GROWTH HORMONE TREATMENT IN ADULTS

Groeihormoon behandeling bij volwassenen

PROEFSCHRIFT

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CONTENTS

LIST OF ABBREVIATIONS

ANOVA	analysis of variance
ANP	atrial natriuretic peptide
BCM	body cell mass
BIA	body impedance analysis
BMI	body mass index
BW	body weight
D_2O	deuteriumoxide
DPA	dual photon absorptiometry
ECW	extra-cellular water
FFA	free fatty acids
FFM	fat-free mass
FM	fat mass
FSH	follicular stimulating hormone
FT4	free thyroxine
GFR	glomerular filtration rate
GH	growth hormone
GHRH	growth hormone-releasing hormone
HBSA	Hank's balanced salt solution
HDL	high density lipoprotein
HSA	human serum albumin
HTL	hepatic triglyceride lipase
ICW	intra-cellular water

IMP	impedance
IGF-I	insulin-like growth factor-I
LBM	lean body mass
LPL	lipoprotein-lipase
MEM	minimal essential medium
PrI	prolactine
PTH	parathormone
RIA	radioimmunoassay
RPF	renal plasma flow
rT3	reversed triiodothyronine
SEM	standard error of the mean
SM-C	somatomedin-C, now called: IGF-I
SMS	somatostatin
SPA	single photon absorptiometry
T3	triiodothyronine
T4	thyroxine
TBW	total body water
TG	triglycerides
TmP/GFR	maximal renal tubular reabsorption of phosphorus corrected for the GFR
TT4	total thyroxine
U	unit

CHAPTER 1

1.1. INTRODUCTION

Growth promoting effects of crude anterior pituitary extracts in hypophysectomized and intact laboratory animals were first demonstrated in 1921 by Evans and Long (1). Following further isolation the diverse physiologic effects of a relative pure growth promoting factor in animals were described by Li et al in 1949 (2). After demonstration of a species specificity among growth hormones (GH) (3,4,5,6) and the development of a new procedure for crystallizing GH, enabling more GH with an increased purity to be obtained from human pituitaries (7), Beck et al (8) were the first to report the effects of human and monkey growth hormone in man. Subsequently many other reports appeared investigating the effects of the administration of pituitary- derived primate GH, for 1 week to 6 months, in adults with GH deficiency, in patients with a variety of other diseases and in normals (9,10,11,12). These effects included increased nitrogen, phosphorus, potassium and sodium retention, increased calcium absorption, hypercalciuria and disturbances in carbohydrate metabolism.

Effects of GH administration in patients with burns (13,14,15) or trauma (16) were also investigated and showed promising results as to the reversal of catabolism/induction of anabolism.

Raben was the first to report the successful stimulation of growth with human GH in a child with GH deficiency (17), and this was followed by many other reports (reviewed in ref 18).

Due to the species specificity, requiring GH to be derived from the limited supply of primate (human) pituitaries obtained at autopsy, GH remained scarce. Its therapeutic use remained, under strict medical supervision by paediatric endocrinologists, limited to the treatment of children with dwarfism due to GH deficiency. Even in these children GH was administered in regimens now known to be insufficient for optimal growth, however.

In 1985 the use of human pituitary-derived GH had to be stopped completely after reports of contamination of batches of GH by one or more pituitaries containing a "slow virus" causing Creutzfeldt-Jacob disease and resulting in several deaths (19,20,21,22,23,24).

Following the development of recombinant DNA technology several pharmaceutical companies developed the ability to produce in limitless quantities human GH in vitro by Escherichiae Coli. Since 1986 Humatrope, bioequivalent and identical to human GH, manufactured by Eli Lilly & Co (Indianapolis, USA) is available for the treatment of children with short stature and research in adults (25). Other pharmaceutical industries also producing human GH are Novo Nordisk (Norditropin), KabiVitrum

(Genotropin) and Genentech (Protropin).
The studies described in this thesis have all been performed with Humatrop, provided for free by Eli Lilly Nederland.

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1.2. PHYSIOLOGY OF GROWTH HORMONE SECRETION AND ASSESSMENT OF GH DEFICIENCY

Physiology of GH secretion

Growth hormone is synthesized, stored and secreted by the somatotrophic cells in the anterior lobe of the pituitary gland. It is a single-chain peptide of 191 amino acids with two intramolecular S-S bounds and without carbohydrate substituents. It has a molecular weight of 22 KDa (1). About 10 % of the GH in the pituitary is in a smaller 20 KDa form, due to aberrant processing of GH mRNA precursor.

Growth hormone is secreted in an intermittent pulsatile fashion (2,3,4) regulated by a balance between stimulatory (Growth Hormone Releasing Hormone, GHRH) and inhibitory (Somatostatin, SMS) hypothalamic hormones (5), which reach the pituitary through the hypophyseal portal circulation, as well as by direct negative feedback (at both the hypothalamic and the pituitary level) by Insulin-like Growth Factor-I/Somatomedin-C (IGF-I).

As discussed in the following chapter most, though not all effects of GH are mediated by IGF-I which is synthesized in various peripheral tissues. IGF-I secreted by the liver is the main determinant of plasma IGF-I levels.

The release of SMS and GHRH by hypothalamic neurons is influenced by a variety of neuropeptides, like Thyrotrophin Releasing Hormone, opioid peptides and Vasoactive Intestinal Polypeptide, as well as by neurotransmitters, including acetylcholine, dopamine, serotonin, norepinephrine which are relaying signals from neurons in other brain areas (6,7).

Physiologic conditions known to influence GH secretion (mostly indirectly via stimulation or inhibition of the release of GHRH and/or SMS) are: (delta-) sleep (8), exercise (9), metabolic signals (like hypoglycemia, proteins (arginine), fatty acids 10,11), fasting (12), body weight (13), gonadal steroids (14,15), and age.

The effects of age on basal and stimulated GH secretion (and IGF-I) levels have been addressed in a number of studies with conflicting results (15,16,17, 18,19,20,21,22,23,24,25). In some studies no age related decline of GH secretion was observed (16-19). Some authors used GH stimulation tests, while stimulated GH secretion may not represent spontaneous age-related GH secretion. Others used groups of patients which might have differed not only in age but also in weight and nutritional status which could offset age-related changes. However, in most studies an age-related decline in spontaneous (and stimulated) GH secretion was found. Accordingly, plasma IGF-I levels also show an age related decline (15,26,27,28,29).

Assessment of GH deficiency

GH secretion in children with short stature possibly due to GH deficiency can be assessed either by the measurement of spontaneous GH secretion or with physiologic (sleep) or pharmacological (clonidine, arginine, insulin, GHRH) stimulation tests. As the 24 hour sampling for the measurement of spontaneous GH secretion is cumbersome and possibly less sensitive (30), most centres use one or more stimulation tests. However, reproducibility of stimulation tests is reportedly low (31,32) and their value to assess endogenous GH secretion is disputed (33,34). In addition GH stimulation by insulin is unpleasant and potentially dangerous.

The use of GHRH as a stimulator of GH secretion has the advantages of being easy to perform and without side-effects (35,36,37,38,39). However, as some patients may be GH-deficient due to hypothalamic or suprahypothalamic disorders, these may not be detected by direct stimulation of the pituitary gland with GHRH.

In older adults the difference between normal and subnormal GH secretion is even more difficult to establish, as physiologic GH secretion at this age also diminishes.

Another approach to the assessment of GH deficiency is the measurement of circulating IGF-I concentrations (40,41). Levels of IGF-I are relatively constant during the day (42). As other factors (nutritional (43,44), hormonal (40), age (15,26-29)) influence circulating IGF-I levels as well, these should also be taken into consideration when interpreting the plasma IGF-I level.

In conclusion the diagnosis of GH deficiency is usually based on a combination of clinical suspicion, two GH stimulation tests showing inadequate (usually a cut off point of 7 ug/l is used) rise of GH as well as a lowered plasma IGF-I level.

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1.3. GROWTH HORMONE FUNCTIONS

1.3.1. INCIDENCE OF GH DEFICIENCY IN ADULTHOOD

Estimated prevalence of GH deficiency in children in the Western World varies, depending on the criteria used, between 1 in 4000-5000 (1) and 1 in 30.000 (2). In the Netherlands an estimated 1 in 12.000 children are diagnosed to be GH deficient (3) and are being treated with (daily) subcutaneous GH injections. Presently GH treatment is stopped after these children have reached their final height.
The incidence of (pan)hypopituitarism, directly or indirectly (after surgical or radiation treatment), due to a pituitary tumor in adulthood is unknown. Based on the incidence in children it may be assumed that in the Netherlands (with a total population of 15 million) between 1000 and 2000 adults have growth hormone deficiency.

Consequences

Until recently growth hormone deficiency in adulthood, either idiopathic or acquired after treatment for a non malignant pituitary tumor, was thought not to be associated with a reduced life expectancy, as long as concomitant other hormonal insufficiencies were adequately substituted. However, in a retrospective study of 333 patients with hypopituitarism diagnosed over a period of 30 years, Rosen and Bengtsson found an increased overall mortality, with a significantly increased risk for death from cardiovascular disorders (4). This observation was independent of variables like age at diagnosis, the degree of hypopituitarism, the presence of hypertension or diabetes mellitus and gender. In addition a reduced risk of death from malignant tumors was suggested. The authors suggest that their findings might be due to untreated GH deficiency in adulthood. In addition, many endocrinologists have the impression that adults with GH deficiency often do not feel well.
GH secretion does not cease after the epiphyses have fused and final height is achieved at the end of puberty. Instead it continues well into the 6th to 7th decade (Chapter 1.2.), suggesting that phylogenetically GH apparently is of use in adulthood. As will be described below GH plays a role in adulthood in maintaining body composition and exercise capacity, in bone, lipid, glucose and thyroid hormone homeostasis, in renal, cardiac and immune function, as well as in the maintenance of subjective well being.

1.3.2. GH AND SUBJECTIVE WELL BEING

Adults with GH deficiency have shown a reduction in social activity and/or psychological well being in most (5,6,7,8,9,10) though not all (11) studies. As some of these studies included patients who had been treated with radiation therapy for their initial disease, and/or were also being treated for other hormonal

insufficiencies, it cannot be ascertained in all studies that the abnormalities described were due to GH deficiency per se. In addition, as many patients who had GH deficiency at childhood had not attained normal height, in spite of GH substitution (which was either in an insufficient dose and or frequency or initiated too late), short stature may be a contributory factor.
Another interesting report noted an increase in cognitive psychometric testing during GH treatment of adults with GH deficiency due to panhypopituitarism (12). Recently a decrease in deep sleep in adults with isolated GH deficiency was observed (13,14). This decrease in deep sleep may be either a cause or a consequence of (isolated) GH deficiency. It may be a contributory factor to the reduced well being and performance in adults with GH deficiency.

1.3.3. GH AND BODY COMPOSITION, MUSCLE MASS AND STRENGTH

Animal data

Sonntag et al demonstrated that GH influences protein metabolism in skeletal muscle in adult rats (15). Administration of excessive amounts of GH to rats, either by implantation of GH-producing tumors (16,17) or by sc injection (18) resulted in increased growth of skeletal muscle. Ullman et al demonstrated GH-induced muscle growth in regenerating muscle after ischaemic injury and diminished muscle atrophy after denervation (19). Another study in adult rats showed a change in skeletal muscle fibre composition after hypophysectomy, which was reversed after 7 daily injections of GH (20). These changes imply a reduction in the capacity of the muscle fibres for aerobic metabolism of substrates and a decreased resistance to fatigue in adult rat muscle after hypophysectomy.

Human data

GH deficiency

In GH deficient children higher skinfold thicknesses and lower muscle masses or muscle widths are found, which are at least partially reversible by GH substitution treatment (21,22,23,24,25,26,27). In addition GH treatment to GH deficient subjects induced redistribution of fat from abdominal to peripheral sites (28). After cessation of GH treatment fat mass has been shown to increase and muscle mass to decrease (21,22,26).
The administration of GH to adults with GH deficiency leads to an increase in thigh muscle volume, measured by cross sectional CT scanning of the quadriceps muscle (29,30), and an increase in fat free mass (FFM) and a decrease in fat mass (as assessed by hydrodensitometry (31) or total-body potassium 32)). Four months of GH treatment (dosage 2 U/m^2/d) led to a significant increase in muscle and a decrease in adipose tissue mass (compared with a placebo treatment group (29)),

while after more than a year of this treatment, the observed changes had further increased (30). In the study by Crist et al (31) an increase in FFM of 1.3 kg was observed after 2 weeks of GH treatment in 6 GH deficient females who received 700 ug GH/d sc (or approximately 10 ug GH/kg BW/d). Within the same period FM decreased 2.3 kg. In the longer-term study by Solomon et al (32) 6 months of GH administration (0.07 U (or ± 30 ug)GH/kg BW/d) led to an increase of FFM of 5.5 kg with a concomitant decrease in fat mass of 5.7 kg. Interestingly the main part of these changes was observed within the first month of the 6 months treatment period.
After cessation of GH treatment in young adults a decrease in thigh muscle volume was observed with a simultaneous decrease in quadriceps muscle strength (33,34). In another study quadriceps muscle strength in GH deficient adults was lower compared with healthy adults, also after correction for body weight and muscle area (35). Exercise capacity, measured by a cycle ergometry test, in GH deficient adults showed a considerable improvement during GH therapy (29,36).

Physiologic GH secretion
As described in Chapter 1.4. exogenous GH has clear anabolic effects in catabolic but non-GH deficient adults. GH administration (dosage 30 ug/kg BW, three times a week for 6 months) to a group of healthy elderly (age >60 yr) men led to a 8.8% increase in fat free mass and a 14.4% decrease in fat mass as assessed by total body potassium, while in a similar placebo treated group no changes were observed (37).

GH hypersecretion
Body composition in acromegalics differs from normal: plasma volume, total body water and extra cellular water (ECW) are increased as is total body potassium, reflecting a higher FFM and lower FM. Upon successful treatment (GH levels below 2.5 ug/l) normalisation of ECW, reflecting FFM, and FM have been observed (38,39).

1.3.4. GH AND BONE HOMEOSTASIS

Growth hormone derives its name from the observation that it is the substance present in pituitary extracts responsible for the stimulation of longitudinal bone (and thereby body) growth in the young (40,41), completed late in adolescence. (As GH deficient children from GH deficient mothers have normal weight and length, fetal growth seems GH independent). Growth hormone also plays a role in the active process of bone remodelling (resorption and formation) in adulthood.
The mechanism by which GH influences bone (re)modelling is complex, consisting of direct effects of GH and IGF-I on bone tissue, as well as indirect through effects on vitamin D, calcium and phosphorus metabolism.

Direct effects of GH on bone.

The stimulation of longitudinal bone growth in childhood by stimulation of the chondrocytes in cartilage and the epiphyseal growth plate, is the result of the combined effects of GH and IGF-I (42). While the somatomedin hypothesis (43) postulated that the growth promoting effects of GH are mediated by serum factors, now known as IGF-I, extensive evidence indicates that IGF-I alone is not capable of maintaining normal growth in GH deficient animals. In addition, in vitro studies showed both the presence of GH receptors on chondrocytes (44), as well as the capability of bone cells to secrete IGF-I (45,46). Results from a study using a rat hind-limb perfusion model also suggests that GH stimulates growth through local production of IGF-I (47). This and other evidence (reviewed in 42) indicate that GH stimulates the differentiation of prechondrocytes, which makes these cells responsive to IGF-I (either from the liver or locally produced). This further stimulates the clonal expansion of differentiating chondrocytes and bone matrix formation (48). Such a sequence of events is in agreement with the "dual effector theory" of GH action postulated by Green et al (49).

The ability of GH to stimulate osteoblasts and bone matrix formation can be observed by measuring circulating levels of osteocalcin (bone gamma-carboxy glutamic acid-containing protein, which is a major non-collagenous protein of bone matrix, secreted by osteoblasts (50)) and type I and III procollagen (51).

In GH deficient children lowered pretreatment levels of osteocalcin are normalized by GH therapy (52,53) and similar observations were made using type I and III procollagen as a parameter (54,55). Also, in GH deficient adults normal osteocalcin levels increase upon GH administration (56). In acromegalics elevated levels of osteocalcin and type III procollagen are normalized after successful treatment (57,58).

Effects of GH on calcium and phosphorus metabolism.

Older studies, using crude pituitary extracts, showed an increase in intestinal calcium absorption and urinary calcium excretion, when GH was administered to hypopituitary patients for several days to weeks (59,60,61).

In acromegalics similar effects were observed (62,63), while others demonstrated increased levels of 1,25-dihydroxyvitamin D, reversible upon treatment (64,65,66). Therefore it was postulated that GH exerts its effect via increased synthesis of 1,25 dihydroxy-vitamin D, possibly by stimulation of renal 1-a-hydroxylase activity (65,66).

However, in GH deficient children both acute (67) and chronic (68,69) administration of relatively low doses of GH, sufficient to induce calcium and phosphorus retention, did not result in a rise of plasma 1,25-dihydroxyvitamin D levels. Another study in GH deficient children showed that a higher GH dosage was capable

to induce a raise in 1,25-dihydroxyvitamin D within 5 days, while chronic administration of a lower dose of GH did not induce changes in 1,25-dihydroxyvitamin D; meanwhile urinary cAMP output increased without measurable changes in iPTH (70).
Therefore, it appears that while physiologic GH levels stimulate intestinal calcium absorption directly and increase the sensitivity of the kidney to PTH (although insufficient to compensate for the increased intestinal calcium absorption, thereby increasing urinary calcium excretion), pharmacological amounts of GH increase intestinal calcium absorption indirectly by raising 1,25-dihydroxyvitamin D levels.
Research in animals and in GH deficient children indicates that both the effects of GH on renal phosphate handling and on 1,25-dihydroxyvitamin D synthesis are mediated by IGF-I (71,72). Furthermore there is also evidence that estrogens and 1,25-dihydroxyvitamin D exert at least some of their anabolic effects on bone through the local stimulation of IGF-I synthesis (73) or via an increase in IGF-I binding sites (74).

Clinical consequences in adulthood.
No data on bone mineral mass in adults with isolated GH deficiency compared with age and sex matched controls are available. Results from a pilot study in 8 GH deficient patients showed a significant increase in bone mineral density of the lumbar spine after 16-24 weeks of daily administration of 6 U of GH over a period of 32 to 40 weeks (75). Circulating osteocalcin and urinary hydroxy-proline excretion increased during GH treatment. In healthy adult men 7 days of GH administration (in a high dose) led to an increase in osteocalcin and bone alkaline phosphatase levels, which persisted for 6 months thereafter. However, elevated urinary OH-proline excretion returned to normal within 4 weeks (76). One week of GH treatment apparently stimulates osteoblasts and activates bone remodelling for a longer period of time.
In an older study of 7 women with postmenopausal osteoporosis no increment in bone mass could be observed after 6 months of daily GH administration (77). More recently GH treatment for 2 months, followed by calcitonin for 3 months and a medication free period of 3 months was shown to increase total body calcium in 7 women (78). However, an increase in bone mineral content at the radius did not reach significance.
The net effect of GH excess on bone mineral mass (assessed by single and dual photon absorptiometry) appears to be site specific: In a study of 24 acromegalics forearm bone mineral mass (mainly cortical bone) was increased, while vertebral bone mass (mainly trabecular bone) was decreased when compared with age and sex matched controls (79).
In summary, the effects of GH on bone are complex and appear partially contradictory.

1.3.5. GH AND CARBOHYDRATE AND LIPID HOMEOSTASIS

As extensively reviewed in ref 80 the in vitro and in vivo effects of GH on carbohydrate and lipid metabolism can be separated in insulin-like and anti-insulin-like effects.

In vitro studies.

In the first hours of incubation of adipose and muscle tissue, derived from hypophysectomized rats, with GH glucose uptake and oxidation increase, while lipolysis decreases. Prior exposure of these tissues to GH diminishes these insulin-like effects. Likewise, tissues from intact rats did not show an insulin-like response to incubation with GH. Anti-insulin-like effects can be observed, however, after 2-4 hours of GH incubation in tissues derived from hypophysectomized rats: glucose uptake and oxidation decrease while lipolysis increases.

