone treatment in adults with growth hormone deficiency. Horm Res 33(Suppl 4):52-54.

12. **Rosén T, Wirén L, Wilhelmsen L, Wiklund I, Bengtsson B-Å.** 1994 Decreased psychological well-being in adult patients with growth hormone deficiency. Clin Endocrinol (Oxf). 40:111-116.

13. **Bojs G, Falkheden T, Sjögren B.** 1961 Studies on glomerular filtration rate and renal plasma flow in pituitary insufficiency in man including measurements of the renal extraction of para-aminohippuric acid. Acta Endocrinol (Copenh) 37:616-622.

14. **Rosén T, Bengtsson B-Å.** 1990 Premature mortality due to cardiovascular disease in hypopituitarism. Lancet 336:285-288.

15. **Wüster C, Slencka E, Ziegler R.** 1991 Erhöhte prävalenz von osteoporose und arteriosklerose bei konventionell substituierter hypophysenvorderlappeninsuffizienz: bedarf einer zusätzlichen wachstumshormonsubstitution? Klin Wochenschr. 69:769-773.

16. **Shahi M, Beshyah S, Hackett D, Sharp P, Johnston D, Foale R.** 1991 Cardiac structure and function in growth hormone deficiency. Br Heart J. 66:58.

17. **Lehman ED, Hopkins KD, Weissberger AJ, Gosling RG, Sönksen PH.** 1993 Aortic distensibility in growth hormone deficient adults. Lancet 341:309.

18. **Davidson MB.** 1987 Effect of growth hormone on carbohydrate and lipid metabolism. Endocrin Rev. 8:115-131.

19. **Juul A, Behrenscheer A, Tims T, Nielsen B, Halkjær-Kristensen J, Skakkebæk NE.** 1993 Impaired thermoregulation in adults with growth hormone deficiency during heat exposure and exercise. Clin Endocrinol (Oxf). 38:237-244.

20. **Salomon F, Cuneo RC, Hesp R, Sönksen PH.** 1989 The effects of treatment with recombinant human growth hormone on body composition and metabolism in adults with growth hormone deficiency. N Engl J Med. 321:1797-1803.

21. **Jørgensen JOL, Pedersen SA, Thuesen L, Jørgensen J, Skakkebæk NE, Christiansen JS.** 1989 Beneficial effects of growth hormone treatment in GH-deficient adults. Lancet 1:1221-1225.

22. **Bengtsson B-Å, Edén S, Bosaeus I, et al.** 1993 Treatment of adults with growth hormone deficiency with recombinant human growth hormone. J Clin Endocrinol Metab. 76:309-317.

23. **Tanner JM, Whitehouse RH.** 1967 The effect of human growth hormone on subcutaneous fat thickness in hyposomatotrophic and panhypopituitary dwarfs. J Endocrinol. 39:263-268.

24. **Sönksen PH, Cuneo RC, Salomon F, et al.** 1991 Growth hormone therapy in adults with growth hormone deficiency. Acta Paediatr Scand. 379:139-146.

25. **Parra AP, Argote RM, García G, Cervantes C, Alatorre S, Pérez-Pasten E.** 1979 Body composition in hypopituitary dwarfs before and during human growth hormone therapy. Metabolism 28:851-857.

26. **Binnerts A, Swart GR, Wilson JH, et al.** 1992 The effect of growth hormone administration in growth hormone deficient adults on bone, protein, carbohydrate and lipid homeostasis, as well as on body composition. Clin Endocrinol (Oxf). 37:79-87.

27. **Cuneo RC, Salomon F, Wiles CM, Hesp R, Sönksen PH.** 1991 Growth hormone treatment in growth hormone-deficient adults. I. Effects on muscle mass and strength. J Appl Physiol 70(2):688-694.

28. **Whitehead HM, Boreham C, McIlrath EM, et al.** 1992 Growth hormone treatment of adults with growth hormone deficiency: results of a 13-month placebo controlled cross-over study. Clin Endocrinol (Oxf). 36:45-52.

29. **Christiansen JS, Jørgensen JO, Møller J, Jørgensen J, Skakkebæk NE.** 1990 Effects of growth hormone on body composition in adults. Horm Res. 33(Suppl 4):61-64.