In vivo, GH deficient states.

Chronic GH deficiency in animals and humans (81,82) causes decreased fasting glucose concentrations (due to decreased hepatic glucose output), decreased insulin secretion and increased insulin sensitivity (due to increased glucose utilization and blunting of the increase in hepatic glucose output in response to falling glucose levels). In addition, GH deficiency is associated with hypercholesterolaemia (83,84,85,86) though, according to one study (87), this occurred only in GH deficient subjects with a positive family history for hyperlipidaemia.

In vivo, insulin-like effects.

Similarly to the above mentioned in vitro effects, GH administration to hypophysectomized dogs, rats and GH deficient children (88) leads to a rapid but transient (lasting an hour) decrease in glucose and FFA concentrations. In normal fasting animals and humans (89) these short-term effects could also be observed.
Under physiological circumstances these short-term insulin-like effects are probably not of great importance, as the endogenous secretion of GH, during daily activity and after the onset of sleep, induces a refractory state of its insulin-like effects during day and night.

In vivo, anti-insulin-like effects.

Chronic GH administration to hypophysectomized animals raised fasting glucose concentrations, further impaired glucose tolerance in spite of increased insulin levels, and decreased insulin sensitivity.
In intact animals the anti-insulin-like effects of GH (e.g. increased fasting glucose and insulin concentrations, as well as increased insulin levels after pharmacological stimulation) can be observed within several hours after the first injection as well as

during daily GH injections. GH administration hampers both the ability of insulin to depress hepatic glucose production as well as to stimulate glucose uptake. In a study in normal dogs GH administration for several months led to permanent diabetes mellitus, which was not reversible after the discontinuation of GH (90).
In GH deficient children given GH fasting glucose levels, insulin levels, hepatic glucose output, as well as FFA levels increased (81,82,91), while cholesterol levels decreased in some (84,85) though not all (87,92,93) studies.
Depending on the GH dose administered to healthy humans fasting glucose concentrations were either normal (94,95,96) or elevated (97), with increased fasting (94,95) or glucose load-stimulated (96) insulin levels. In normal subjects the anti-insulin-like actions of GH occur both at hepatic and peripheral sites. Cholesterol levels decreased (98) or did not change (99) during GH administration in healthy humans.
In acromegalics abnormal GH secretion causes hyperinsulinism (100,101, 102) and insulin resistance, both peripheral (103) as well as at the hepatic level (104). After surgical (101) or medical (102) therapy of acromegaly, carbohydrate metabolism improved. The degree of improvement was inversely related to the duration of the disease as well as to the height of both pre- and post- operative GH levels. Mediated by the elevated insulin (105) and decreased lipoprotein lipase (106) levels found in acromegalics serum triglycerides are usually increased, and return to normal after therapy (105).

Conclusions.
As prior exposure to GH hampers the insulin-like effects (enhanced glucose utilization and antilipolysis) of GH, they are of little physiologic importance, although hypoglycaemia may develop in long-term GH deficient patients after the first GH injection.
The anti-insulin-like effects observed during chronic exposure to GH, involve impaired glucose utilization and increased hepatic glucose output. These are of clinical importance in chronic states of elevated GH levels such as acromegaly, diabetes mellitus and during chronic administration of GH to non-GH deficient adults.
The effects of GH on circulating cholesterol and triglycerides are of little physiologic importance, though they may contribute to elevated cholesterol levels in GH deficiency and elevated triglyceride levels in acromegaly.

1.3.6. GH AND OTHER HORMONAL FUNCTIONS

Thyroid hormones.
Thyroid function is probably influenced in several ways by the presence or absence of GH. Firstly the TSH response to TRH is increased and prolonged in children with

idiopathic GH deficiency (107,108,109,110,111,112), while hypothyroidism and reduced TSH response to TRH have been reported after therapeutic administration of GH (108-111). In addition, in acromegalics the TSH response to TRH is low (113,114,115,116). The paradox of pituitary disease (inducing GH deficiency) and an increased TSH response to TRH was further demonstrated in a study by Cobb et al (117), which suggested that endogenous GH depresses the TSH response to TRH while enhancing thyroid secretion of T3 in response to the evoked TSH release.

Secondly, IGF-I, not GH, has distinct growth promoting effects on thyroid follicular cells, though it is more likely that paracrine mediated IGF-I (118,119) (probably under the influence of TSH and not of GH) and not plasma IGF-I levels (mediated by GH) are of importance in this regard.

Thirdly, there is evidence, both from the older (110,120, 121) and more recent (122,123) literature, that GH influences the peripheral metabolism of T4 and T3. In a short term study in healthy adults given 125 ug GH/kg/day for four days a GH-induced increase in peripheral deiodination of T4 to T3 was observed (122). Similar observations were reported when GH deficient adults were treated with a somewhat lower dose of 2 U (667 ug) GH/m^2 for 4 months (123).

GH may exert this effect either directly or indirectly through the generation of IGF-I. In normals fasting induces an increase in GH (124) and a decrease in IGF-I levels (125,126, 127,128), while T3 levels decrease due to a reduction in the peripheral conversion of T4 to T3 (129). Refeeding induces an increase in IGF-I (126-128), as well as an increase in T3 levels and a decrease in rT3 production (129). Therefore IGF-I and not GH itself might stimulate the peripheral conversion of T4 to T3 during GH treatment of GH deficient adults. It is tempting to speculate about which influence GH deficiency and treatment thereof have on body composition via the changes in circulating T3 levels mentioned above.

Gonadal hormones

Gonadal function in patients with isolated GH deficiency is normal (86). However GH treatment of non-GH deficient women who did not respond to gonadotrophin treatment in an in vitro fertilization program, leads to super-ovulation (130), related to marked increases of the IGF-I levels in follicular fluid (131).

Extensive literature has appeared which shows an important modulating (para- and autocrine) role, of IGF-I in the function of the ovaries. Fertility in GH deficient mice is restored by GH treatment (132). In vivo GH enhances IGF-I production by human granulosa cells (133), while in vitro IGF-I stimulates the production of estradiol (134) by granulosa cells. Granulosa cells have specific IGF-I receptors (135), which can be upregulated by FSH (136).

In males, similar observations indicating that testicular IGF-I levels are regulated by

locally acting GH rather than by hepatic IGF-I production (137,138), have been made.
In conclusion, it appears that GH deficiency itself is not of major importance in normal gonadal function in adulthood and that most effects GH may have are mediated by locally produced IGF-I.

1.3.7. GH AND THE CARDIOVASCULAR SYSTEM

Cardiac function

Animal data

In hypophysectomized rats hearts fail to respond adequately to aortic binding, as those rats do not develop cardiomegaly (139). In contrast, normal rats bearing a GH-secreting tumor develop an increase in ventricular weight (140,141). Haemodynamic studies, performed 2-4 months after tumor implantation in normal rats, showed an improvement in cardiac function, also when corrected for cardiac weight (140). Cardiac contractility increased in parallel to changes in myosin composition (141). Evidence from studies by Guler et al indicates that the effects of GH on cardiac weight in hypophysectomized rats are mediated by, probably locally produced (142), IGF-I (143). However, the GH induced fluid retention may contribute to the observed haemodynamic changes.

Human data

In a group of 333 hypopituitary patients (from which patients with acromegaly and Cushing's disease were excluded), who had received adequate replacement therapy with thyroid, gonadal and/or adrenocortical hormones, premature mortality due to cardiovascular disease was noted, which was possibly attributable to longstanding GH deficiency (4).
GH therapy led to a dramatic improvement of cardiac function in a 53 yr old patient with severe cardiac failure and probable (though not proven) GH deficiency (144), while in a group of 22 GH deficient adults treated for 4 months, an improvement in cardiac function was suggested (29). In normal adult volunteers (145) an increase in echocardiografically assessed myocardial contractility was noted after 1 week of GH administration, which had reversed within 1 week after stopping GH. In humans treatment with GH enhances myosin heavy chain MRNA accumulation in skeletal muscle (146). As in the rat data it is difficult, at present, to determine which effects are caused by GH itself, which are mediated by locally produced IGF-I and which are secondary to an increase in preload.
Acromegalics have left ventricular hypertrophy (147,148,149), while in longstanding acromegaly heart failure, secondary to hypertension and coronary artery disease, is a frequent complication (150,151,152). In a study comparing 12

patients with recently developed active acromegaly (mean duration estimated at 6 years) without hypertension or diabetes mellitus, with an age- and sex-matched control group an increase in myocardial contractility, cardiac output (due to both increased heart rate and stroke volume) with a reduced afterload and normal preload was observed in the acromegalics (153). Treatment with octreotide of 9 acromegalic patients led within 6 months to a decrease in systolic and diastolic blood pressure and to a reduction of left ventricular wall thickness, though after 12 months of medical therapy left ventricular wall thickness was still increased compared with controls (154).

Growth hormone excess apparently causes an initial improvement in myocardial function (so called "hyperkinetic heart syndrome"), which is in the long run followed by an increase in blood pressure. This seems similar to the model according to which essential hypertension starts out with increased cardiac output with normal or low peripheral resistance, progressing by adaptive changes to increased peripheral resistance (155,156) and eventually cardiovascular disease.

Renal function

Animal data

The stimulating effect of GH on kidney growth in normal and hypophysectomized rats as well as the compensatory growth after unilateral nephrectomy is at least partially mediated by locally produced IGF-I (143,157,158). In the rat IGF-I also plays a role in the regeneration of the proximal tubuli after ischaemic injury (159). As extensively reviewed by Hammerman, GH and IGF-I regulate glomerular filtration rate (GFR), renal plasma flow (RPF), phosphate transport and gluconeogenesis in the intact kidney (160). Receptors for GH have been found in the proximal tubule, IGF-I receptors are found in the proximal tubule and glomerulus.

Human data

Studies in GH deficient humans demonstrated normalization of previously reduced (when compared to an age-matched control group (29)) GFR and RPF, as well as an increase in creatinine clearance

(32) after 4-6 months of GH replacement therapy. In normals given GH for 1 week (raising GH levels to the level observed in diabetics) GFR and RPF increased to a similar extent as observed in the early stages of diabetic nephropathy (161). In normals ingestion of protein stimulates GH secretion (162) and induces an increase in GFR (163), while the latter effect can be blocked by octreotide (164), presumably by reducing GH (or glucagon) secretion. As in rats, evidence exists that the effects of GH on the human kidney are mediated by auto- and paracrine acting IGF-I (160,165,166,167). Involvement of the renin-angiotensin system is unproven as one study in normal men showed that the GH-induced rise in GFR could

not be blocked by enalapril (168).
In patients with acromegaly GFR and RPF expressed per body surface area are reported to be increased by 28%, when compared with a group of normal age- and sex-matched subjects (169), while after hypophysectomy a decrease in RPF, GFR and renal mass was reported (170).

Fluid and sodium homeostasis
From the early beginning of GH research four decades ago a marked sodium- and fluid-retaining effect of GH administration in man has been observed (59,60,61,171, 172,173,174). While initially GH-induced mineralocorticoid release was postulated to account for the sodium retention (173), others could not detect an increase in aldosterone after GH injection in healthy men (172). In addition other studies showed similar sodium-retaining effects in adrenalectomized rats (171) and man (174), thereby making this theory less likely.
A direct effect of GH itself was shown by Ludens et al who observed transient sodium retention in the first 6 hours after GH injection to adrenalectomized rats, an effect which could be blocked by the administration of actinomycin-D, an RNA synthesis inhibitor (175). However, Rabkin et al found no acute effects (within 3 to 5 hours) of highly purified GH in rats and normal men (176).
The limitations of the early studies (partly performed before the species specificity of GH was known, using impure pituitary extracts potentially containing vasopressin, as well as insensitive aldosterone assay's) led to renewed interest into the "aldosterone hypothesis": Ho and Weisberger showed in 6 healthy young men given a large GH dose (0.2 U (75 ug) /kg/d) for 5 days, a clear increase in plasma renin activity and a seven fold increase in aldosterone levels (177). A recent report by Moller et al demonstrated in 8 healthy males after two weeks treatment with 12 U (4 mg) GH/day, increased extracellular volume and unchanged plasma volume when compared with placebo treatment (178). Simultaneously circulating Atrial Natriuretic Peptide (ANP) levels decreased and aldosterone levels increased in 7 of the 8 subjects (though the mean increase did not reach significance). As ANP is known to inhibit aldosterone production, these observations might fit into the same picture. These effects, using relatively short term GH excess, could not be demonstrated in acromegaly: untreated acromegalics were found to have lowered plasma renin activity (179), and normal levels of ANP were found in the presence of elevated plasma volume, when compared with treated acromegalics and normal controls (180).
Evidence for a direct effect of GH on the cellular (renal and elsewhere) Na/K pump has been reported by Ng and Evans (181). They demonstrated an increase in ouabain-sensitive Na efflux in leucocytes from patients with active acromegaly, while after successful treatment no difference with normal controls was observed. In addition, the ouabain-sensitive Na efflux of normal leucocytes could be stimulated after

incubation with 25 mU GH/l for 1 hour. An older study in rats receiving GH for 7 days had shown a GH-induced stimulatory effect on Na/K ATP-ase in kidney, brain and liver homogenates (182). Perhaps secondary to this primary effect of GH, which leads to volume expansion, an increase in endogenous digitalis-like factor was observed in untreated acromegaly (183). This could normalise the cellular sodium pump activity (184), a mechanism which might be similar to that reported in essential hypertension (185).
In conclusion the pathophysiology of short term GH excess on sodium and fluid retention, as well as the chronic situation of volume overload and/or hypertension in acromegaly (186) are not yet completely understood.
For the GH deficient adult, a reduced risk of hypertension apparently is of lesser importance in the development of cardiovascular disease (4) than the increase in cholesterol levels.

1.3.8. GH AND IMMUNE FUNCTION

Introduction

In mice GH deficiency is causally related with a wasting disease with recurrent and ultimately fatal infections. As extensively reviewed (187) both in vivo (in animals) and in vitro GH deficiency and GH administration have been shown to affect various components of the immune system like thymic growth (188), the number of peripheral T and B cells (189), antibody synthesis (190), natural killer cell activity (191), the priming of macrophages for the release of superoxide anion (192) and neutrophil differentiation (193).

Immune function in GH deficient adults.

In humans no clinically apparent immunedeficiency syndrome leading to increased risk of infectious diseases is associated with isolated GH deficiency. Levels of circulating immunoglobulins, proportion of B cells and T cell subsets and mitogenic response are reported to be normal in GH deficient humans (194,195,196,197, 198,199). Several alterations in the immune system of GH deficient humans have been reported, however: a reduction in natural killer cell activity (194,195), defective synthesis of antibodies in response to antigen (200), defective antibody- or cell- mediated immunity in vivo (201) and in vitro (198) and the inability of lymphoid cells to stimulate cells in an allogenic mixed lymphocyte reaction (202) among others (187).
There is evidence for an auto- or paracrine interaction as there are not only GH receptors on human peripheral mononuclear cells (203), but there is also evidence

for the capability of mononuclear leucocytes to secrete GH (204) and B lymphocytes to secrete IGF-I (205).

Effects of GH administration to GH deficient subjects.
GH administration to GH deficient children with normal pre-treatment T lymphocyte subsets led to a temporary increase (after 3-6 months of therapy) in CD3 and CD8 and a decrease in CD4 cells, while after 9-12 months of treatment lymphocyte subsets had returned to normal pretreatment distributions (198). Simultaneously in vitro IgM production steadily increased from subnormal to normal levels after 9-12 months of GH treatment (198). In another study (199) GH substitution of GH deficient children induced minimal effects (a decrease in the number of B cells and a transiently increased reactivity to phytohemagglutinin stimulation), while in another report (195) short-term administration of neither GH nor GHRH induced an increase in natural killer cell activity, which in that study was found to be decreased before the initiation of therapy. In yet another study natural killer cell activity in women with impaired GH secretion increased after 14 days of GH administration (31), while GHRH was found to inhibit chemotaxis of peripheral blood leucocytes obtained from normal subjects (206).

Effect of immune function on the GHRH-GH-IGF-I-axis.
Apart from effects of GH and GHRH on immune function there is also evidence for effects of immune activity on the GH-IGF-I axis. In septic patients IGF-I production after exogenous GH is reduced (207), while in vitro cytokines (tumor necrosis factor and interleukin-1b) reduce spontaneous and stimulated GH secretion by cultured rat pituitary cells, either directly at the pituitary level (208), and/or indirectly by increased somatostatin release from the hypothalamus (209).

In conclusion, while GH deficiency in man causes no clinically apparent dysfunction of the immune system, GH appears to modulate various functions of the immune system.

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1.4. GROWTH HORMONE AS AN ADJUNCT TO THE NUTRITIONAL TREATMENT OF CATABOLIC DISEASES

Effects of (par)enteral (hyper) alimentation.

The administration of nutrients to a catabolic individual (be it due to infection and sepsis, trauma, burns or cancer) to improve his/her nutritional status appears logical, as loss of body protein is associated with immunosuppression (thereby increasing the incidence of infection) (1), poor wound healing (2), respiratory muscle weakness (3) and impaired muscle function causing decreased ambulation (4) and consequently increased mortality.

The problem of delivering the nutrients to the severely ill individual, often incapable of normal food intake or gastric tube feeding due to (often temporary) intestinal dysfunction was solved after the development of long-term total parenteral nutrition by the infusion of hypertonic nutrient solutions via a central venous catheter (5). Improved nitrogen balance and patient outcome were reported in a number of open studies and reviews (6,7,8). However, over the last decade the efficacy of nutritional support in reducing morbidity and mortality has been questioned, while complications of the placement of central venous canulae, although infrequent in experienced hands, and complications due to bacterial contamination of these canula's leads to serious morbidity (9,10,11,12).

In a study of 8 adult intensive care (ventilator dependent) patients, 10 days of aggressive intravenous nutrition did not prevent protein loss, while body weight increased due to gains in body fat and water (13). In another, larger, study (14) the incidence of postoperative (within 90 days) complications was compared in 395 malnourished adult patients undergoing major abdominal or thoracic surgery randomised to receive perioperative (10-18 days) total parenteral nutrition (TPN), or no perioperative TPN. The rates of complications and mortality were identical in both groups. In subgroup analysis a reduction of postoperative complications could only be observed in the most severely malnourished patients. The use of GH to enhance the efficiency of nutrient utilization and to promote protein anabolism in malnourished and/or metabolically stressed individuals might lead to reduced morbidity, shortened reconvalescence and reduced mortality (15).

Effects of GH administration on nitrogen balance.

Normal volunteers

In healthy volunteers on hypocaloric feeding (500 kcal/day) it could be demonstrated that 10 mg, recombinant DNA-derived, GH daily, stimulated protein synthesis and fat oxidation, enhanced nitrogen and mineral metabolism and resulted in a positive nitrogen balance (16,17). In another study the acute muscle protein response to a 6 hr infusion of GH was investigated in the limbs of nutritionally depleted subjects

during a period of intravenous refeeding. A significant increase in limb aminoacid uptake and in muscle myosin heavy-chain mRNA were observed (18).
In obese, but otherwise healthy women who were fed hypocaloric diets with adequate protein administration of GH (0.05 mg/kg ideal body weight (IBW) every other day or 0.1 mg/kg IBW/d) for several weeks led to improved nitrogen retention (19,20,21). The higher GH dose led to a higher and more prolonged effect on nitrogen retention, though after 5 weeks of this treatment no nitrogen retention was observed anymore in subjects receiving 18 kcal/kg IBW/d.

Patients with pituitary disease

Using pituitary derived GH, Henneman et al observed nitrogen retention, to a maximum of 5-8 g N/day, in all 10 investigated subjects, some of whom had hypopituitarism (22). In similar populations and also using pituitary derived GH others also observed nitrogen retention, though to a lesser extent (23, 24). Mean nitrogen retention was two fold higher in a group of hypopituitaric dwarfs when compared with control children when both were given 2 mg GH/kg BW for 5 days (25).

Patients with burns

In 5 patients with severe burns, Liljedahl et al observed an increased nitrogen retention, which was mainly due to an increased dietary intake, when pituitary GH (10-20 mg/d) was administered for a period of 7 to 9 days, 2 to 3 weeks after injury (26). However, in a later study it was found that pituitary GH (4-8 mg/d) administration for 1 week was of little benefit (as to nitrogen retention) in the catabolic phase (+/- 3 weeks) after injury, while later, in the anabolic phase (+/- 10 weeks), nitrogen retention did increase during GH administration (27). Wilmore et al gave 5 mg GH/d for 1 week to nine severely burned patients 2-35 weeks after injury and observed retention of nitrogen and potassium in the same ratio as the ratio in lean tissue (28).
More recent results from a randomized, double-blinded, placebo controlled trial in 17 adult patients showed that the daily administration of 10 mg GH enhanced the healing of the split-thickness skin graft donor sites (29). Furthermore GH, 0.2 mg/ kg/d, was shown to improve donor-site healing and to reduce mean hospital stay by 14 days in a double blind-placebo controlled study in severely (60% of body surface area) burned children (30).

Patients with (surgical) trauma

Using 2.5-10 mg, pituitary-derived GH for 5 days in 4 patients Johnston and Hadden found no effect on protein catabolism in the immediate postoperative period after

herniorrhaphy (31). More recently, however, an anabolic effect of recombinant DNA derived-GH was demonstrated in the post-surgical state in several studies (32,33,34,35,36,37). In the first placebo controlled study by the group of Sim in patients after major gastrointestinal surgery, receiving 5% glucose infusions (to a total of 500 kcal/d), 0.1 mg GH/kg/d in the first 6 postoperative days was shown to reduce nitrogen loss, increase fat oxidation and reduce protein oxidation while protein turnover (both synthesis (by 209%) as well as breakdown (by 170%)) increased (32). In a second study by the same group, mainly differing from the first in the fact that the patients received by vein 7 g of nitrogen and 450 kcal of fat in addition to the 500 kcal glucose, comparable results were observed. The main difference between both studies was that even on this limited amount of nutrition GH was able to induce nitrogen retention (33). In the third study by this group, now studying the effect of GH (0.1 mg/kg/d for 1 week) in patients on full intravenous nutrition, again increased nitrogen retention was observed. However, no increase in fat oxidation was demonstrated, perhaps due to the higher insulin levels (which tend to inhibit lipolysis) occurring in this study (36). In the first study by Wilmore's group (34) 10 mg GH/d for 1 week (compared with a placebo week in the same patients) in 11 severely malnourished post-surgical patients receiving hypocaloric (60%) intravenous nutrition was shown to induce nitrogen (3.5 g/d) and phosphorus (220 mg/d) retention. In a follow-up study in 6 of the original 11 patients similar effects were observed during the whole GH treatment period of 13-25 days. In a double blinded placebo controlled study in 18 post gastrectomy/colectomy patients, receiving 1 g protein and 20 kcal/kg BW, using less GH (0.06 mg/kg/d during the first postoperative week) nitrogen loss was reduced (though not abolished), lean body mass and hand grip strength were maintained vs a reduction of both in the controls (35). In a mixed (severe burn/vehicle trauma) study population, receiving full intravenous nutritional support, 10 mg GH/d for 1-6 consecutive weeks led to sustained nitrogen, phosphorus and potassium retention, when compared with the previous control week in the same patients (37).
It should be kept in mind, however, that sofar no study (except ref 30) demonstrated improved clinical outcome. As 1 gram of nitrogen retention, if completely used by the body for muscle conservation/regeneration, stands for only 50 gram of lean tissue, a positive nitrogen balance of for example 3 g/d for 1 week translates into circa 1 kg of lean body mass, or only 5% of lean tissue.
Lastly, an interesting observation for gastrointestinal surgeons was made in a rat model where (human) GH, 2 mg(!) /kg/d started 1 week before left colonic resection and continued until death, was shown to increase the strength of colonic anastomosis (38).

Other possible useful indications
In healthy volunteers on a normal enteral diet GH, 0.1 mg/kg/d, was able to prevent

the catabolic side-effect of prednisone, 0.8 mg/kg/d. Prednisone alone induced a nitrogen balance of -0.25 mg/kg LBM/d, due to increased protein degradation, while in controls nitrogen balance was +0.25 mg/kg LBM/d. GH alone caused a balance of +0.60, while GH and prednisone together led to a nitrogen balance of +0.25 mg/kg LBM/d (39).
In another study GH (30 ug/kg/d for 3 days followed by 60 ug/kg/d for 3 days) was administered to malnourished elderly adults with chronic obstructive pulmonary disease on total parenteral nutrition. The higher GH dosage induced an increase in fat oxidation and a nitrogen retention of circa 2 g/d. No changes in hand grip or respiratory muscle function occurred in this short term study (40).

The mechanism of GH induced anabolism
Probably several mechanisms contribute to the anabolic effect of GH. Firstly, GH itself has a direct effect on lipolysis (see chapter 1.3.5) and on cellular protein synthesis (18). Secondly IGF-I (which in practically all studies described above rose during GH treatment), either released from the liver in the circulation or locally produced in the tissues (41,42,43,44) mediates the anabolic effects. Thirdly, some of the anabolic effects of GH treatment could also be explained by increased (mainly in the higher GH dosages used) insulin levels (43). However, since the increase in insulin levels is at least partly caused by insulin resistance, this increase in levels may not represent increase in insulin effects.

Side effects
As discussed in chapters 1.3.5. and 1.3.7. hyperglycaemia and fluid retention appear to be the major adverse effects during relatively short-term GH treatment, while the use of GH in patients with cancer seems unwise at present, as discussed in chapter 1.5.

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1.5. GROWTH HORMONE AND CANCER

As described in chapter 1.4. GH can be used to induce an anabolic state in many catabolic conditions. Cancer-induced cachexia diminishes the quality of life significantly and the amount of weight loss in cancer patients is positively correlated with their overall mortality. In addition, improvement of nutritional status may reduce chemotherapy-related toxicity, as has recently been shown in a rat model (1). The usefulness of (par)enteral (hyper)alimentation in the management of cancer patients is controversial (2,3,4,5,6,7). Since one of the causes of cancer-induced muscle wasting appears to be reduced protein synthesis combined with unchanged degradation (8), GH treatment might be tried as an adjunct to nutrition to prevent or diminish cancer-induced cachexia. However, GH administration might stimulate tumorogenesis and tumor growth. In a rat model hypophysectomy led to decreased tumor growth, reversible by suppletion with thyroxine and GH. One report by Svaninger et al (9) demonstrated no improvement of body composition and muscle wasting (nor an increase in tumor growth) by GH administration in adult, non-growing, sarcoma- bearing mice, while in hypophysectomized rats GH administration stimulated body and tumor growth to a similar extent. In their sarcoma tumor model in intact animals GH levels were elevated from day 8 after tumor implantation, which was explained by the authors as a way to promote endogenous substrate mobilization. Unfortunately IGF-I levels were not reported.

Human data

Follow up for 5-30 years of a group of over 300 hypopituitary patients (substituted for thyroid, adrenal and gonadal deficiencies) showed a reduced mortality by malignant diseases (10). According to some (11,12) GH treatment in GH-deficient children might be followed by an apparent increased incidence of leukaemia, though disputed by others (13). Growth hormone therapy in children, who became GH deficient due to radiation therapy for brain tumors or leukemia, apparently does not induce an increase in tumor relapse (14,15). The incidence of neoplasms (colon, breast) in retrospective surveys of relatively large numbers of patients with acromegaly of varying activity after treatment, is found to be increased in most (16,17,18, 19,20,21) though not all (22) reports. Several reports indicate that at least some tumors cause GH levels to increase (23,24).

In vitro data

Evidence is accumulating that GH influences tumorogenesis and tumor growth indirectly through locally and/or systemically produced IGF-I. Specific binding sites for IGF-I have been found in several breast tumor cell lines and tissues (25,26,27,28,29), colonic (28), and glial (30) tumor tissue, hematologic

(31,32,33), and other cancer cell lines (34,35,36). Tumor cells were shown to have increased binding capacity to IGF-I receptors when compared with less dedifferentiated cell lines or with normal surrounding tissue (26,30,32,33,35). IGF-I is produced by breast (37,38), lung (39,40) and colonic (41) tumor tissue or cell lines.

Increased DNA synthesis and cell growth in response to IGF-I has been reported for many tumor types (26,28,31,33,34,37,42,43,44). In sarcomas, liver and renal tumors IGF-II appears to have similar para- and/or autocrine effects, as reviewed in ref 45.

In addition, a direct tumorgrowth stimulatory effect of GH itself was demonstrated when it was found that GH directly induces the expression of the c-myc oncogene in a lymphoma cell line (46).

In conclusion, strong arguments can be derived from the studies mentioned above to argue against the use of GH (or IGF-I) to promote anabolism in patients with a recent history of cancer, tempting as it may be from a clinical standpoint during cancer cachexia.

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1.6. METHODS OF BODY COMPOSITION ASSESSMENT

Body composition can be assessed using models in which the body is viewed as consisting of either two or four compartments. Most methods use the two compartment model in which the body consists of a chemically distinct fat and fat free (or lean body) mass. The fat mass is anhydrous, potassium free and has a density of 0.900 g/ml at 37C, while the fat free mass is assumed to have a relative constant density of 1.1 g/ml at 37C, a water content of 72-74%, a potassium content of 60-70 mmol/kg in men and 50-60 mmol/kg in women. Fat free or lean body mass can be divided in extracellular mass (ECM) and body cell mass (BCM, the metabolic active compartment). In the four compartment model the body consists of water, protein, bone mineral or fat.

Methods of body composition assessment can be classified as traditional, using the two compartment model or as recent, some of which are capable to determine the four compartments described above (1).

Traditional methods use the determination of total body water, total body potassium, urinary creatinine excretion, densitometry and anthropometry.

Recent and newer methods include neutron activation analysis, measurement of muscle metabolites (plasma creatinine or urinary 3 methyl-histidine), single or dual photon absorptiometry, bioelectrical impedance, computerized tomography and magnetic resonance imaging.

In the following only those methods used in Chapter 5 and 6 are described in some detail. These, as well as the other methods have been extensively reviewed in ref 1 and 2.

Total body water

Total body water (TBW) was found to be a relatively fixed fraction (73.2%) of LBM (3). TBW can be determined by isotope dilution using isotopes of hydrogen, tritium or deuterium (4,5). Prerequisites of this technique are that the isotope a) has the same distribution volume as water, b) is exchanged by the body similar to water and c) is not toxic in the amounts used. Deuterium has the advantage of being relatively cheap and easy to determine with infrared absorptiometry, while it is not radioactive. After an equilibration period (two hours) has passed after the oral administration of a fixed amount of deuteriated water, serum is sampled for the determination of deuterium concentration. Thereafter TBW can be calculated: a correction should be made for urinary loss of deuterium and for the exchange of deuterium with nonaqueous hydrogen (6).

An important problem of the isotope dilution method as a way of determining body composition in patients is the assumption that TBW is 73.2% of LBM, which may be incorrect in edematous or dehydrated states.

In a similar fashion, using ^{22}Na as the isotope, extra-cellular water or mass (ECM) can be determined after an equilibration period of 24 hours after the iv administration of 8 uCi ^{22}Na, as described in more detail by Shizgal (7). When both isotope dilution methods are used simultaneously the metabolically active body cell mass (BCM) can be calculated by subtracting ECM from LBM.

Densitometry

Based on the assumptions concerning the densities of the fat and fat free masses described at the beginning of this chapter, it is possible to calculate the fat mass of the body from its density (specific gravity), using a formula from Siri (8). Body density can be determined by underwater weighing, according to the Archimedes principle (9,10). A correction for residual lung volume must be made, which can be determined with a helium dilution method, while another problem with this method is the assumption that the density of the lean body mass is 1.100 g/ml, which may be incorrect as lean body mass is a mixture of several components (unlike fat which is homogeneous) with densities varying from 3.00 (bone) to 0.993 (water).

Bioelectrical impedance

The bioelectrical impedance measurement (with a portable tetrapolar impedance plethysmograph which introduces a painless signal (50 kHz, 800 uA) to the body of the supine subject) is a swift, noninvasive and relatively new method to assess fat free mass, which requires little effort from subject or researcher. Results have been validated against more cumbersome, traditional methods of body composition assessment (11,12,13,14). The principle of this method lies in the induction of an impedance by an applied, low level alternating, electrical current in the human body. As the impedance at a given signal frequency is related to conductor length and volume, the volume of the conducting mass can be calculated from measured impedance and height. In living organisms the conducting mass consists of fat free mass as this (as opposed to the fat mass) contains virtually all the water and conducting electrolytes within the body.

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1.7. SCOPE OF THE THESIS

With the development of recombinant DNA technology a practically limitless amount of GH became available for clinical use.
Against the background of the sizable literature, aims of the work in this thesis were to further investigate:

a) optimal dose regimens regarding beneficial and harmful effects of the administration of pharmacological doses of GH in catabolic elderly adults.
b) the possible role of GH as a stimulator of cancer growth or in the treatment of cancer induced cachexia in a tumor-bearing rat model.
c) body composition of GH deficient adults.
d) the effects of chronic GH replacement in GH deficient adults.

CHAPTER 2.1.

THE EFFECTS OF HUMAN GROWTH HORMONE ADMINISTRATION IN ELDERLY ADULTS WITH RECENT WEIGHT LOSS

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SUMMARY

The effects of human growth hormone (GH) administration in elderly adults with recent weight loss were investigated in a metabolic ward study. Four patients were studied for 20 days. In addition to a constant caloric and nitrogen sufficient diet, consisting of the recommended amounts of protein and energy plus 20%, the patients received GH in dosages of 25 and 50 ug/kg/day for two four day periods (days 5-8 and 13-16, respectively). Significant increases in nitrogen (N) retention of 1.6 (114.2) and 1.4 g/day (100 mmol/d), respectively, occurred when compared with the control periods. No difference was found between the two GH dosages but the nitrogen-retaining effect of the higher dose appeared to last for several days after its administration was stopped. Plasma IGF-I levels rose during both treatment periods. No important disturbances in carbohydrate metabolism occurred. Body weight increased 2.3 kg during each treatment period probably due to water retention. We conclude that even during more than adequate nutritional intake low GH doses cause considerable nitrogen retention in underweight adults.

INTRODUCTION

Human growth hormone (GH) has anabolic effects in surgical and burn patients (1-5). In normal subjects (6), GH-deficient adults (7,8) and patients recovering from gastrointestinal surgery (9), daily administration of 100-140 ug GH/kg body weight combined with a normal (6) or an energy-restricted (6,9) diet resulted in a positive nitrogen balance. However, several adverse effects which might prevent long-term (eg weeks) GH administration were noted. In particular raised blood glucose and insulin levels (6,9), as well as fluid and sodium retention (6), were reported. In this study we investigated whether administration of much lower dosages of GH to underweight elderly adults given optimal nutritional support induces nitrogen retention without important changes in carbohydrate tolerance and fluid balance.

MATERIALS AND METHODS

Subjects

Two men (age 59 and 70 years) and two women (age 51 and 80) were studied. All required enteral nutritional support for weight loss of approximately 5 kg in recent weeks to months, brought about by nonmalignant illness. Their serum albumin levels were normal, no triceps skinfold thickness measurements were performed. These patients had no evidence of malignant, renal or hepatic disease, or diabetes mellitus.

One patient (no 3) had partial hypopituitarism for which he was receiving cortisone. We suspect he had also growth hormone deficiency, in this respect differing from the other patients. The aims and methods of the study were explained and informed consent was obtained before the study, which was approved by the Medical Ethics Committee of the University Hospital Dykzigt-Rotterdam.

Protocol

The patients were admitted to the Metabolic Ward for a period of 24 days. During the entire study period they were given (enterally) a mixed diet containing 120% or more of the recommended amounts of energy (10) and protein (11) for a person of their age, height and weight. The diets were prepared in accordance with the wishes of the patients, weighed and frozen prior to admission. The patient's own accounts and visual confirmation by the Ward staff were used to check completeness of food intake. Two diets were diluted with distilled water and homogenized for measurements of their nitrogen and mineral content.

The first three days after admission were used to achieve metabolic balance (these days are not included in the figures, the fourth day was day 1 of the study). From day 5 through day 8 the patients received, in addition to their diet, 25 ug GH/kg body weight/day sc at 0900 h (Period 2) and from day 13 through 16 50 ug GH/kg/day (Period 4). HGH (Humatrop) was supplied by Eli Lilly (Indianapolis, IN).

The following measurements were done daily at 0800 h: weight, temperature, blood pressure and pulse rate. On days 1,4,9,12,17 and 20 routine hematological and clinical chemical parameters were determined, as were blood glucose and plasma insulin levels before and 30,60,90,120,150 and 180 minutes after the standardized breakfast. On the days the patients received GH blood glucose levels were measured fasting and also at 2200 hours. Plasma IGF-I (Somatomedin-C) levels were determined at least ten times during the study.

All urine was collected in 24 hour samples. In each sample nitrogen (N), creatinine, sodium (Na), potassium (K), calcium (Ca), phosphorus (P), and glucose was measured quantitatively and ketone bodies qualitatively. Faeces was collected in 4 day periods from day 1 onwards. The 4 day periods were demarcated by the administration of 500 mg carmine red, while the faeces volume was checked by the daily administration of 1200 mg polyethyleneglycol (PEG). Faecal N, Na, K, Ca and P was measured in each pooled 4 day collection.

Methods

Routine methods were used for the hematological and chemical measurements in blood. Urinary, faecal and dietary N was determined with the use of an automatic nitrogen analyzer, type 1400 Carlo Erba (Milan). The electrolytes were determined by flame photometry. Faecal PEG was measured by a turbimetric method. Nitrogen

balance was calculated by subtracting urinary, faecal and integumental N excretion from the dietary intake. Integumental N loss was estimated to be 0.5 g/day (12). Results are shown as grams or mmol (amino-) N, not N2. Insulin levels were determined by RIA in EDTA plasma using kits obtained from the Incstar Corporation (Stillwater, Mn ,U.S.A.). Normal fasting levels were found to be less than 20 mU/ml. The intra-assay cv was 4.2% and the inter-assay cv 10.0%. IGF-I was measured by RIA in EDTA plasma using kits obtained from the Nichol's Institute of Diagnostics (San Juan Capristano, CA ,U.S.A.) (13). Normal values were 0.34-1.90 U/ml in men and 0.45-2.2 U/ml in women. The intra-assay cv was 7.2% and the inter-assay cv was 12.8%. The statistical significance of the differences between mean values was determined using analysis of variance (ANOVA). When significant overall effects were obtained by ANOVA, multiple comparisons were made with the Newman-Keuls test. All data are shown as the mean +/- SE. P values <0.05 were considered significant.

RESULTS

GH was well tolerated by all four patients. No changes in kidney or liver function tests, in electrolytes, cholesterol, triglycerides, total protein and albumin, or in leucocyte count were found.

A significant increase in nitrogen retention occurred during both GH administration periods in all four patients. This increase was of similar magnitude with both dosages used. However no decrease in nitrogen retention occurred in period 5, indicating a prolonged effect of the higher GH dosage (Fig 1; upper panel). An example of the effect of GH administration on nitrogen balance in an individual patient (no 3) is shown in Fig 1, lower panel.

Plasma IGF-I levels rose during both GH treatment periods from (sub)normal values to high/normal values, and the values tended to be higher in period 4 than in period 2. The plasma IGF-I levels in all 4 patients is shown in Fig 2.

Body weight increased during both treatment periods and then decreased within days after cessation of GH (Fig 2). The mean maximal increase in body weight was 2.3 +/- 0.6 kg, with a variation between 1 and 4 kg. Temporary fluid retention was most likely the main cause of the weight increase, even though no peripheral edema was evident at any time during this investigation.

No significant changes in sodium balance occurred during GH treatment, because of large individual fluctuation. Significant sodium retention occurred in patients 1, 3 and 4 in comparison with the previous control period ($p<0.05$ in all instances; data not shown).