30. **Degerblad M, Elgindy N, Hall K, Sjöberg H-E, Thorén M.** 1992 Potent effect of recombinant growth hormone on bone mineral density and body composition in adults with hypopituitarism. Acta Endocrinol (Copenh) 126:387-393.

31. **Valk NK, van der Lely AJ, de Herder WW, Lindemans J, Lamberts SWJ.** 1994 The effects of human growth hormone (GH) administration in GH-deficient adults: a 20-day metabolic ward study. J Clin Endocrinol Metab. 79:1070-1076.

32. **National Diabetes Data Group.** 1979 Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 28:1039.

33. **Cederholm J, Wibell L.** 1985 Evaluation of insulin release and relative peripheral resistance with the use of the oral glucose tolerance test: a study in subjects with normoglycaemia, glucose intolerance and non-insulin-dependent diabetes mellitus. Scand J Clin Lab Invest. 45:741-751.

34. **Blum WF, Albertsson-Wikland K, Rosberg S, Ranke MB.** 1993 Serum levels of insulin-like growth factor I (IGF-I) and IGF binding protein 3 reflect spontaneous growth hormone secretion. J Clin Endocrinol Metab. 76:1610-1616.

35. **Strasburger CJ, Dressendörfer RA, Lee PDK.** 1994 Non-isotopic two site immunoassay for IGFBP-3. Growth Reg. 4 (Suppl 1):138.

36. **Seibel MJ, Cosman F, Shen V, et al.** 1993 Urinary hydroxypyridinium crosslinks of collagen as markers of bone resorption and estrogen efficacy in postmenopausal osteoporosis. J Bone Miner Res. 8:881-889.

37. **Mazess RB, Barden HS, Bisek JP, Hanson J.** 1990 Dual-energy x-ray absorptiometry for total-body and regional bone-mineral and soft-tissue composition. Am J Clin Nutr. 51: 1106-1112.

38. **Stewart SP, Bramley PN, Heighton R, et al.** 1993 Estimation of body-composition from bioelectrical impedance of body segments: comparison with dual-energy x-ray absorptiometry. Br J Nutr. 69:645-655.

39. **Durnin JVGA, Womersley J.** 1974 Body fat assessed from total body density and its estimation from skinfold thickness : measurements on 481 men and women aged from 16 to 72 years. Br J Nutr. 32:77-97.

40. **Hunt SM, McEwen J, McKenna SP.**1986 In: Measuring health status, London:Croom Helm.

41. **Hunt SM, McKenna SP, McEwen J, Williams J, Papp E.** 1981 The Nottingham Health Profile: subjective health status and medical consultations. Soc Sci Med. 15A:221-229.

42. **Hunt SM, McEwen J, McKenna SP, Backett EM, Pope C.** 1984 Subjective health assessment and the perceived outcome of minor surgery. J Psychosom Res. 28:105-114.

43. **Essink-Bot M-L, van Agt HME, Bonsel GJ.** 1992 NHP of SIP: een vergelijkend onderzoek onder chronisch zieken. T Soc Gezondheidsz. 70:152-159.

44. **Mazess RB, Barden HS.** 1988 Measurement of bone by dual-photon absorptiometry (DPA) and dual-energy x-ray absorptiometry (DEXA). Ann Chir Gynaecol. 77:197-203.

45. **Nilas L, Borg J, Gotfredsen A, Christiansen C.** 1985 Comparison of single- and dual-photon absorptiometry in postmenopausal bone mineral loss. J Nucl Med. 26: 1257-1262.

46. **van Berkum FNR, Birkenhäger JC, van Veen LCP, et al.** 1989 Non-invasive axial and peripheral assessment of bone mineral content: A comparison between osteoporotic women and normal subjects. J Bone Miner Res. 4:679-685.

47. **Rosén T, Johansson G, Hallgren P, Caidahl K, Bosaeus I, Bengtsson B-Å.** 1994 Beneficial effects of 12 months replacement therapy with recombinant human growth hormone to growth hormone deficient adults. Endocrinol Metab. 1:55-66.

48. **Runge VM, Eisenmann PA, Johnson SC.** 1987 Effects of exercise induced dehydration on BIA to determine body composition. Med Sci Sports Exerc. 19:S38.