Low dose GH administration caused no significant potassium retention (7, 6, 2 and -3 mmol/d, respectively, in patients 1 to 4). In period 4, at 50 ug hGH/kg/d, significant retention occurred in all 4 patients (26, 16, 14 and 9 mmol/d, $p<0.01$ versus period

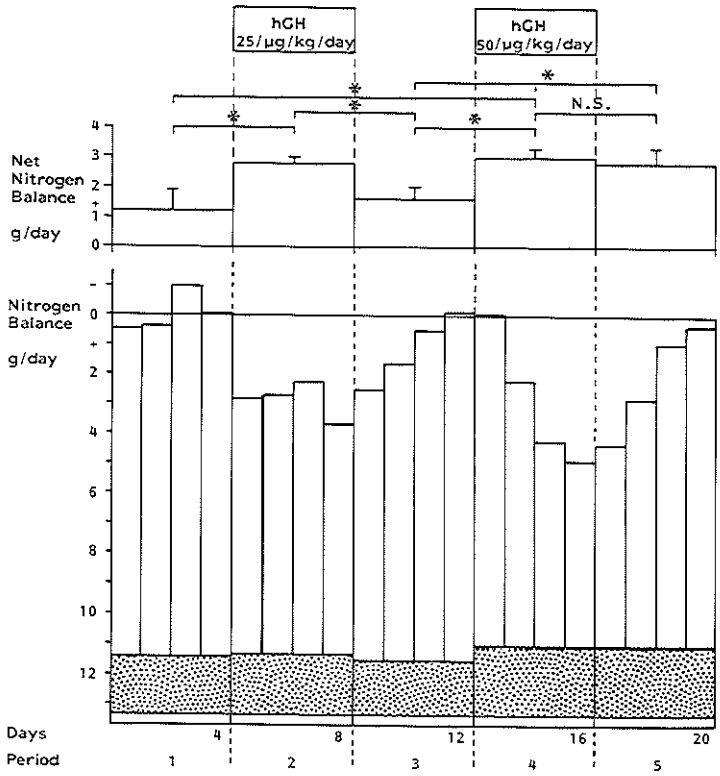

Figure 1: Upper panel: mean (+/- SE) net nitrogen balance of the four patients during the five treatment periods.(* = $p<0.01$, N.S.= not significant) Lower panel: nitrogen balance in patient 3. The daily N intake in this patient was 12.8 g. Integumental N loss was estimated to be 0.5 g/day. Dotted area: average daily fecal N loss in each four day period. Vertical open bars: daily urinary N loss. To convert to mmol/day multiply by 71.4.

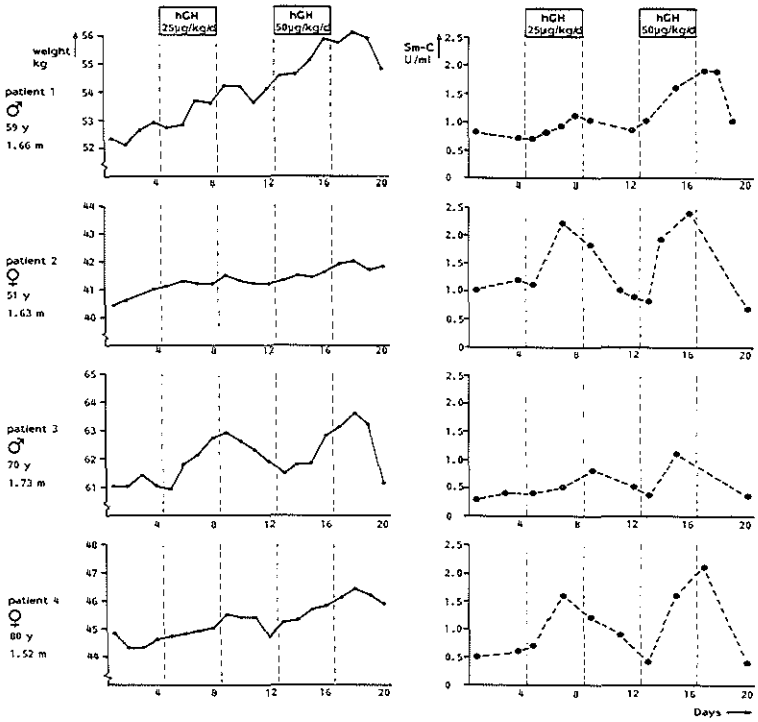

Figure 2: Left panel: Sex, age (years), length (cm) and body weights (kg) and ,right panel, plasma Sm-C (IGF-I) levels measured in four patients treated with GH during a 20 day study period. (normal adult plasma Sm-C (IGF-I): 0.3-2.2 U/ml).

3). Significant phosphorus retention and/or decreased excretion occurred during GH administration, at both dosages. This retention diminished abruptly after cessation of the lower but not after cessation of the higher GH dosage (See Fig 3 for group means and as an example the balance of patient 2). Calcium balance was negative during the entire study period in all four patients and tended to become more negative during GH administration although group means did not reach significance (data not shown).
In all patients fasting blood glucose levels remained normal (< 6.0 mmol/l). At 2200 h glucose levels were normal or slightly elevated to a maximum of 5.9 mmol/l on day 6 in patient 1, 6.5 mmol/l on day 14 in patient 2, 7.2 mmol/l on day 15 in patient 3, and 10.7 mmol/l on day 15 in patient 4. Post-breakfast plasma insulin levels did not change during the study. One patient (no 3) had slight glucosuria twice before GH was given (17 mmol/24h on day 3 and 4) and twice in period 2 (90 mmol/24h on day 7 and 28 mmol/24h on day 8). Since no glucosuria occurred during period 4 we consider the glucosuria not to be hGH-related. No patient developed ketonuria.

DISCUSSION

Plasma GH and IGF-I levels decrease with age (14,15). Elderly adults with weight loss due to illness or surgery might therefore benefit from the anabolic effects of exogenously administered GH. This study was undertaken to determine if low dosages of GH stimulated nitrogen retention without harmful side effects on carbohydrate and fluid regulation.
We found that a low dose (25 ug/kg/d) of GH induced a significant increase in nitrogen retention (from 1.2 g/d (85.7 mmol/d) during period 1 to 2.8 g/d (199.9 mmol/d) during period 2) in elderly patients with recent weight loss despite the fact that they received an optimal diet + 20%. Interestingly, a higher dose of 50 ug/kg/d of GH did not exert a more pronounced effect on nitrogen retention, (which increased from 1.6 g/d (114.2 mmol/d) in period 3 to 3.0 g/d (214.2 mmol/d) in period 4), although at this dosage a prolonged effect on nitrogen retention appeared to occur. Ward et al, using 100 ug GH/kg/d for 6 days in postoperative patients receiving only 400 kcal/day, found a decrease in cumulative nitrogen excretion from 42.7 +/- 3.1 in the control group to 31.5 +/- 2.4 g nitrogen in the treated group, indicating an average nitrogen retention of 1.9 g/d (135.7 mmol/d) (9). Manson et al gave normal subjects eating a low caloric diet with adequate amounts of nitrogen 10 mg/day or approximately 140 ug GH/kg/d, for 7 days. In these individuals about 3.8 g/d (271.3 mmol/d) nitrogen was retained (6). It should be noted that in our study, as opposed to those other studies, the patients received a more than adequate diet and yet, despite the sufficient nitrogen and caloric intake, low dose GH had a marked effect. An increase in nitrogen retention of 1.6 g/d (114.2 mmol/d) accounts for

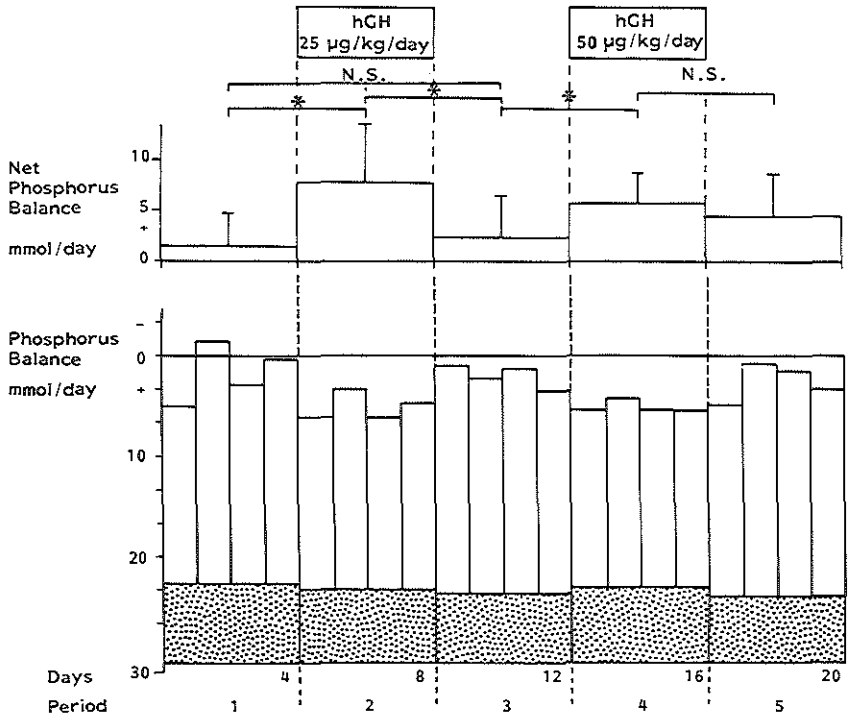

Figure 3: Upper panel: Mean (+/- SE) net phosphorus balance of the four patients during the five treatment periods.(* = $p<0.01$, N.S.= not significant). Lower panel: Phosphorus balance in patient 2. Daily P intake 30 mmol. Dotted area: average fecal loss in each 4 day period, vertical bars: daily urinary loss.

approximately 10 g protein, which represents about 50 grams of muscle (16).
Concerning the possible adverse side effects of high dose GH on glucose metabolism and water retention, fasting glucose levels did not increase, nor did blood glucose or plasma insulin levels change after normal breakfast. Only late in the evening (2200 h) did slight hyperglycemia occur in one patient, without the occurrence of glucosuria. However, even at these low GH dosages, sudden weight changes did occur, which were probably caused by water retention. Consistent sodium retention did not occur at the low GH dose, the higher dose caused sodium retention in 3 patients. Earlier workers demonstrated sodium-retaining effects of GH in rats (17,18) and in man (4,19-21), whereas others found opposite results in rats (22,23), dogs (24) and humans (5,23). More recently water retention was reported during administration of biosynthetic methionyl-hGH to normal subjects (6). GH stimulates Na-K ATPase activity in several tissues including the kidney (25). Therefore direct stimulation of tubular reabsorption of sodium is possible. In accordance with the reports of others (17,19,20), potassium and phosphorus retention increased during GH treatment. On average the increases in potassium and phosphorus retention were proportional to the increase in nitrogen retention, which might indicate that both were used for muscle protein synthesis (muscle contains approximately 3 mmol potassium and 2 mmol phosphorus per gram nitrogen, (16)).
GH administration increased plasma IGF-I levels, as reported in several studies (9,26,27). Even the GH dosage of 50 ug/kg/day raised plasma IGF-I levels to the upper limit of normal. At 25 ug/kg/day the IGF-I response was smaller in two patients, the cause of which is unknown. We cannot draw conclusions concerning the question whether nitrogen retention is a direct effect of GH itself or whether it is mediated by IGF-I. The anabolic effect appears to last for several days after cessation of GH administration. Therefore it might be possible to decrease the GH dosage even further, or to administer GH every two or three days rather then daily, thereby decreasing the frequency and magnitude of adverse effects, while retaining the anabolic effects. Indeed Clemmons et al found that alternate day GH treatment does promote nitrogen retention in obese adults (28).
In conclusion, even with a more than adequate nutritional intake, relatively low amounts of GH cause considerable nitrogen retention in underweight adults, and the nitrogen retaining effect appears to last for several days after GH administration was discontinued. Other treatment regimens using even lower dosages of GH or alternate day administration should be investigated in order to determine if they can promote nitrogen retention with fewer side effects.

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CHAPTER 2.2.

GROWTH HORMONE ADMINISTRATION IN ADULTS: MINIMAL DOSE TO INDUCE NITROGEN RETENTION

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Submitted

SUMMARY

In a previous study we showed that the administration of 25 and 50 ug/kg/day of growth hormone (GH) induced a similar anabolic effect in elderly men with recent weight loss, if GH administration was accompanied by optimal enteral feeding. In the present study we compared the effect of two GH dosages, 12.5 and 25 ug/kg/day for four days on nitrogen balance. The results show that GH administration in a dose of 25 ug/kg/day is the lowest dose in these four patients which induces nitrogen retention and that this dose did not induce carbohydrate intolerance or significantly increased insulin levels.
GH in a dose of 25 ug/kg/day in combination with optimal enteral feeding appears the optimal dose to induce nitrogen retention in adults.

INTRODUCTION

Several reports in the sixties and seventies indicated possible beneficial effects of human GH administration in adult surgical and burn patients (1-5). The availability of synthetic GH was followed by renewed interest about the possible clinical use as an anabolic agent, in surgical (6,7), obese (8-10), and prednisone treated (11) patients, as well as in elderly adults with malnutrition and chronic obstructive pulmonary disease (12), and/or recent weight loss (13). Several, possibly dose-related, adverse side effects have been noted, in particular raised blood glucose and insulin levels (6-10), as well as fluid retention (6,9,10). In these studies GH was administered daily (6,7,10,11) or every other day (8,9) in a dosage of 100-140 ug GH/kg to patients on normal or energy restricted diets, but in recent studies also a lower amount of 30 ug/kg GH per day or every other day was used (12,14). Our previous work indicated that GH in a dosage as low as 25 ug/kg in combination with optimal enteral nutrition (120% of the recommended daily amounts of protein and energy) induces similar nitrogen (N) retention, when compared with the previous high-dose GH studies, without significant disturbances in carbohydrate metabolism (13). In a recent study by Marcus et al (15) GH in a dose of 30 ug/kg/day was shown to have a significant N retaining (measured as a decrease in urinary N excretion) effect which was smaller than the N retention induced by 120 ug GH/kg/day, when administered for 7 days to healthy elderly people. At the lower GH dose no changes in glucose but an increase in insulin levels were observed, while at the higher GH dose both glucose and insulin levels increased significantly.
In order to further investigate the lowest effective dose of GH we compared the effects on N balance of 12.5 vs 25 ug GH/kg/day in combination with an optimal enteral diet, in four adults with a non-malignant illness.

SUBJECTS AND METHODS

Subjects

Two men (aged 71 and 54) and two women (aged 50 and 63) were studied. They required enteral nutritional support for chronic or recent weight loss of 2-30 kg in recent months, brought about by non-malignant illness. These patients had no evidence of malignant, renal or hepatic disease or diabetes mellitus.

Body mass index was considerably lowered in two and low normal in the other two patients. Their serum albumin levels were normal. The aims and methods of the study were explained to the patients and informed consent was obtained before the study, which was approved by the Medical Ethics Committee of the University Hospital Dijkzigt-Rotterdam.

Protocol

The patients were admitted to the Metabolic Ward for a period of 24 days. During the entire study period they were given (enterally) a mixed diet containing 120% of the recommended amounts of protein (16) and energy (17) for a person of their age, height and weight. The individual diets were prepared in accordance with the wishes of the patients and were weighed and frozen before admission of the patients to the hospital. The patients own accounts and visual confirmation by the ward staff were used to check completeness of food intake. Two diets were diluted with distilled water and homogenized for measurements of nitrogen (N) and Na contents.

The first three days after admission were used to achieve metabolic balance (these days are not included in the figures, the fourth day was day 1 of the study). From day 5 till 8 the patients received in addition to their diet, 12.5 ug GH/kg BW/day, s.c. at 09.00 h (period 2) and from day 13 till 16 25 ug GH/kg/d. GH (Humatrop) was supplied by Eli Lilly Co (Indianapolis, IN).

The following measurements were made daily at 0800 h: weight, blood pressure and pulse rate. On days 1, 4, 9, 12, 17 and 20 routine hematological and clinical chemical parameters were determined, as were blood glucose and plasma insulin levels before and 30,60,90,120,150 and 180 min after standardized breakfast.

Every day plasma insulin-like growth factor-I (IGF-I) was measured as were blood glucose levels fasting and at 2200 h.

All urine was collected in 24-h samples. In each sample N, creatinine, Na and glucose were measured quantitatively and ketone bodies were measured qualitatively. Feces was collected in 4-day periods from day 1 onward. The 4 day-periods were demarcated by the administration of 500 mg carmine red, while the feces volume was checked by the daily administration of 1200 mg polyethylene glycol. Fecal N and Na were measured in each pooled 4-day collection.

Methods

Routine methods were used for the hematological and chemical measurements in blood. Urinary, fecal and dietary N 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. N balance was calculated by subtracting urinary, fecal and integumental N excretion from the dietary intake. Integumental N loss was estimated to be 0.5 g/day (18). Insulin levels were determined by RIA in EDTA plasma using kits from Incstar Corp (Stillwater, MN). The intraasay coefficient of variation (cv) was 4.2%, the interassay cv was 10%. IGF-I was measured by RIA in EDTA plasma using kits obtained from the Nichol's Institute of Diagnostics (San Juan Capristano, CA) (19). Normal values were 0.34-1.9 U/ml in men and 0.45-2.2 U/ml in women. The intraassay cv was 7.2% and the interassay cv was 12.8%. The statistical significance of the differences between mean values was determined using analysis of variance. When significant overall effects were obtained by analysis of variance, multiple comparisons were made with the Newman-Keuls test. $P<0.05$ was considered significant.

RESULTS

GH was well tolerated by all four patients. No changes in kidney or liver function tests, electrolytes, cholesterol, triglycerides, total protein, albumin or leucocyte count occurred during the study period.

The effects of GH administration on body weight and plasma IGF-I levels in the four patients are depicted in Figure 1. Weight changes during both GH treatment periods were minimal in 3 patients (Fig 1, left panel). In patient 3 a GH-induced (and partially reversible) weight gain occurred which might be attributed to fluid retention. In this patient no oedema was found at physical examination, however. Sodium balance showed a tendency for increased sodium retention during both treatment periods in all patients, though the changes did not reach significance (data not shown). Plasma IGF-I levels remained unchanged in patient 1. In patient 2 IGF-I levels increased slightly during GH treatment, while in patients 3 and 4 a more marked increase in IGF-I levels was observed, which was highest during the second period of (higher) GH administration (Fig 1, right panel).

Figure 2 shows the effect of GH administration on nitrogen balance: for the group as a whole significant nitrogen retention occurred only at the higher GH dose of 25 ug/kg/day.

During both GH treatment periods blood glucose levels measured after standardized breakfast remained unchanged in all patients, while insulin levels showed a tendency to increase, not reaching statistical significance (data not shown). However, in patient

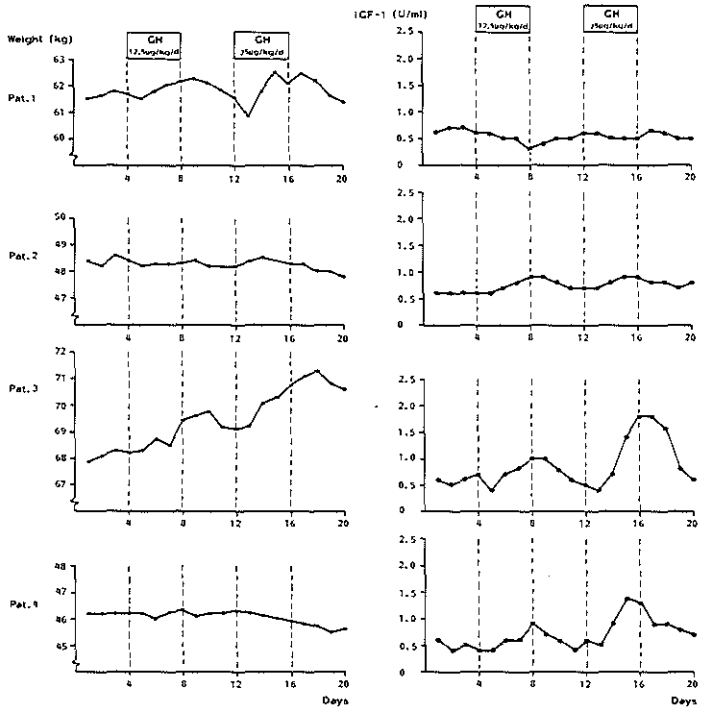

Figure 1: Left panel: Body weights (kg) and, right panel: plasma IGF-I levels measured in four patients treated with GH during a 20 day study period. (Normal IGF-I: 0,3-2.2 U/ml).

Period	1	2 GH 12.5 ug/kg/d	3	4 GH 25 ug/kg/d	5
Patient					
1	-0.45	1.64	1.70	2.72	1.72
2	-0.58	0.23	-0.18	1.40	0.66
3	2.16	2.94	3.87	7.06	1.77
4	-0.70	-0.64	-1.07	-0.06	-1.09
Mean	0.11	1.04	1.08	2.78*	0.77
SEM	0.99	0.90	0.86	1.20	0.26

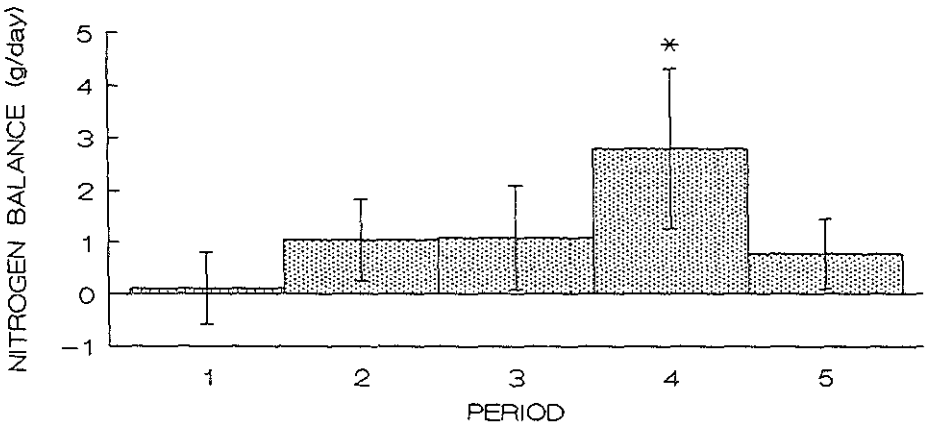

Figure 2: Nitrogen balance in the individual patients and mean ± SEM in each treatment period. Each period consists of 4 days. GH dose in period 2: 12.5 ug/kg/d, in period 4: 25 ug/kg/d. Periods 1, 3 and 5: control periods. *: $p<0.05$

3 and 4 both GH treatment periods induced carbohydrate intolerance with blood glucose levels at 22.00 h of 7.9 and 8.6 mmol/l on day 8 and 8.4 and 8.3 mmol/l on day 16, respectively. No patient developed glucosuria or ketonuria.

DISCUSSION

GH treatment in adults with (recent) catabolism has been shown to reduce catabolism/induce anabolism. However the optimal dosage of GH which induces these positive effects without adverse side effects (and at reasonable cost) has not been established. In our previous report (13) we demonstrated that 25 ug GH/kg/d was equipotent to 50 ug/kg/d in its power to induce nitrogen retention, while this low dose had fewer side effects. The results from the present study show that a further decreased GH dosage (to 12.5 ug/kg/d) does not result in a significant nitrogen retention any more. In agreement with our previous results we observed a pronounced interindividual variation in the reaction of body weight and IGF-I levels to GH administration. In contrast to our previous results we observed in two of the present patients at the GH dosage of 25 ug/kg/d the induction of an, albeit minor, glucose intolerance. One patient had a marked increase in weight, without the development of overt oedema. Disappointingly even the lowest GH dose tended to induce sodium retention during this short-term study.

In contrast to our short term study in adults with optimal enteral nutrition, a report by Snyder et al (10) showed that the anabolic effect of growth hormone in obese diet-restricted subjects was optimal when a dosage of 100 ug/kg/d was used. However this dose caused fluid retention amounting to about 2.5 kg, as well as significant disturbances in carbohydrate metabolism, even in severe diet restricted subjects.

In conclusion we show that in adult patients given more than adequate enteral feeding (120% of the recommended daily requirements of calories and protein), 25 ug GH/kg/day is the lowest dose which induces anabolism as measured by the nitrogen balance technique. In our previous study we showed that the nitrogen retaining effects of 25 and 50 ug GH/kg/day were similar. This suggests that a daily dose of 25 ug/kg of GH is the optimal dose to induce anabolism in patients during optimal enteral nutrition, with the least adverse effects on carbohydrate and fluid homeostasis. It has to be stressed, however, that the considerable interindividual variation in the response to GH administration noted in our study, as well as the fact that only four patients were investigated limits the value of our conclusions, making them preliminary.

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CHAPTER 3

THE EFFECT OF HUMAN GROWTH HORMONE ADMINISTRATION ON TUMOR GROWTH, BODY WEIGHT AND CIRCULATING INSULIN-LIKE GROWTH FACTOR I LEVELS OF RATS BEARING A TRANSPLANTABLE RAT PITUITARY TUMOR (7315b)

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SUMMARY

The direct effects of human GH and IGF-I on PRL secretion and cell proliferation were studied on PRL secreting rat pituitary tumor 7315b cells in vitro, as well as the effects in vivo of human GH administration on body weight, IGF-I levels and tumor size in rats bearing this transplantable tumor. In the in vitro studies IGF-I levels above 5 nM stimulated PRL release in a dose-dependent manner while GH, in concentrations of 0.23-45 nM, did not affect PRL release. Cell proliferation was stimulated by IGF-I in a dose dependent manner from 0.5 nM onwards, while GH did not have an effect. The in vivo studies showed that 1 mg GH/rat/day reversed tumor-induced cachexia and normalized the suppressed IGF-I levels without stimulating tumor growth. It is concluded that tumor-induced cachexia can be prevented or reversed by exogenous GH administration without an increase in tumor mass, even if a tumor model is used whose cultured tumor cells respond to exposure to IGF-I with a mitotic response.

INTRODUCTION

In animals ,as well as in man, growth hormone (GH) exerts a wide variety of effects. Most of its growth-promoting actions are mediated via the formation of Insulin-like Growth Factor I (IGF-I), while it also exerts a direct lipolytic action (1). Knowledge about the effects and possible therapeutic uses of GH in adults has been limited by the lack of available supplies. Therefore during the past decades its use has mainly been restricted to the treatment of children with short stature.

After human GH, which is identical and bioequivalent to endogenous GH, has been produced by recombinant DNA technology, new reports concerning the usefulness of GH therapy in adults with various catabolic disorders (trauma, surgery) have been published (2-5). To date no studies have been carried out on the potential beneficial anabolic effects of GH therapy in cancer patients.

Cancer-induced cachexia diminishes the quality of live significantly and may be attenuated by GH therapy. The amount of weight loss in cancer patients is positively correlated with the overall mortality. In addition improvement of nutritional status may reduce chemotherapy related toxicity, as has recently been shown in a rat model (6). The usefulness of (par)enteral (hyper)alimentation in the management of cancer patients is controversial (7-10).

Controversy exists also concerning the possible carcinogenic and/or tumor growth-stimulating effects of GH treatment in humans with GH deficiency, either direct or mediated by circulating or locally produced IGF-I (11-13). According to some investigators the incidence of neoplasms in acromegaly is increased (14-17), though others did not observe this relation (18-19)

In the present study we investigated the direct effects of human GH and IGF-I on Prolactin (PRL) secreting rat pituitary tumor 7315b cells in vitro, as well as the effects of human GH administration to rats bearing this transplantable rat pituitary tumor 7315b. In the in vitro experiments the effects on cellular DNA content and PRL secretion were investigated, while in the in vivo experiments changes in tumor size, body weight, plasma IGF-I, PRL and GH levels were studied.

METHODS

In vitro tumor cell experiments.

Female Buffalo rats (R.B.I., Rijswijk, the Netherlands), weighing 150-170 g, were kept in an artificially illuminated room (08.30-20.30 h) with food and water ad libitum. The animals were inoculated subcutaneously between the scapulae with a cell suspension of the transplantable, PRL secreting 7315b rat pituitary tumor as described in detail elsewhere (20). Three to four weeks after inoculation of the tumor cell suspension a tumor of approximately 20 cm^2 has grown on the back of the animals. At this moment the animals were killed by an overdose of ether anaesthesia and the tumor was carefully removed and collected in a sterile saline solution (9 g/l NaCl).

7315b pituitary tumor cells were isolated by mechanical dispersion. The isolated tumor was washed twice with calcium-, magnesium- free Hank's Balanced Salt Solution (HBBS) supplemented with 1% human serum albumin (HSA), penicillin (10^5 U/l), streptomycin (100 ug/l), fungizone (0.5 mg/l) and sodium bicarbonate (0.4 g/l final concentration). The capsula of the tumor was carefully removed, after which the tumor was minced into small pieces. The remaining suspension of tumor tissue was gently vortexed for 30 seconds. After vortexing the suspension was centrifuged at 600 g for 5 min and the pellet was washed twice with HBSS+HSA. The remaining pellet was resuspended in HBSS+HSA and the suspension was filtered over a nylon gauze. In order to separate vital from non-vital cells the suspension was layered on Ficoll-Isopaque (density 1.077 g/ml; prepared by the Dijkzigt Hospital Pharmacy, Rotterdam, the Netherlands) and centrifuged at 500 g for 20 min. The interphase containing vital cells was collected and washed twice with HBSS+HSS. Finally, the cells were resuspended in culture medium.

The culture medium used in all experiments consisted of Minimal Essential Medium with Earle's salts (MEM) supplemented with MEM non-essential amino acids, sodium pyruvate (1 mmol/l), 10% fetal calf serum, penicillin (10^5 U/l), streptomycin (100 ug/l), fungizone (0.5 mg/l), L-glutamine (2 mmol/l) and sodium bicarbonate (2.2 g/l final concentration). The medium was adjusted to pH 7.4 with 1 mol/l NaOH. The 7315b pituitary tumor cells were seeded at a density of 20.000 cells per well in 1 ml of culture medium in 24 well plates (Costar, Cambridge, Mass, USA) without or with IGF-I or GH.

After 6 days of culture the media and cells were collected and stored at -20 C until analysis. Medium and supplements were purchased from Grand Island Biological Co. Europe (Paisly, Scotland). IGF-I was obtained from Bachem (Bachem Feinchemicalien ASG, Bubendorf, Switzerland) and human recombinant growth hormone (GH, Humatrope) from Eli Lilly & Co, Indianapolis.
Rat PRL concentrations in the culture media were measured by a double antibody RIA using materials and protocols supplied by the distribution officer of the NIADDK. All results are expressed in rat prolactin reference preparation-1 (RP-1).
The DNA content of the tumor cells was determined as described in detail elsewhere (21). The method is based on a DNA dependent fluorescence enhancement of a fluorochrome. In short, cultured tumor cells, which did not attach to the floor of the wells, were collected at the end of the incubation period and washed twice with an ice-cold saline solution. The remaining cell pellet was stored at -20 C until analysis. The cells were extracted with 300 uL ammonia solution (1 mol/l) + Triton x 100 (0.2% v/v) by sonification during 5 seconds at amplitude 15 (Soniprep 150; MSE). Thereafter 2 ml assay buffer (100 mmol/l NaCl, 10 mmol/l EDTA, 10 mmol/l Tris; pH 7.0) was added. The remaining solution was centrifuged at 2000 g during 5 min and 100 uL aliquots of the supernatant were mixed with 1.5 ml Hoechst dye H33258 (100 ng/ml). Fluorescence was measured after 15 min with the exication and emission wavelengths set at 350 nmol/l and 455 nmol/l respectively. Fluorescence of experimental samples was referenced to a standard curve of calf thymus DNA (type II, no D-3636; Sigma Chemical Company, StLouis, Mo, USA), All data are expressed as means +/- SE.

In vivo tumor experiments.
In two experiments the effect of GH administration on the growth of the 7315b tumor and on reversal (experiment 1) or prevention (experiment 2) of tumor-induced cachexia was evaluated. Tumor growth was evaluated by expressing tumor size in centimetres squared (maximum length x maximum width) which has been shown to be closely correlated with tumor weight (22). In the first experiment the effects of administration of 1 mg GH/rat/day (Humatrope, Eli Lilly & Co, Indianapolis) subcutaneously in 0.25 ml diluent starting on day 13 after tumor implantation until the end of the experiment (day 23) were evaluated, while in the second experiment the effects of GH administration for 15 days starting on the day of inoculation were evaluated. In both experiments tumor bearing controls received daily injections of diluent only while in the second experiment the effects of either GH or diluent was evaluated in non tumor bearing controls as well. Each group of animals consisted of 6 rats.
Plasma IGF-I levels were measured in EDTA plasma obtained from the tail vein during the experiment or after decapitation at the end of the experiment. The commercial kit for the determination of IGF-I from the Nichol's Institute of Diagnostics (San Juan Capistrano, CA, USA) was used. The intra-assay cv was 7.2 and the inter-assay cv

12.8%. PRL was determined in the same samples by double antibody RIA as described above.

Statistical evaluation

Statistical analysis was done by analysis of variance, followed by Duncan's test for determining the differences between control and experimental groups. In the in vivo experiments changes in body weight were evaluated by Students unpaired t-test.

RESULTS

The effects of IGF-I and GH on PRL secretion and DNA content of cultured 7315b tumor cells

The 7315b tumor cells were cultured for a period of 6 days. The effects of a wide concentration range of IGF-I (0.5-50 nmol/l) and GH (0.23-45 nmol/l) were investigated (fig 1). Low concentrations of 0.5 and 1 nmol/l IGF-I did not affect PRL release, while 5,10 and 50 nmol/l IGF-I stimulated PRL release in a dose dependent manner (5 nmol/l vs control: $p<0.01$; 10 vs 5 nmol/l: $p<0.05$; 50 vs 10 nmol/l: $p<0.05$). A low concentration of IGF-I (0.5 nmol/l) stimulated the DNA content of the cells after 6 days by 12% ($p<0.05$ vs control), while higher concentrations of IGF-I stimulated the DNA content in a dose dependent manner, 10 nmol/l being the maximal stimulatory concentration (stimulation by 1, 5, 10 and 50 nmol/l IGF-I being 24, 37, 50 and 55% respectively, 1 and 5 vs 0.5 nmol/l: $p<0.05$; 10 and 50 vs 1 nmol/l: $p<0.01$). We also measured the IGF-I concentration of the culture medium used in these experiments: the final IGF-I concentration of the medium to which the control cells were exposed amounted to 0.2 nmol/l.

Human GH in a concentration between 0,23 and 45 nmol/l did not affect PRL release, while it did also not influence the DNA content of the tumor cells after 6 days of culture (fig 1).

The effect of the administration of human GH in vivo on 7315b tumor growth, body and organ weights, serum PRL and IGF-I levels in rats.

The daily, subcutaneous administration of a pharmacological dose of 1 mg GH/rat per day for 10-15 days was investigated on 7315b tumor growth in two separate experiments.

In the first experiment GH was injected daily from day 13 till 23 after tumor implantation. GH administration did not affect pituitary tumor growth: tumor size (as expressed in cm^2) did not differ from that found in tumor bearing animals which received the diluent only (fig 2). In contrast, however, the GH treated tumor bearing

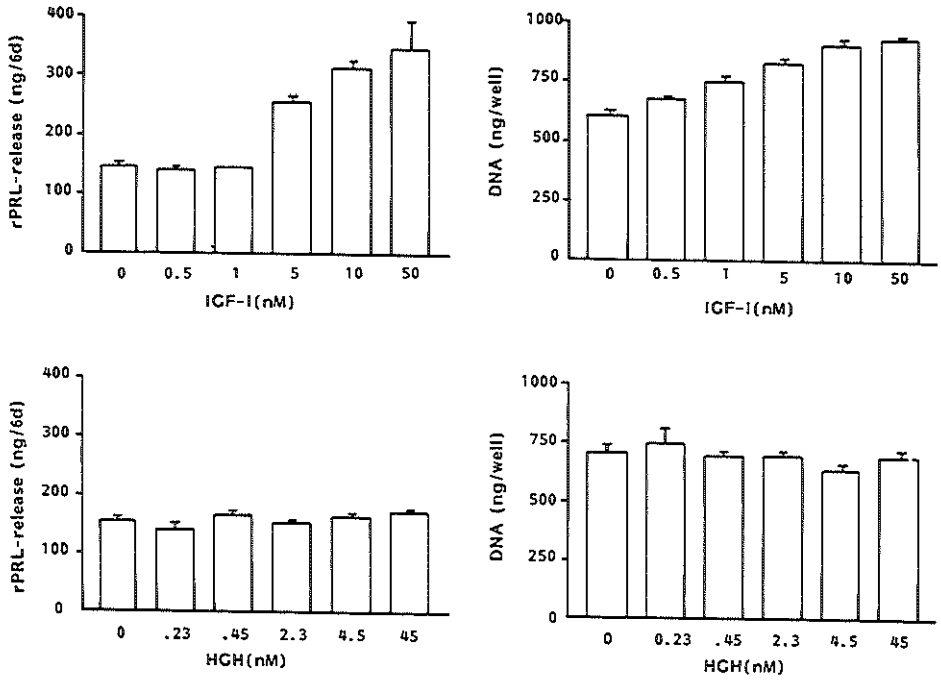

Figure 1: The effects of increasing concentrations of IGF-I (upper panels) and GH (lower panels) on rat PRL release (ng/6 days) and DNA content (ng/well) of 7315b tumor cells cultured for 6 days.

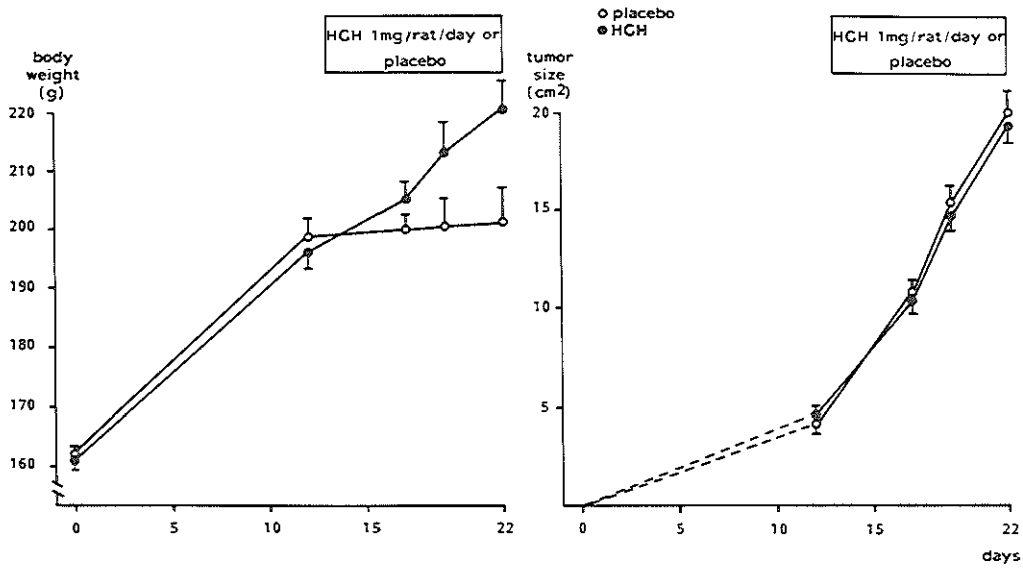

Figure 2: The effects on body weight (left panel; g) and tumor size (right panel; cm^2) of the administration of human GH (1 mg/rat/day) or placebo for 10 days to rats bearing the transplantable rat pituitary tumor 7315b starting on day 13 after tumor implantation. N=6 for each group; mean $\pm$ SEM.

rats had gained weight considerably. In fig 2 it is shown that during the 10 day placebo treatment period tumor bearing control animals gained 4.5 ± 6 g (mean ± SEM) in weight. However, the GH treated tumor bearing rats gained 24.3 ± 1 g. After deduction of the mean tumor weight (35.1 ± 5 g in the placebo treated and 40.5 ± 2 g in the GH treated animals; NS) the weight of the placebo treated animals amounted to 166 ± 6 g, indicating a mean body weight gain of only 3 ± 2 g, in comparison with the mean body weight prior to tumor implantation. The actual mean body weight of the GH treated group at the end of the experiment amounted to 180 ± 4 g (220.5 ± 5.4 minus 40.5 ± 2). Therefore the GH treated animals had gained 18 ± 2 g in weight during the 22 day investigational period. This weight gain did not differ from that observed in non-tumor bearing controls (starting weight 161 ± 2.8, final weight 175.3 ± 3.7) but was higher than that seen in the tumor bearing control rats ($p<0.01$).