49. **Zabetakis GW, Gleim GW, Vitting KE, Gardenswartz MH.** 1987 Volume changes effect electrical impedance measurements of body composition. Med Sci Sports Exerc. 19:S40.

50. **Svendsen OL, Haarbo J, Heitman HL, Gotfredsen A, Christiansen C.** 1991 Measurement of body fat in elderly subjects by dual-energy x-ray absorptiometry, bioelectrical impedance and anthropometry. Am J Clin Nutr. 53:1117-1123.

51. **Deurenberg P, van der Kooij K, Leenen R, Weststrate JA, Seidell JC.** 1990 Sex and age specific prediction formulas for estimating body composition from bioelectrical impedance: a cross validation study. Int J Obesity 15:17-25.

52. **Mazess RB.** 1991 Do bioimpedance changes reflect weight, not composition? Am J Clin Nutr. 53:178-179 (letter).

53. **Deurenberg P, Weststrate JA, Hautvast JGAJ, van der Kooij K.** 1991 Is the bioelectrical-impedance method valid? Am J Clin Nutr. 53:179 (letter).

54. **Forbes GB, Simon W, Amatruda JM.** 1992 Is bioimpedance a good predictor of body composition change? Am J Clin Nutr. 56:4-6.

55. **Pace N, Rathbun EN.** 1945 Studies on body composition. III. The body water and chemically combined nitrogen content in relation to fat content. J Biol Chem. 158:685-691.

56. **Sheng HP, Huggins RA.** 1979 A review of body composition studies with emphasis on total body water and fat. Am J Clin Nutr. 32:630-647.

57. **Haarbo J, Gotfredsen A, Hassager C, Christiansen C.** 1991 Validation of body composition by dual energy x-ray absorptiometry (DEXA). Clin Physiol. 11:331-341.

58. **Fuller NJ, Laskey MA, Elia M.** 1992 Assessment of the composition of major body regions by dual-energy x-ray absorptiometry (DEXA), with special reference to limb muscle mass. Clin Physiol. 12:253-266.

59. **Vandeweghe M, Taelman P, Kaufman J-M.** 1993 Short and long-term effects of growth hormone treatment on bone turnover and bone mineral content in adult growth hormone-deficient males. Clin Endocrinol (Oxf). 39:409-415.

7 Summary, synthesis and future

Summary

After the development of GH biosynthesis by recombinant DNA techniques the number of studies with human GH has greatly increased. The prior restrictions of its use due to scarcity were lifted, and studies of GH action and therapy other than in childhood growth hormone deficiency (GHD) were initiated (**chapter 1.1 & 1.2**).

The human body consists for a substantial part of proteins mainly in the form of muscle. In GHD patients muscle mass is decreased. Also, general diseases in man greatly affect body protein turnover by causing a negative nitrogen (protein) balance. Since GH acts as an anabolic hormone and induces a positive nitrogen balance, nitrogen metabolism is important when studying the effects of GH therapy (**chapter 1.3**).

The assessment of body composition can be achieved by several principally different techniques. These methods are influenced by several parameters of disease and cannot be applied routinely in all patients without taking the disease characteristics into account (**chapter 1.4**).

In the late 1980s and early 1990s the results of the first long-term studies on GH replacement therapy in adults were reported and the features of the GHD syndrome in adulthood were described. These include among others an increase in body fat and a decrease in lean body mass, an impaired physical performance, a decrease of bone mineral mass, disorders in lipid metabolism, the development of premature atherosclerosis, and a decreased quality of life (**chapter 1.5**).

The unlimited availability of hGH resulted in a considerable expansion of studies into potential new indications for GH treatment other than replacement therapy (**chapter 1.6**).

Long-term safety reports concerning GH therapy are not available in adults. Experience, however, is increasing since large numbers of GHD adult patients take part in large multi-center trials on GH replacement therapy, mainly in Europe. No deleterious side-effects of long-term GH therapy have been observed so far. They are also not expected to occur, as the GH dose administered is within the physiological "replacement" range (**chapter 1.7**).