In comparison with non-tumor bearing controls plasma IGF-I levels were slightly lowered on day 12 after tumor implantation (NS, table 1). This decrease was statistical significant in the tumor bearing controls 18 and 22 days after tumor implantation ($p<0.05$) , while GH treatment in tumor bearing animals resulted in a significant stimulation of IGF-I to levels which were comparable with those observed in non-tumor bearing controls (both on day 18 and 22: $p<0.05$ vs tumor bearing controls).

Table 1: *The effect of GH (1 mg/rat/day) or placebo administration on the total plasma IGF-I concentration (in nmol/l) of rats with the pituitary tumor 7315b, for ten days starting on day 12 after tumor implantation (mean ± SEM; n=6 per group). a:P<0.05 vs control non tumor; b:P<0.05 vs control tumor.*

	IGF-I (nmol/l)		
	day 12	18	22
control non tumor	25.3 +/- 1.6	23.6 +/- 1.0	27.2 +/- 2.4
control tumor	23.1 +/- 1.7	17.2 +/- 1.1[a]	19.7 +/- 0.9[a]
tumor + GH	23.2 +/- 0.8	27.7 +/- 1.7[b]	28.5 +/- 0.7[b]

Prolactin levels in the GH treated tumor bearing group did not differ significantly from the placebo treated tumor bearing animals (1133 ± 156 vs 771 ± 163 ng/ml). As to be expected after implantation of a PRL secreting tumor, PRL levels in both tumor bearing groups were significantly higher compared with the non tumor bearing controls (81 ± 32).

In the second experiment GH administration was started on the day of tumor implantation and continued for 15 days. Again no significant effect of GH administration

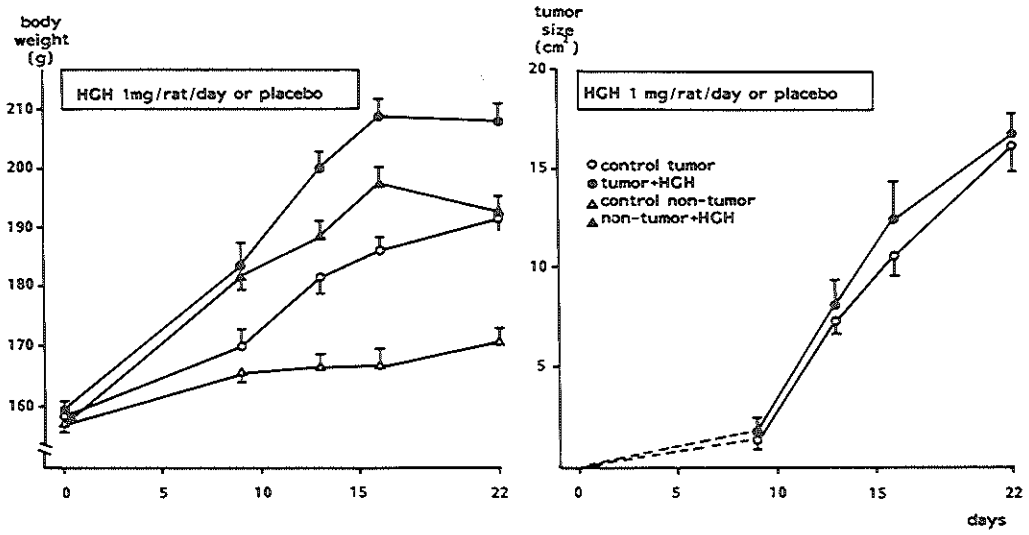

Figure 3: The effects on body weight (left panel; g) and tumor size (right panel; cm^2) of the administration of human GH (1 mg/rat/day) or placebo for 15 days to rats with or without the transplantable rat pituitary tumor 7315b implanted on day 1. N=6 for each group; mean ± SEM.

on tumor growth was observed (fig 3). GH exerted powerful stimulatory effects on body growth both in the non-tumor and in the tumor bearing animals. The placebo treated non-tumor bearing rats gained 14 ± 2 g in weight, while in the GH treated control group this amounted to 33 ± 3 g ($p<0.01$). The tumor bearing placebo treated rats gained 34 ± 4 g in weight, but after deduction of tumor weight (26 ± 4 g) net weight gain was only 8 ± 2 g. Growth hormone administration to tumor bearing animals resulted in a total weight gain of 49 ± 4 g; after deduction of tumor weight (29 ± 7): 20 ± 2 g ($p<0.01$ vs tumor bearing-placebo treated rats).

After 15 days of GH treatment plasma IGF-I levels in the GH treated non-tumor bearing rats were significantly elevated compared with the placebo treated non-tumor bearing animals ($p<0.05$; table 2). The consequences of tumor implantation on IGF-I levels again became evident: both on day 16 and 22 they were significantly suppressed in comparison with the placebo treated non-tumor bearing rats ($p<0.05$ in both instances). Administration of GH to tumor bearing rats resulted already on day 9 but also on day 16 in a stimulation of IGF-I levels, which were significantly higher than in the placebo treated tumor bearing animals ($p<0.05$). These levels were, both on day 9 and 16, similar to those observed in GH treated non-tumor bearing rats, and on day 16 also higher than in placebo treated non-tumor bearing controls ($p<0.05$). On day 22 the stimulatory effects of GH administration in the first 15 day period had ceased and IGF-I levels had indeed decreased to levels comparable with those in the placebo treated tumor bearing group. PRL levels in the GH treated tumor bearing group again did not differ significantly from the placebo treated tumor bearing rats (618 ± 195 vs 520 ± 103 ng/ml).

Table 2: *The effect of GH (1 mg/rat/day) or placebo administration on the total plasma IGF-I concentration (in nmol/l) of rats with the pituitary tumor 7315b, for 15 days starting on the day of tumor implantation (mean ± SEM; n=6 per group). a:P<0.05 vs non tumor-placebo; b:P<0.05 vs tumor-placebo.*

	IGF-I (nmol/l)		
day	9	16	22
non tumor-placebo	20.6 +/- 1.0	22.2 +/- 0.5	21.4 +/- 1.0
non tumor+GH	23.9 +/- 1.1	28.1 +/- 0.6[a]	25.0 +/- 1.0[a]
tumor-placebo	18.3 +/- 0.7	16.9 +/- 0.5[a]	14.1 +/- 1.1[a]
tumor+GH	23.8 +/- 1.1[b]	25.9 +/- 0.7[b]	15.8 +/- 1.1

PRL levels were in both tumor bearing groups were elevated compared with both non tumor bearing groups (35 $\pm$ 6 in the placebo treated and 26 $\pm$ 7 ng/ml in th GH treated rats).

DISCUSSION

Evidence is accumulating that IGF-I plays a role in tumorgenesis and tumor growth. In vitro investigation of several tumor cell lines demonstrate specific binding sites for IGF-I (23-31), increased binding to IGF-I receptors when compared with less dedifferentiated cell lines or with normal surrounding tissue (24,29-31), production of IGF-I by tumor cell lines or tissue (32,33) and increased DNA synthesis and cell growth in response to IGF-I (23,25,27-29,32,34-36).

From these observations one might derive strong arguments against the therapeutic use of GH to reduce catabolism and/or induce an anabolic state in patients with cancer-induced cachexia, since it might simultaneously stimulate growth of the cancer itself. In addition evidence was also presented from studies by Friesen's group that GH directly induces the expression of the c-myc oncogene in a lymphoma cell line (37) Preliminary results (J. Foekens, personal communication) from investigations into the presence of IGF-1 binding sites on the rat pituitary tumor cell line 7315b used in this study indicate indeed the presence of specific high affinity IGF-1 binding sites on this tumor.

The results of the in vitro studies are in accordance with similar studies in other cancer cell lines, in that it was shown that IGF-1 induces cell proliferation and protein (in this case PRL) secretion. GH itself did not induce either effect.

The results of the in vivo studies show that GH administration for 10 to 15 days reverses (first experiment) or prevents (second experiment) tumor-induced cachexia without stimulating tumor growth measured in cm^2 or PRL secretion. On weight basis we used 120-240 times the GH dosage used in human studies to induce an anabolic state (38).

Our results are in disagreement with the results of Svaninger et al (39), who demonstrated no improvement of body composition and muscle wasting (nor an increase in tumor growth) by GH administration in adult, non growing, sarcoma bearing mice, while in hypophysectomized rats GH administration stimulated body and tumor growth to a similar extent. In their sarcoma tumor model in intact animals GH levels were elevated from day 8 after tumor implantation, explained by the authors as a way to promote endogenous substrate mobilization. The exogenous GH dosage was only 100 ug/100 g BW/day (as opposed to our GH dose of 1 mg/rat, which corresponds to 600 ug/100 g BW/day) which was insufficient to stimulate growth in freely fed control mice. In their study no IGF-I levels were reported.

In our study the decrease of IGF-I levels in tumor bearing rats reflects the tumor induced cachexia (40-41) and was reversible by GH administration. IGF-I levels decreased to the low level observed in untreated tumor bearing rats within days after cessation of GH administration. It might be speculated that due to tumor-produced cytokines (like interleukins, tumor necrosis factor), a catabolic pathophysiologic condition occurs, perhaps both by a direct effect on peripheral tissues and indirectly by reducing GH and IGF-I levels. Exogenous GH appears to be capable to reverse these tumor induced effects.

In conclusion tumor-induced cachexia can be prevented or reversed by exogenous GH administration without an increase in tumor mass, even when a tumor model is used which contains specific binding sites for IGF-I and the cultured tumor cells respond to exposure to IGF-I with a mitotic response.

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CHAPTER 4

BODY COMPOSITION IN GROWTH HORMONE DEFICIENT ADULTS

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SUMMARY

Body composition in a group of GH deficient adults was compared with a control group, matched for age, sex, body height, body weight and body mass index, using bioelectrical impedance and deuteriumoxide dilution methods and hydrodensitometry. The body fat percentages of GH deficient adults were higher in spite of comparable weights, heights, age and body mass index. Body impedance was higher in the GH deficient adults, also after correction for differences in height and fat free mass. As a consequence, prediction formulas for body composition from body impedance, developed in normal subjects can not be applied to GH deficient subjects. The higher body impedance in the GH deficient subjects can be ascribed to a smaller amount of extracellular water in these subjects compared to the controls.

INTRODUCTION

At present growth hormone (GH) deficient adults (both GH deficient children who have reached their final height and adults who have developed GH deficiency as part of hypopituitarism) are not substituted with GH for two reasons. Firstly the limited supply of pituitary derived GH has restricted its use to children with short stature, and secondly GH deficient adults, if adequately substituted for other hormonal deficiencies (if present), have a normal life expectancy. Since recombinant DNA technology has increased GH availability, a more widespread use of GH has become feasible. Therefore the possible adverse effects of GH deficiency in adult life have become a matter of more than academic interest. Normally spontaneous and stimulated GH secretion continues into adult life, although it decreases with age (1-10).
GH has been shown to affect cardiac function (11,12), renal perfusion (13,14), the immune system (15,16), metabolic homeostasis (17), and body composition (18). Reduced social performance in adults with GH deficiency has been reported (19,20), albeit not by all investigators (21).
We report the results of an investigation of body composition by bioelectrical impedance analysis (BIA) and D_2O dilution measurements in a group of adults with GH deficiency in comparison with body composition measurements by BIA and hydrodensitometry in a group of age, sex, height and body weight matched (healthy) controls.

Patients and methods

The study group consisted of 13 subjects who were known with isolated GH deficiency or (incomplete) hypopituitarism for at least 2 years (Table 1). If previously treated with pituitary derived GH (patients 4,7,8 and 13) this substitution treatment had

been stopped at least 2 years before the study. In 11 of these 13 patients (partial) hypopituitarism was present, which was adequately substituted. Adrenal insufficiency, which was present in 10 patients, was treated with either cortisone acetate (15-37.5 mg/day, divided over 2-3 doses) or hydrocortisone (15-30 mg/day, divided over 2-3 doses). The absence of adrenal insufficiency in patients 2,4 and 5 had been confirmed with a metyrapone test. Insufficiency of the pituitary-thyroid axis was present in 10 patients, who all received substitution with L-thyroxine (75-175 ug daily). This had resulted in normal to high-normal free thyroxine levels in all patients. The male patients received either 250 mg testosterone-enantate intramuscularly once every 3 weeks (patients 3,6,7,8) or 80 mg testosterone-undecanoate 2-3 times daily (pat 1,12,13). The four female patients with hypogonadism all received cyclic estrogen/ progestagen substitution therapy. If present adrenal, thyroid, and/or gonadal insufficiencies had been treated for at least 2 years with continuous, unchanged medication. All patients were studied on an outpatient base. Although not generally accepted, we omitted therefore an insulin test to prove the presence of GH deficiency. The presence of GH deficiency in these 13 patients was confirmed however by the presence of a basal plasma IGF-I level of less than 0.5 U/ml in combination with a peak GH of less than 7.0 ug/l after stimulation with human Growth Hormone Releasing Hormone (GHRH, Somatobiss, Ethifarma, The Netherlands). After an overnight fast a catheter was inserted into an antecubital vein and blood was sampled for basal IGF-I and GH levels (t=-15 min). At t=0 min, 1 ug GHRH/kg body weight was injected intravenously after which blood was sampled for GH determination at t= 5, 10, 20, 30, 45, 60 and 90 minutes. IGF-I levels were determined in EDTA plasma using the commercial RIA kit from the Nichols Institute of Diagnostics (San Juan Capristano, Ca, USA). Normal ranges (between 18 and 65 yr) are 0.34-1.90 U/ml in men and 0.45-2.20 U/ml in women. These are based on 95% confidence intervals for a group of more than 100 controls. This assay has an intra- and inter-assay coefficient of variation (cv) 7.2 and 12.8 % respectively. Serum GH was measured using a commercially available IRMA from Eurodiagnostics (Apeldoorn, The Netherlands). The intra- and inter-assay cv were 10.2 and 11.1 % respectively.

In four patients (no 4,7,8 and 13) GH deficiency had already previously been demonstrated by the presence of an insufficient response of GH to an insulin-induced hypoglycaemia as well as to arginine-infusion.

Body composition was measured (in the study and control group) after an overnight fast by the tetrapolar bioelectrical impedance method as described previously (22-25) with a body composition analyzer (RJL-systems BIA-101 Detroit, MI, USA). Fat-free mass (FFM) was calculated from bioelectrical impedance (R) and body height (H, m) using the equation of Deurenberg ($FFM = 0.652 \times 10^4 \times H^2/R + 3.8 \times S$ (S= gender: male=1, female=0) +10.9; (26)), or Lukaski ($FFM = 0.838 \times 10^4 \times H^2/R + 4.2$; (23)). Additionally in the GH deficient group body composition (total body water, TBW) was

determined by D_2O dilution as described elsewhere (27,28). In short, after an overnight fast and insertion of a catheter into an antecubital vein blood was sampled and the patient was given, by mouth, a dose of 0.24 g 99.7% D_2O/kg body weight diluted in 200 ml H2O. After 150, 180 and 210 min 10 ml heparinized blood was withdrawn, and the plasma stored at -20 C until analysis. After sublimation, D_2O concentration was measured by infrared spectrophotometry and TBW was calculated. A correction of 4 % was made to compensate for exchange of deuterium with non-water compartments (29). FFM was calculated from TBW by dividing TBW by 0.73 (30). Body composition measurement by underwater weighing was not performed in the patients.

The controls consisted of 23 healthy adult volunteers matched with the study group for age, sex, body height and weight and derived from a much larger population of healthy subjects.

In the controls body composition was, apart from bioelectrical impedance, also measured densitometrically by underwater weighing (to the nearest 0.05 kg; 3826 MP 81 Sartorius, Gottingen, Germany) with simultaneous determination of the residual lung volume by helium dilution. Siri's formula (31) was used to calculate fat mass (FM) and FFM from total body density. The equipment has a precision of 1% (0.002 kg/l).

Aims and methods of the study were explained to patients and controls and informed consent was obtained before the study, which was approved by the Medical Ethics Committee of the University Hospital Dijkzigt-Rotterdam and the Ethical Committee of the Department of Human Nutrition, University of Wageningen.

Statistical analyses were performed with the SPSS PC-programm (1988). Analysis of (co-)variance techniques and Student t-tests were performed to test for differences between groups or for differences in variables within groups. Regression lines were tested for differences in slopes and/or intercepts by the method described by Kleinbaum et al (32). All values are expressed as mean ± SEM.

RESULTS

Table 1 shows some individual characteristics of the GH deficient patients. As can be seen in Table 2 the patient and control groups did not differ regarding sex distribution, age, height, weight and body mass index (kg/m^2). However percentage body fat was significantly higher in the group of GH-deficient patients. Also body impedance (R) was significantly higher in the patients ($P < 0.01$, see Fig 1), also after correcting (by analysis of covariance) for differences in FFM and height (data not shown). Fat free mass calculated with the formula's of Deurenberg (FFM-De) or Lukaski (FFM-Lu) was significantly lower ($P < 0.05$) in the patients in comparison with the controls. Similarly fat mass (FM) and body fat percentage in the patients was higher compared to the

Table 1: Patient characteristics.

PATIENT	SEX	AGE yr	HEIGHT cm	WEIGHT kg	GH DEFICIENCY CAUSE	GH DEFICIENCY DURATION yr	IGF-I U/ml	PEAK GH ug/l	HORMONE SUBSTITUT C/T/G	GH DOSIS ug/kg/d
1	F	46	137	61	a	20	0.2	1.7	+/+/+	12
2	F	32	165	92	b	15	0.1	1.1	+/+/+	16
3	F	27	145	65	a	12	0.2	3.1		6
4	M	32	182	90	b	14	0.1	2.3	+/+/+	16
5	M	34	161	47	a	11	0.3	4.2	+/+/+	25
6	M	25	176	94	b	12	0.2	3.2		15
7	F	33	153	56	b	11	0.2	2.9	/+/	18
8	F	28	164	56	b	12	0.1	0.7	+/+/+	25
Mean		32	160	70		13	0.2	2.4		17
SEM		2	5	6		1	0.0	0.4		2

a: idiopathic GH deficiency, b: after surgery for a craniopharyngeoma. Peak GH=maximal GH level after the iv injection of GHRH (1 ug/kg BW). C:(gluco)corticosteroids, T: L-thyroxine, G: gonadal steroids. Patients 2, 4 and 6 were treated with 1.48 mg (4 IU) of GH/day, which corresponded with a dose of 15-16 ug/kg/day.

Table 2: *Some physical characteristics for patients and controls.*

	Controls Mean ± SEM		Patients Mean ± SEM	
N (males/females)	23 (15/8)		13 (8/5)	
Weight (kg)	70.7	3.7	72.7	5.3
Height (m)	1.70	0.02	1.67	0.04
Body Mass Index (kg/m^2)	24.3	0.8	26.0	1.4
Age (years)	32.2	2.4	32.6	2.9
Impedance (Ohm)	474	14	602	34 *

*:P<0.01

Table 3: *Impedance, fat-free mass, fat mass and fat percentage in controls and patients calculated with the formula's of Lukaski (23) or Deurenberg (26).*

	Controls Mean ± SEM		Patients Mean ± SEM		P value
Impedance (Ohm)	474	14	602	34	<0.001
FFM-Lu (kg)	56.5	2.3	45.4	3.7	<0.05
FM-Lu (kg)	14.2	1.7	27.3	2.4	<0.001
Fat Percentage-Lu (%)	19.3	1.6	37.6	2.2	<0.001
FFM-De (kg)	54.1	2.1	45.3	3.2	<0.05
FM-De (kg)	16.6	1.8	27.4	2.8	<0.01
Fat Percentage-De (%)	22.6	1.5	37.1	2.3	<0.001

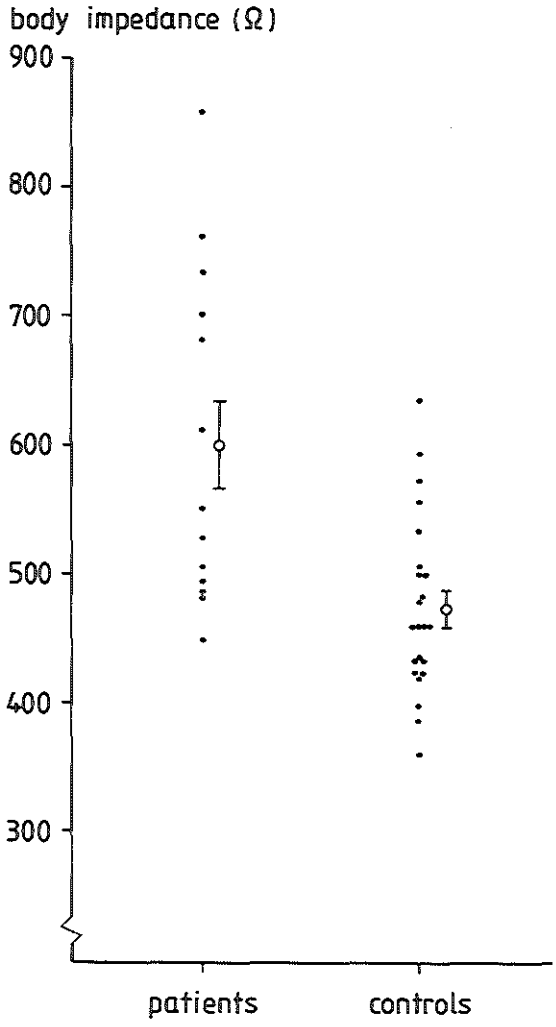

Figure 1: Impedance (Ohm) in the individual controls and patients as well as mean ± SEM for both groups.

controls (Table 3).
In the GH deficient patients FFM calculated from total body water was markedly lower than FFM derived from hydrodensitometry in the control subjects, but the difference did not reach statistical significance (P= 0.20). However, the fat mass (FM) and the body fat percentage were significantly higher in the GH deficient patients compared with the control subjects (P= 0.012 and P= 0.001 respectively; Table 4).

Table 4: *Fat-free mass, fat mass and fat percentage calculated from the body density in the controls and from total body water in the patients.*

	Controls Mean ± SEM		Patients Mean ± SEM		P value
Fat-free Mass (kg)	: 52.4	2.3	46.6	3.9	NS
Fat Mass (kg)	: 18.4	1.8	26.2	2.3	<0.01
Fat Percentage (%)	: 25.3	1.6	36.2	2.3	<0.001

In the controls FFM determined densitometrically was lower than the FFM calculated from body impedance, whereas in the patients no differences were observed between predicted FFM by impedance and FFM as determined by deuterium oxide dilution (Table 5).

Table 5: *Comparison between fat free mass calculated from densitometry in the controls and deuteriumoxide dilution in the patients (FFM) with the fat free mass calculated from impedance with the formula's of Lukaski (FFM-Lu) and Deurenberg (FFM-De).*

	FFM	FFM-Lu	FFM-De
Controls	52.4 ± 2.3	56.5 ± 2.3*	54.1 ± 2.1*
Patients	46.6 ± 3.9	45.4 ± 3.7	45.3 ± 3.2

*:P<0.01 compared to FFM

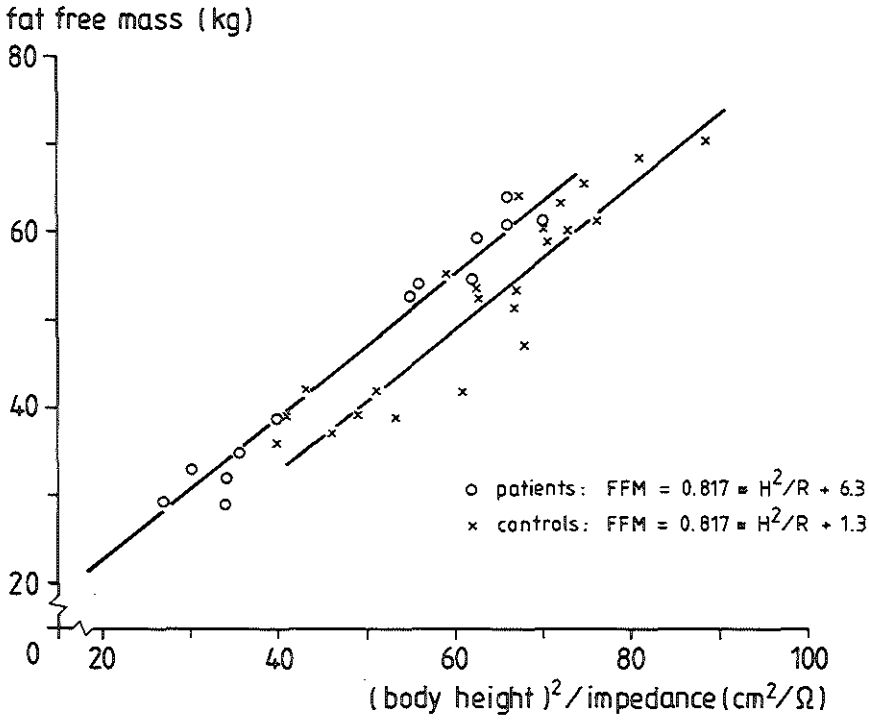

Figure 2: Regression between impedance (H^2/R) and FFM (densitometry) in the controls and FFM (Deuteriumoxide dilution) in the patients. The slopes of the two regression equations are not significantly different (P=0.15), but the intercept in the patients is significant higher (P=0.0001).

Fig 2 shows the relation between body impedance (expressed as H^2/R) and FFM (determined by way of underwater weighing) in the individual controls and the relation between impedance and FFM (determined by way of deuterium dilution) in the individual patients. The calculated prediction formula's, for controls and patients, for FFM from impedance are different: the slope of the regression line is similar ($P=0.15$) but the intercept in the patients is higher ($P=0.0001$).

DISCUSSION

Reported differences in body composition in GH deficiency mainly concern children in whom higher skinfold thicknesses and lower muscle masses or muscle widths have been found. These changes are at least partially reversible after GH treatment (33-38). After cessation of GH treatment fat mass has been shown to increase and muscle mass to decrease (33,34,38).

In this study we investigated 13 adult patients with GH deficiency. In 11 of them (partial) hypopituitarism was present, which had been substituted already adequately in all instances for more than 2 years. We confirmed the presence of GH deficiency in these patients by the combination of a low peak serum GH level after stimulation of the pituitary by GHRH together with the low circulating IGF-I level (which in these patients was not caused by malnutrition, diabetes mellitus or liver disease). Despite of the fact that this combination of investigations might be considered as unusual and not generally accepted in the diagnosis of GH deficiency, we feel confident that these tests were sufficient to support this diagnosis, both against the background of the simultaneous presence of insufficiency of other anterior pituitary functions in virtually all patients, as well as on the basis of the previous demonstration of GH deficiency with classical stimulation tests in 4 patients.

The administration of GH to adults with GH deficiency has been reported to lead to an increase in thigh muscle volume (39) and in FFM (18,40), while after cessation of GH treatment a decrease in thigh muscle volume was observed (41).

To our knowledge no study has been reported comparing body composition in GH deficient adults with a matched control population. Since body composition is influenced by age and sex it is a prerequisite to have a control group matched for these variables, before differences in body composition can be attributed to other variables, such as GH deficiency. Since we have such a control group, we do present our data here, despite the disadvantage that the validation of the BIA equation in our groups had been performed with different techniques, underwater weighing in the controls and deuterium-oxide dilution in the patients.

Table 2 shows that controls and patients in this study were identical for age, sex, height and weight, while Tables 3 and 4 demonstrate significant differences in body

composition: a significant higher FM and percent body fat, while FFM tended to be lower (not reaching significance) in the GH deficient patients.
As is shown in Table 5 in the controls the impedance formula's of Lukaski et al (23) and Deurenberg et al (26) overestimate the FFM as measured by underwater weighing. This can be explained by the fact that the controls are older compared to the populations from which both formula's are derived. In older subjects FFM is generally overestimated when prediction formulas derived in younger reference populations are used, due to an altered body water distribution (42,43).
The fact that in the patient population the impedance formulas did not overestimate the FFM found by D_2O-dilution, as a reference method, can be explained by the fact that in GH deficient patients the relative amount of intracellular water is increased (34), thus counteracting the lowering effect of age.
The relation between impedance and FFM as depicted in Fig 2 shows a different regression line between H^2/R and FFM in the patient and controls, implying that a GH deficient patient has a higher impedance compared with a control after adjusting for differences in FFM and height. Since it has been demonstrated that the impedance is dependent on the intra cellular water (ICW) and extra cellular water (ECW) distribution (42), the increased impedance in GH deficiency can be attributed to a relative decrease in ECW. Indeed Novak et al (34) found a decrease in ECW as a percentage of body weight after cessation of GH treatment in GH deficient children. In acromegaly ECW is increased (44), while after successful treatment of acromegaly ECW decreases (45). During GH treatment of non-GH deficient adults acute fluid retention is observed (46,47).
Unfortunately we were not in a position to use the same reference technique for body composition measurements in both patients and controls. It could be argued that the differences in the relation between FFM and body impedance, as given in figure 2, might be due to an invalid assumption of the constant mean hydration factor of 0.73 in the FFM in the GH-deficient patients. However to overcome the difference in intercept between the two regression equations, a mean hydration factor as low as 0.68 would have to be used for the calculation of the FFM from TBW in the patient group. This value seems unreasonable low. The difference between the two prediction formulas for FFM from bioelectrical impedance for the patient and the control group (Figure 2) indicates that FFM tends to be underestimated by BIA in GH-deficient patients. Yet, using either one prediction equations would be suitable to follow-up changes in body composition in untreated GH deficient people. However, the prediction equation used in our patients when employed to evaluate possible changes in body composition of GH-deficient patients before and during GH treatment, could result in an overestimation of the increase in FFM. Therefore impedance data in the evaluation of therapeutic effects of GH in GH-deficient patients have to be used carefully.

In conclusion GH-deficient subjects have higher FM and lower FFM compared to age, sex, height and weight matched controls. Body impedance values in GH deficient subjects are higher, compared to controls, even after correction for FFM and height, probably due to an altered body water distribution. This implies that prediction formulas for body composition from body impedance derived in normal healthy subjects will underestimate FFM in GH deficient subjects.

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CHAPTER 5

THE EFFECT OF GROWTH HORMONE (GH) ADMINISTRATION IN GH-DEFICIENT ADULTS ON BONE, PROTEIN, CARBOHYDRATE AND LIPID HOMEOSTASIS, AS WELL AS ON BODY COMPOSITION.

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SUMMARY

The effect of growth hormone administration to adult patients with growth hormone deficiency was studied on bone, protein, carbohydrate and lipid homeostasis as well as on body composition.
Growth hormone was administrated at a dose of 25 ug/kg/day with a maximum of 1.48 mg (4 IU) a day, for 6 months in 8 adults. Studies were done before the start and at 1, 3 and 6 months during therapy, as well as 3 months after treatment had been stopped.
Subjective well being as assessed by a short psychological test showed an improvement in 6 and no change in 2 patients. Body composition, as assessed by body impedance assessment and D_2O dilution both showed an increase in lean body mass of 4 kg (5% of body weight), accompanied by a decrease in mean fat mass of 3 kg. Nitrogen turnover studies showed a transient increase in fed state nitrogen balance due to an increase in the rate of protein synthesis, which exceeded a smaller increase in the protein degradation rate.
Growth hormone treatment did not affect the circulating levels of 25(OH)-vit D, and PTH 1-84, while $1,25(OH)_2$-vit D had significantly increased after 6 months as well as 3 months after GH treatment ended. Osteocalcin and procollagen I levels as well as 24 h urinary excretion of OH-proline and calcium rose during, but subsequently decreased rapidly after GH administration had been stopped, while the increase in alkaline phosphatase persisted. This increase in markers of both bone resorption and bone formation indicate an activation of bone remodelling, but this was not reflected by an increase in bone density.
Glucose levels measured before and during a normal breakfast increased during GH treatment, but serum insulin levels did not. Total cholesterol levels decreased by 0.5 mmol/l. Levels of T4 and free T4 as well as rT3 decreased, while T3 increased during GH treatment.
Therapy with growth hormone for 6 months in a dose varying between 6 to 25 ug/kg/d increased lean body mass and decreased fat mass. The sense of general well-being improved in most patients. Furthermore GH therapy increased bone turnover without a measurable increase in bone density, caused some minor changes in lipid and carbohydrate metabolism, and increased the breakdown of thyroxine to T3.

INTRODUCTION

Growth hormone (GH) deficient adults are currently not substituted with GH. Spontaneous and stimulated GH secretion continues into normal adult life, however, although it decreases over time in later age (1-3). Recent reports by Salomon et al (4)

and Jorgensen et al (5) have demonstrated that GH treatment in GH deficient adults causes effects on body composition and exercise capacity which are of potentially clinical importance. We report the results of GH treatment for 6 months of 8 long-term GH-deficient adults, as well as the changes 3 months after the end of GH substitution on subjective well being, body composition and bone, carbohydrate and lipid homeostasis.

PATIENTS AND METHODS

Patients

Eight adults with hypopituitarism (congenital or after surgery for craniopharyngioma) or isolated GH deficiency were studied (Table 1). In all patients the initial diagnosis of hypopituitarism or isolated GH deficiency had been made at least 10 years before the present study. The patients had not received (pituitary-derived) GH for the past 10 years. If present, other hormonal insufficiencies had been adequately substituted for at least one year before the start of the study. Before entering the study the presence of GH deficiency was confirmed by the combination of a plasma IGF-I level of 0.3 U/ml or less (normal ranges: 0.34-1.90 U/ml in men and 0.45-2.20 U/ml in women), and a peak GH level of less than 10 mU/l after 1 ug/kg GHRH iv. None of the patients suffered from kidney or liver disease, diabetes mellitus or had a history of a malignancy. One patient (nr 5) had osteogenesis imperfecta.

After aims and methods of the study were explained both orally and in writing, written informed consent was obtained from each patient before the start of the study, which was approved by the Ethics Committee of the University Hospital Dijkzigt.

Methods

All individuals were studied for a period of 9 months, during which the conventional hormone substitution regimen was not changed. They received GH during the first 6 months only.

All patients were asked not to change their diet and level of physical activity. After intensive instruction GH (Humatrope; supplied by Eli Lilly & Co, Indianapolis, Ind) was injected by the patients themselves sc at bedtime, in a dose of 25 ug/kg/day, with a maximum of 1.48 mg (4 IU). Immediately before, 1, 3 and 6 months after the start as well as 3 months after the end of the 6 months of GH administration all patients were admitted to the clinical research unit for the following studies:

a. Clinical chemical investigations, including haematology and assessment of kidney function, electrolytes, liver enzymes, 25(OH)-vit D, $1,25(OH)_2$-vit D, PTH 1-84, osteocalcin, procollagen I, total T4, free T4, T3 and rT3, testosterone or oestradiol and

Table 1: Some physical characteristics of the patients.

PAT. nr	AGE yr	DIAGNOSIS	AGE	SEX M/F	HEIGHT m	WEIGHT kg	BMI kg/m2	TBW kg	FFM kg	RESIST Ohm	PITUITARY A	PITUITARY G	INSUFF. T	PEAK GH ug/l	IGF-1 U/ml
1	25	Cranio	16	M	1.86	83.8	24.2	45.3	61.9	552	+	+	+	2.8	0.3
2	30	Cranio	19	F	1.54	62.0	26.1	24.3	33.2	700	-	+	-	2.9	0.3
3	39	Chromof ad	30	M	1.80	87.7	27.1	46.8	63.9	488	+	+	+	3.8	0.2
4	23	Cranio	9	M	1.72	92.8	31.4	41.6	56.8	527	-	-	-	3.2	0.4
5	25	Id GH Def	11	F	1.44	56.0	27.0	22.3	30.5	610	-	-	-	3.1	0.3
6	30	Cranio	17	M	1.82	94.8	28.6	48.5	66.3	504	+	+	+	2.3	0.3
7	54	Cranio	45	M	1.74	74.0	24.4	41.2	56.3	490	+	+	+	4.3	0.4
8	46	Cranio	40	M	1.77	99.0	31.6	47.7	65.2	445	+	+	+	2.4	0.4
9	31	Cranio	16	F	1.65	87.0	32.0	40.8	55.7	496	+	+	+	1.4	0.1
10	19	Cranio	16	F	1.66	48.0	17.4	26.5	36.2	763	+	+	+	6.6	0.4
11	45	Id hypopit	15	F	1.37	57.0	30.4	21.8	29.8	686	+	+	+	1.7	0.4
12	23	Id hypopit	10	M	1.69	56.5	19.8	29.3	39.9	710	+	+	+	1.0	0.2
13	34	Id hypopit	13	M	1.61	46.6	18.0	25.4	34.7	862	+	+	+	4.2	0.5
Mean:	33				1.67	72.7	26.0	35.5	48.5	603				3.1	0.3
SEM :	3				0.04	5.1	1.3	2.8	3.9	34				0.4	0.0

Present age, diagnosis (Cranio= craniopharyngioma; Chromof ad= Chromophobe adenoma; Id GH def= Idiopathic GH deficiency; Id hypopit= Idiopathic panhypopituitarism); age at diagnosis; BMI= Body Mass Index (kg/m^2); TBW= total body water; FFM= fat-free mass (kg); impedance (Ohm); Pituitary insufficiency causing secondary A: adrenal-, G: gonadal-, T: thyroid-insufficiency; peak GH (ug/l, highest GH level after stimulation with 1 ug GHRH/kg BW); IGF-I (U/ml, basal plasma insulin like growth factor-I).