In this thesis several aspects of the GHD syndrome in adulthood, as well as of GH replacement therapy and GH therapy in adults were studied.

The potential differences in the signs and symptoms of GHD in adulthood were studied in 173 patients. The childhood onset (CO) GHD patients (n=74) were younger and smaller than the adult onset (AO) patients (n=99). CO as well as AO patients tended to be obese, with a preference of increased adipose tissue mass around the waist in AO patients. Lean body mass was especially lowered in CO GHD patients. Osteocalcin levels were elevated in most CO patients, while lipid abnormalities (increased cholesterol and lowered HDL-cholesterol levels) especially occurred in AO GHD individuals. Quality of life, as evaluated with the Nottingham Health Profile questionnaire showed abnormal dimension scores in all six subscales for both CO and AO GHD patients, but the degree of distress for physical mobility and energy levels was significantly higher in AO individuals. These results demonstrate considerable differences in the clinical and biochemical presentation of the adult GHD syndrome. Adult patients with idiopathic CO GHD show somatic underdevelopment similar to pituitary dwarfism: they remain too small, have generalized obesity and a lowered muscle mass, elevated osteocalcin levels, marginal abnormalities in their lipid metabolism and are relatively content with their life. It seems as if they have not fully completed development yet. Somatic development is complete in AO GHD patients, however, who previously have experienced the full advantage of health and physical maturation. Most of them are obese, with a central abdominal localization, the majority of them have lowered HDL-cholesterol levels indicating the risk of premature atherosclerosis. Subjectively, they experience a greater loss of their quality of life (**chapter 2**).

Thereafter the potent anabolic effects of GH on nitrogen metabolism were investigated in four GHDA patients in a metabolic ward study. Measurement of IGFBP-3 levels seems to offer no additional information as to defining the optimal GH replacement dose. Sodium retention in the first days after the initiation of GH replacement therapy is considerable and largely influences the assessment of body composition by BIA, rendering this technique in fact unsuitable in evaluating the initial changes in body composition during GH replacement therapy. BIA does not measure body composition

but gives information reflecting the extracellular volume and its electrical conductivity, expressed as resistance measured between two ipsilateral extremities. This value is abnormal in untreated GHD adults and normalizes within a few days after the initiation of GH replacement therapy (**chapter 3**).

GH has both direct and indirect effects on bone turnover and bone mineral mass. GHD patients have a lowered bone mineral content (BMC). In a previous study we observed, an initial (temporary) further decrease of BMC during the first six months of GH replacement therapy. This can be explained by an increase in bone turnover and remodeling. In the present study, the effects of the combined treatment of GH replacement therapy with an inhibitor of bone resorption, pamidronate, was investigated in six GHD adult patients. It was hypothesized that the initial decrease as found with GH therapy only could be prevented by the bisphosphonate. After six months of combined treatment, indeed an increase in BMC was observed while bone turnover remained activated. This suggests a net positive effect on the coupling of bone resorption and bone formation and might favor initial adjuvant pamidronate to GH replacement in GHD patients with a lowered bone mineral mass. Furthermore the effects of the addition of pamidronate to GH replacement on renal phosphate handling were studied. GH (or IGF-I) induces phosphate retention independently from PTH and only partially antagonizes the phosphaturic effect of PTH (**chapter 4**).

Since inhibition of bone resorption by pamidronate causes an increase in BMC, while GH activates bone turnover, it was hypothesized that combined treatment with both compounds might also be of benefit in postmenopausal osteoporosis. Twenty-one osteoporotic elderly women were randomly assigned to two groups and were studied in a placebo-controlled manner with GH vs. placebo for six months, while all patients received pamidronate for twelve months. Bone mass increased in the placebo group (pamidronate only), while bone mass did not change in the patients treated with GH and pamidronate. Bone turnover decreased below baseline in the placebo group, while it did not change in the GH group. It can be concluded that the combined treatment of GH and pamidronate offers no new approach to the treatment of postmenopausal osteoporosis. However, six months might in retrospect have been a too short treatment period and other treatment schedules might prove more beneficial (**chapter 5**).

Finally the effects of long-term GH replacement in patients with *adult onset* GHD were studied. Twenty patients entered an eighteen month GH replacement study, of which the first six months were placebo-controlled. Beneficial effects were found on body composition and lipid profile. Bone turnover increased but did not result in an increment of bone mass at twelve and eighteen months, respectively. Side effects were mild and in most causes transient or reversible by dose reduction. There was a mild deterioration of glucose tolerance which had a tendency to improve toward the end of the study. Interestingly it was found that skinfold anthropometry was a reliable indicator of body composition change, this in contrast with BIA. This further corroborates our findings in chapter 3. Since skinfold anthropometry is an easy, patient-friendly and cheap procedure, it is suggested that this method should be used in the evaluation of the efficacy of GH replacement therapy in GHDA patients (**chapter 6**).

Synthesis and Future

GH is a potent anabolic hormone. GH replacement in adults with GHD is beneficial to the health status of these patients. GH causes a positive nitrogen, potassium and phosphate balance and increases lean body mass, meanwhile decreasing fat mass. Also the effects on sodium retention and extracellular volume are substantial. GH has stimulatory effects on bone turnover and when combined with an inhibitor of bone resorption it causes an increase of bone mass, occurring already during the first six months of therapy. No potential role for GH was found in the treatment of postmenopausal osteoporosis. Long-term GH replacement in GHD adults leads to significant favorable changes in body composition and in the lipid profile, thereby diminishing risk factors for the development of premature atherosclerosis. In the evaluation of changes of body composition during GH replacement, assessment by BIA is not suitable and skinfold anthropometry is to be preferred.

The registration by the authorities of human GH for treatment in GHDA patients is only a matter of time. However, this does not necessarily implicate that all GHD patients will benefit from, or should receive GH replacement therapy.

By definition all patients which suffer from the GHD syndrome are GHD, as determined by an inadequate response to GH stimulation tests. The opposite, however, that all

GHD adult patients suffer from the GHD syndrome remains uncertain. Also, there is a possibility of a third group of patients with signs and symptoms of the GHD syndrome, with a normal GH response to a stimulation test, but lowered IGF-I levels, reflecting impaired 24-hours GH secretion. More studies should be done on these aspects. The considerable differences in the clinical and biochemical symptomatology between adult GHD of childhood and adult onset underline that especially in the AO GHD who had previously led a normal life, individual GH replacement therapy improves the subjective psychological distress and increases HDL-cholesterol levels, thereby preventing premature atherosclerosis. The group of CO GHD adults demonstrate a number of features of pituitary dwarfism. Several signs and symptoms, as well as biochemical parameters, suggest that most individuals have not yet reached full somatic and psychological development, despite previous GH therapy in order to reach their adult height. Therefore it is anticipated that our observations support that, at the end of puberty, GH therapy in this category of GHD individuals should be continued into adulthood, without interruption in order to induce full maturation.

8 Samenvatting voor de niet-medicus

In het begin van deze eeuw werd aangetoond, dat groei wordt gereguleerd door een stof uit de hypofyse (hersenaanhangsel), die momenteel groeihormoon (GH) wordt genoemd. GH werd gezuiverd uit de hersenen van overledenen en was daarom schaars. Dit GH werd vooral gereserveerd voor kinderen met achtergebleven lengtegroei tengevolge van groeihormoondeficiëntie (GHD) . Sinds de ontwikkeling van kunstmatig menselijk groeihormoon (GH) met behulp van recombinant DNA technieken is het aantal studies met GH enorm toegenomen. De eerder opgelegde beperkingen tengevolge van de schaarste werden opgeheven en met onderzoek naar de werking van GH en GH behandeling voor andere indicaties dan GHD bij kinderen werd een begin gemaakt. (**hoofdstuk 1.1 & 1.2**).

Het menselijk lichaam bestaat voor een groot deel uit eiwitten, voornamelijk in de vorm van spierweefsel. Bij patiënten met GHD is er sprake van een afname van de spiermassa. GH heeft een belangrijke taak bij het handhaven van de eiwit (stikstof) balans. Veel ziekten beïnvloeden de eiwitstofwisseling en veroorzaken een negatieve eiwit balans met allerlei negatieve effecten op vele lichaamsfuncties. Daar GH een anabool (opbouwend) effect heeft en een positieve eiwitbalans kan bewerkstelligen, is deze balans van belang, wanneer de effecten van GH behandeling worden bestudeerd (**hoofdstuk 1.3**).

De lichaamssamenstelling (waaronder de spiermassa) kan op meerdere manieren worden gemeten. Bij deze meetmethoden worden verschillende technieken gebruikt. Daar de methoden door vele factoren (o.a. ziekte) worden beïnvloed kunnen ze niet zonder meer willekeurig worden toegepast (**hoofdstuk 1.4**).

Aan het einde van de jaren tachtig en in het begin van de jaren negentig, werden de eerste resultaten van langdurige GH behandeling bij GHD volwassenen beschreven. Tevens werden de belangrijkste kenmerken van GHD bij volwassenen gedefinieerd en werd het beeld van het groeihormoondeficiëntiesyndroom bij volwassenen duidelijk. Dit omvat o.a. een toename van lichaamsvet en een afname van spiermassa, een verminderd inspanningsvermogen, een vermindering van

botmassa, verstoring van de vet (cholesterol) stofwisseling, vroegtijdige aderverkalking en een verminderde kwaliteit van leven (**hoofdstuk 1.5**).

De welhaast onbeperkte beschikbaarheid van GH, heeft ook onderzoek naar nieuwe toepassingsmogelijkheden van GH mogelijk gemaakt. Dit betreft o.a. kleine kinderen zonder GHD, patiënten met overgewicht, ouderen, patiënten met ernstige ziekten zoals bv. na brandwonden, ernstige bloedvergiftiging, ontstekingen, na ongevallen of grote operaties, patiënten met heupfracturen, patiënten die met bijnierschorshormoon (bv. prednison) worden behandeld, patiënten met kanker, patiënten met AIDS, vrouwen met botontkalking na de menopauze (**hoofdstuk 1.6**).

Er zijn nog geen resultaten bekend van onderzoek naar de veiligheid van GH behandeling op de lange termijn. De ervaring op dit gebied neemt echter snel toe, door de grote aantallen patiënten, die deelnemen aan onderzoek naar de effecten GH behandeling op de volwassen leeftijd. Er zijn tot nog toe geen nadelige effecten van GH behandeling aangetoond. De belangrijkste klachten, die soms kort na de start van de behandeling optreden zijn spierpijn en "vocht vasthouden". Deze klachten verdwijnen spontaan of na geringe verlaging van de hoeveelheid toegediend GH. Ook op theoretische gronden is het niet te verwachten, dat er belangrijke bijwerkingen zullen optreden, daar het gaat om "het vervangen van wat op onnatuurlijke wijze ontbreekt".

In dit proefschrift worden resultaten van onderzoek naar het GHD syndroom op de volwassen leeftijd, GH behandeling bij GHD volwassenen en GH behandeling bij vroegtijdige botontkalking bij vrouwen beschreven.

De mogelijke verschillen in GHD op de volwassen leeftijd werden bestudeerd in 173 patiënten. Patiënten die reeds als kind GHD waren (childhood onset; CO) waren jonger en kleiner dan de patiënten die op volwassen leeftijd GHD werden (adult onset; AO). Zowel CO als AO patiënten toonden een neiging tot overgewicht, met een voorkeur voor vetophoping rond de buik bij AO patiënten. In het bijzonder bij de CO GHD patiënten was er een vermindering van het vetvrije gedeelte van het lichaam, hetgeen voor een groot deel uit spier bestaat. Afwijkingen in de cholesterolspiegels komen vooral bij AO GHD voor. De kwaliteit van leven, zoals deze werd gemeten met uitgebreide vragenlijsten, toonden afwijkingen op alle gemeten

gebieden voor beide patiënt groepen, maar de beperkingen in lichaamsbeweegbaarheid en energie waren beduidend ernstiger in AO GHD. Deze resultaten tonen belangrijke verschillen tussen CO en AO GHD. Volwassen patiënten met CO GHD tonen verschijnselen van lichamelijke onderontwikkeling: ze blijven te klein, ze hebben gegeneraliseerd overgewicht en een verminderde spiermassa, ze hebben geringe cholesterol afwijkingen en zijn relatief tevreden met hun leven. In AO GHD is de lichamelijke ontwikkeling voltooid. Meeste patiënten hebben overgewicht, welk meer rond de buik is gelokaliseerd. De meerderheid heeft afwijkende cholesterolwaarden met een verhoogd risico op vroegtijdige aderverkalking. Deze patiënten ervaren een groter verlies in kwaliteit van leven (**hoofdstuk 2**).

Daarna worden de sterke anabole effecten van GH op de stikstofstofwisseling beschreven, zoals deze werden bestudeerd in een balans studie bij vier GHD patiënten. Voor dit onderzoek werden de patiënten 24 dagen opgenomen. Gedurende 15 dagen werden de patiënten met GH behandeld. Tijdens deze opname nuttigden zij elke dag dezelfde maaltijd. Een van deze maaltijden werd volledig gemengd en onderzocht op de samenstelling. Alle urine en ontlasting werd verzameld. Door het verschil tussen in (maaltijd) en uit (urine en ontlasting) te meten kon het effect van GH op o.a. de stikstofstofwisseling en de zout en water huishouding worden bestudeerd. Er blijkt een zeer snel effect te zijn op het vasthouden van water en zout als gevolg van GH behandeling. Ook het positieve effect op de stikstofstofwisseling is al na twee dagen aantoonbaar. Uit dit onderzoek werd tevens duidelijk dat de lichaamssamenstelling meting die gebaseerd is op de elektrische weerstand van het lichaam ongeschikt is bij GHD patiënten die met GH worden behandeld. De meting geeft meer informatie over de hoeveelheid lichaamswater dan over de hoeveelheid lichaamsvet (**hoofdstuk 3**).

GH heeft een positieve invloed op de botstofwisseling en de botmassa. GHD patiënten hebben een verlaagde botmassa. Een verlaagde botmassa vormt een verhoogd risico op wervelinzakkingen en botbreuken van de heup. In een eerdere studie werd door ons aangetoond, dat in het begin van GH behandeling de botmassa iets daalt. Dit lijkt te kunnen worden verklaard door een toename van de

botombouwactiviteit. Anderen toonden reeds aan dat een voorgezette GH behandeling de botmassa doet stijgen. Er bestaan tevens medicijnen die de botafbraak remmen. In een onderzoek dat werd uitgevoerd bij zes GHD patiënten werd deze remmer van de botafbraak gecombineerd met de stimulator van de botstofwisseling GH. De veronderstelling was, dat het hierdoor mogelijk zou kunnen zijn, de botmassa direct na aanvang van GH therapie te laten stijgen. Dit bleek inderdaad het geval na zes maanden behandeling. Dit zou kunnen betekenen dat een gecombineerde behandeling het evenwicht van botafbraak en botaanmaak op een gunstige manier beïnvloed (**hoofdstuk 4**).

Omdat het remmen van de botafbraak op zich al een toename van de botmassa bewerkstelligt en GH de botombouw stimuleert, zou een gecombineerde behandeling ook van betekenis kunnen zijn bij vrouwen met een verlaagde botmassa na de overgang (postmenopauzale osteoporose). Een en twintig vrouwen werden bestudeerd, gedurende een jaar. Zij kregen allen een remmer van de botafbraak. In het eerste half jaar kreeg de helft van de vrouwen GH behandeling, de andere helft een niet werkzame injectie vloeistof (placebo). Zowel de patiënt als de onderzoeker waren pas na afloop van de behandeling op de hoogte van de ingestelde behandeling (GH of placebo). In de groep met placebo behandeling was er een stijging van de botmassa (zoals te verwachten) met een vermindering van de botombouw. In de groep met GH behandeling bleef de botmassa echter gelijk met een gelijkgebleven botombouw. Er was dus geen positief effect van GH. Achteraf kan mogelijk worden verondersteld, dat de behandeling te kort heeft geduurd of dat het behandelingschema anders had moeten zijn (**hoofdstuk 5**).

Tenslotte worden de resultaten beschreven van langdurige GH behandeling bij twintig AO GHD patiënten. Er werden gunstige effecten gevonden op lichaamssamenstelling en vetstofwisseling. De botombouw nam toe maar resulteerde niet in een toename van de botmassa bij alle patiënten. De bijwerkingen waren gering en van voorbijgaande aard. Er was een geringe stoornis in de suikerstofwisseling, die tegen het einde van de studieperiode weer herstelde. Een interessante bevinding was bovendien, dat lichaamssamenstelling meting met behulp van het meten van onderhuidse vetplooien betrouwbaarder resultaten opleverde dan meting via de

elektrische weerstand van het lichaam. Vetplooimeting is goedkoop en simpel en kan daarom goed gebruikt worden om de effectiviteit van GH behandeling bij GHD patiënten te meten (**hoofdstuk 6**).

GH en de toekomst

GH is een krachtig anabool hormoon. GH behandeling bij volwassenen met GHD is gunstig voor de gezondheid van deze patiënten. GH veroorzaakt een positieve stikstof, kalium en fosfaatbalans en doet de spiermassa toenemen terwijl de vetmassa afneemt. Ook de effecten op water- en zouthuishouding zijn aanzienlijk. GH heeft een stimulerend effect op de botombouw en, indien gecombineerd met een remmer van de botafbraak, doet de botmassa toenemen bij GHD patiënten. Er werd geen rol aangetoond voor GH therapie in postmenopauzale osteoporose. Lange termijn GH behandeling van GHD patiënten leidt tot positieve effecten op lichaamssamenstelling, cholesterol en daarmee tot een gunstiger vooruitzicht met betrekking tot vroegtijdige aderverkalking. De veranderingen in lichaamssamenstelling kunnen zeer goed worden gemeten met een simpele huidplooimeter.

Inmiddels werd menselijk GH geregistreerd voor de behandeling bij GHD volwassenen. Dit betekent echter niet zonder meer dat alle patiënten voordeel zullen ondervinden van GH therapie en ook niet dat al deze patiënten met GH zouden moeten worden behandeld. Per definitie zijn alle patiënten die aan het GHD syndroom lijden GHD. Dat wil echter nog niet zeggen dat alle GHD patiënten lijden aan het GHD syndroom. Daarnaast is er mogelijk een derde groep patiënten met kenmerken en klachten van het GHD syndroom zonder dat ze GHD zijn.

De aanzienlijke verschillen tussen CO en AO GHD zoals wij deze hebben waargenomen, ondersteunen de inschatting dat in de toekomst, aan het einde van de puberteit, GH behandeling dient te worden voortgezet om volledige ontwikkeling mogelijk te maken en de complicaties van GHD te doen voorkomen. Daarnaast bestaat er inmiddels voldoende aanleiding om bij patienten die op volwassen leeftijd GHD zijn geworden GH substitutie te overwegen.

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The chapters presented in this thesis are based upon the following publications:

1. **Attanasio AF, Valk NK, Strasburger CJ, Birkett M, Matranga AMC, Lamberts SWJ.** 1995 Adult growth hormone deficiency syndrome patients demonstrate heterogeneity between childhood onset and adult onset. (submitted).

2. **Valk NK, van der Lely AJ, de Herder WW, Lindemans J, Lamberts SWJ.** 1994 The effects of human growth hormone (GH) administration in GH-deficient adults: a 20-day metabolic ward study. J Clin Endocrinol Metab. 79:1070-1076.

3. **Valk NK, Erdtsieck RJ, Algra D, Lamberts SWJ, Pols HAP.** 1995 Combined treatment of growth hormone (GH) and the bisphosphonate pamidronate vs. treatment with GH alone in GH-deficient adults: the effects on renal phosphate handling, bone turnover and bone mineral mass. Clin Endocrinol (Oxf) 43:317-324.

4. **Erdtsieck RJ, Pols HAP, Valk NK, van Ouwerkerk BM, Lamberts SWJ, Mulder P, Birkenhäger JC.** 1995 Treatment of postmenopausal osteoporosis with a combination of growth hormone and pamidronate. A placebo-controlled trial. Clin Endocrinol (Oxf) (accepted for publication).

5. **Valk NK, Attanasio AF, van der Lely AJ, Lamberts SWJ.** 1995 Treatment of patients with adult onset growth hormone (GH) deficiency with recombinant human GH: An eighteen months placebo controlled study. (submitted).

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