IGF-I levels were performed. 24 Hour urine was collected for the measurements of creatinine, calcium and OH-proline. These determinations were carried out with commercially available RIA or IRMA kits. All assays used had an intra-assay variation of less than 8% and an inter-assay variation of less than 12%, except for the inter-assay variation of the $1,25(OH)_2$-vit D assay which amounted to 19.8%.

b. After an overnight fast basal cholesterol, HDL-cholesterol and triglycerides (TG) were determined as well as lipoprotein lipase (LPL) and hepatic triglyceride lipase activity (HTL) 20 minutes after the injection of 50 U heparin/kg BW as described previously (6).

c. After another overnight fast blood glucose and plasma insulin levels were measured before and 30, 60 ,90, 120, 150 and 180 min after a standard breakfast. In the results section the sum of these 7 values has been reported.

d. Subjective well being was assessed using a short (self-rating) questionnaire where the patients had to answer 10 questions using a 7 point scale. The questions regarded fatigue, physical and psychological fitness, activity and the ability to concentrate. The score ranges from 10 (no fatigue) to 70 (maximal fatigue).

e. Body composition was determined by body impedance analysis (BIA) (model BIA 101, RJL Systems Detroit MI). Total body water (TBW), lean body mass (LBM) and fat mass were calculated from the measured impedance and reactance (7-10) by a RJL Systems supplied computer programme.
In addition body composition was measured with the use of a multiple isotope dilution technique using D_2O (11,12) and ^{22}Na (13). After an overnight fast and insertion of a catheter into an antecubital vein blood was sampled and the patient was given, by mouth, a dose of 0.24 g 99.7 % D_2O/kg body weight diluted in 200 ml H2O and an iv injection of 8 uCi of $^{22}NaCl$. After 150, 180 and 210 min 10 ml heparinised blood was withdrawn, and the plasma stored at -20 C until analysis. After sublimation, D_2O concentration was measured by infrared spectrophotometry and TBW was calculated (11,12). LBM was calculated with the equation LBM=TBW/0.73 (14). Extra-cellular water (ECW) (and intra-cellular water, ICW) were calculated from the level of ^{22}Na activity in blood drawn after 60, 70, 80 and 90 min, as described previously (13).

f. Protein turnover was studied with a two-compartment model(15) using a single oral dose of 200 mg of [^{15}N]glycine 99% (KOR Chemical, Boston, USA) (16) as the isotope to label the amino acid pool while urinary nitrogen (N) excretion products (urea and ammonia) were used to measure the nitrogen flux (17). During the study the patients were in a steady metabolic state, and received a liquid formula diet containing 1 g of

natural protein and 25 kcal/kg BW/d, divided into 11 identical hourly portions. The steady state was assumed to be reached 2 h after the first liquid meal, with a subsequent collection period of 9 h (17). The results are expressed as mg of N/9 h.

g. Bone mineral density was determined every 3 months using both single photon absorptiometry (SPA) at the forearm (proximal and distal sites) and dual photon absorptiometry (DPA) of the second to fourth lumbar vertebrae. For the SPA measurements the method described by Nilas et al (18) with a Nuclear Data 1100a bone density scanner was used, the DPA measurements were done with a Novo BMC-lab 22a scanning device as described by Krollner and Nielsen (19).

RESULTS

In 3 patients (nos 1, 3 and 7) the initial dose of GH (25 ug/kg/d) had to be reduced within two weeks because of marked fluid retention of 3-5 kg and complaints related to carpal tunnel nerve pressure (which was confirmed with electromyography) in one of these patients (no 3). The actual dosages of GH used during the study period are listed in Table 1.
Except for patient no 2, all patients reported a considerable improvement in well being, endurance and physical fitness. This subjective information was reinforced by the (individual and) group mean results of the psychological testing (Fig 1), which shows a gradual reduction in fatigue and virtually no fatigue after 6 months of GH treatment.

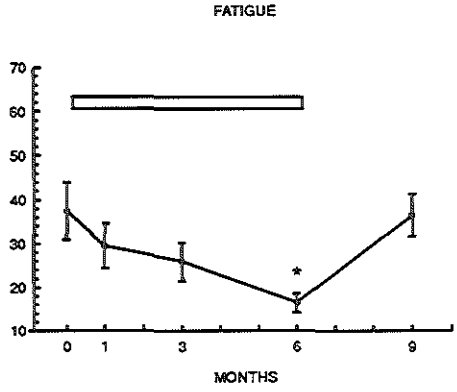

Figure 1: Self assessment score regarding fatigue (10: no fatigue; 70 maximal fatigue). The horizontal bar denotes the GH treatment period. * $p<0.05$ vs 0 and 9 months. Mean $\pm$ SEM, N=8.

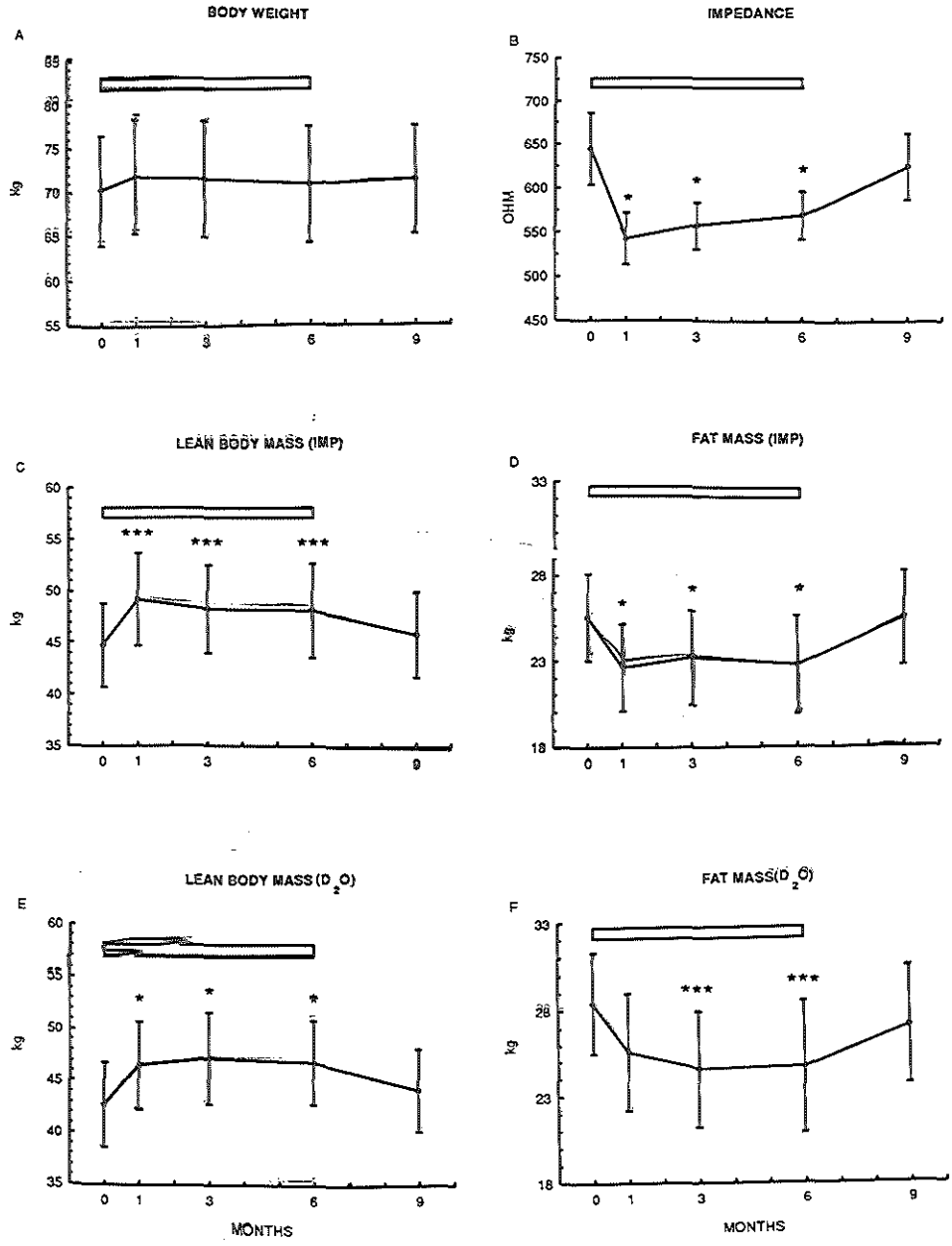

Figure 2: Body composition parameters. a: body weight, b: body impedance; c,d: lean body mass and fat mass calculated from impedance; e,f lean body mass and fat mass determined by D_2O dilution. * $p<0.01$ vs 0 and 9 months, ** $p<0.01$ vs 0 and $P<0.05$ vs 9 months, **$p<0.05$ vs baseline. Mean $\pm$ SEM, N=8.

The effects of GH administration on body composition are depicted in Fig 2. Body weight (BW) did not change significantly (Fig 2a). Body composition as assessed by body impedance measurement showed significant changes: body impedance decreased sharply (Fig 2b), representing an increase in LBM ranging from 3.6-4.6 kg (Fig 2c). In parallel fat mass (FM) decreased by 2.4-3.0 kg (Fig 2d). The maximal change in LBM and fat mass had occurred already 1 month after the start of GH therapy. Three months after GH substitution ended, body composition was unchanged compared to baseline. Body composition as studied by D_2O dilution showed similar changes in LBM and FM (Fig 2e and f). The results from the ^{22}Na dilution showed that the changes in TBW were due to changes in ECW, while ICW did not change (data not shown).

The results of the ^{15}N-glycine studies (Fig 3a-c) showed an initial increase in rates of protein synthesis one month after the start of GH administration while this effect seem to level off thereafter. Indeed nitrogen balance became significantly positive ($p=0.02$; two-tailed paired Student's t-test) after one month, mainly as a consequence of increased synthesis ($p=0.05$), while nitrogen degradation had non-significantly increased. If these parameters were expressed per kg LBM, however, no significant changes were observed (data not shown).

Low pre-treatment serum IGF-I levels increased to normal during GH substitution and returned to baseline 3 months after GH administration was stopped (Fig 4a). During GH treatment IGF-I values ranged from 0.4 to 3.8 U/ml. Values above normal were noted in patients 6 (at 1,3 and 6 months) and 7 (at 1 and 3 months). Serum phosphorus levels showed similar changes (Fig 4b). The maximal renal tubular reabsorption of phosphorus corrected for the glomerular filtration rate (TmP/GFR) increased from 0.98 ± 0.05, before GH treatment started, to 1.57 ± 0.10 ($p<0.01$) after one month of GH treatment. Three months after GH treatment ended TmP/GFR had decreased to 1.05 ± 0.05.

Serum calcium levels tended to increase after 1 month of GH treatment (Fig 5a), but the change did not reach statistical significance and thereafter calcium levels returned to pretreatment levels. Levels of (25)OH-vit D and PTH 1-84 did not change during the study period. The $1,25(OH)_2$-vit D values amounted to 83.0 ± 8.0 pmol/l before the start of GH therapy and had increased to 118.2 ± 15.8 pmol/l at six months ($p<0.05$), while they had increased further to 136.4 ± 11.1 pmol/l three months after stopping GH administration ($p<0.01$ vs pretreatment and $p<0.05$ vs after 6 months of therapy). Alkaline phosphatase levels increased slowly during GH therapy and had not yet returned to baseline 3 months after treatment ended (Fig 5b). Mean circulating osteocalcin and procollagen I levels increased sharply compared with baseline during the 6 months GH treatment period (Fig 5c and d). Hydroxyproline excretion, measured in 24 h urine collections, increased and had returned to baseline 3 months after ending GH administration (Fig 5e). Calcium excretion in 24 h urine increased similarly, though

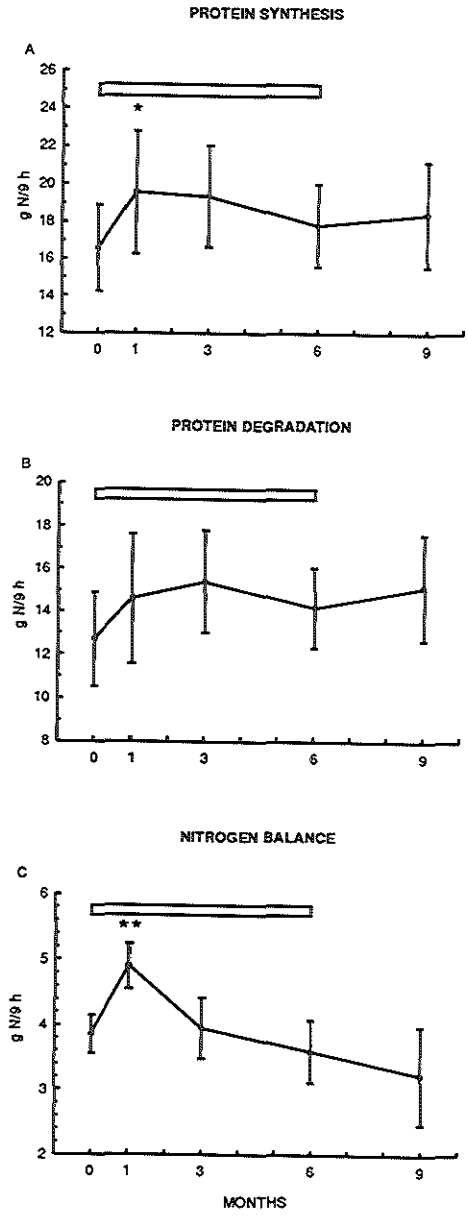

Figure 3: Nitrogen (N) turnover studies. a:protein synthesis, b:degradation and c:balance. Results expressed as gram N/ 9 hr (the length of the nitrogen turnover study period). * p=0.05 vs 0 and ** p=0.02 vs 0 by a paired two-tailed Student t test. Mean $\pm$ SEM, N=8.

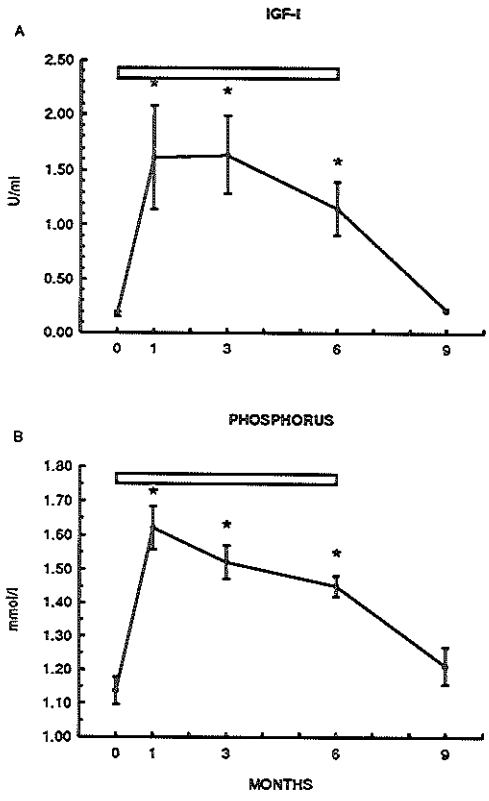

Figure 4: Serum IGF-I (a) and phosphorus (b) levels.
* $p<0.01$ vs 0 and 9 months. Mean $\pm$ SEM, N=8.

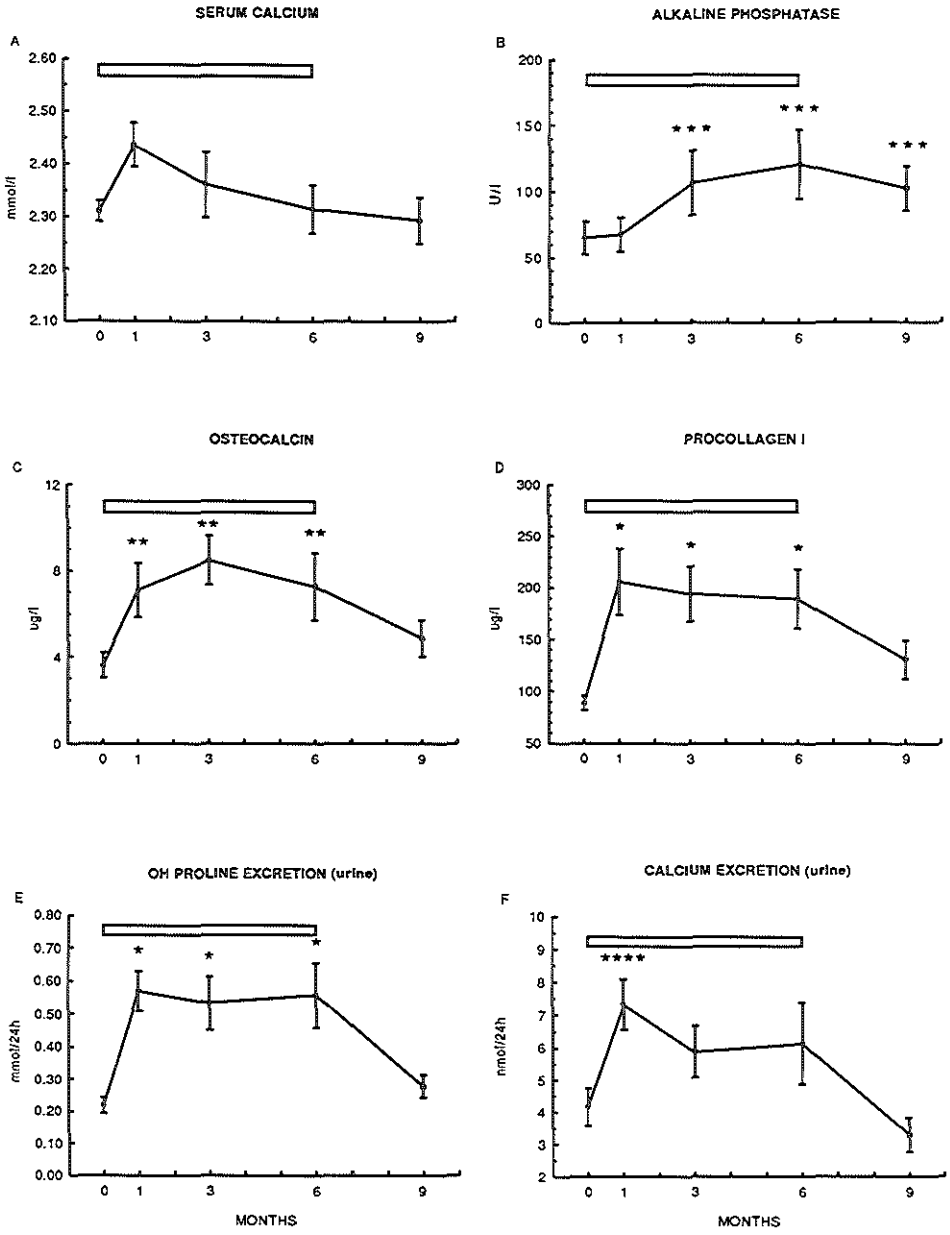

Figure 5: Parameters of bone metabolism. * $p<0.05$ vs 0 and 9 months, ** $p<0.01$ vs 0 and $p<0.05$ vs 9 months, *** $p<0.01$ vs 0 and 1 months, **** $p<0.05$ vs baseline. Mean $\pm$ SEM, N=8.

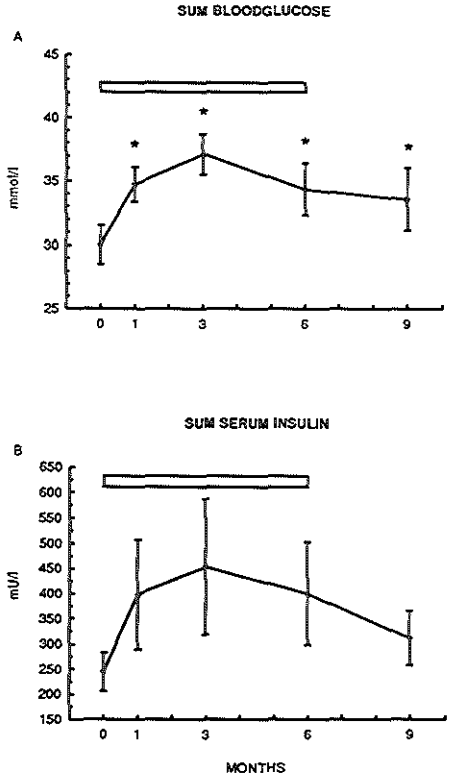

Figure 6: Sum of 7 bloodglucose (a) and 7 serum insulin (b) levels measured before and after a normal breakfast. * $p<0.05$ vs 1 month. Mean $\pm$ SEM, N=8.

only statistically significant at 1 month (Fig 5f). Pretreatment bone densities were lowered in the 5 female patients as compared with age-matched controls (20). The results of the DPA of the spine after 3 and 6 months of GH treatment showed a small but statistically significant decrease in bone density in comparison with pretreatment values (from 0.81 ± 0.05 to 0.79 ± 0.05 g hydroxyapatite/cm^2, $p<0.05$). Three months after GH treatment had been stopped DPA remained unchanged compared to pretreatment (0.81 ± 0.05). SPA measurements showed no changes during the 9 month study period.

The sum of the 7 blood glucose levels measured before and after breakfast increased during GH administration (Fig 6a), while an increase in the sum of the 7 insulin levels did not reach statistical significance (Fig 6b). Glucose levels remained significantly elevated 3 months after stopping GH administration. The mean fasting and 2 h postprandial glucose levels maximally increased after 3 months from 3.8 ± 0.2 and 4.2 ± 0.3 to 4.3 ± 0.2 and 5.8 ± 0.4 mmol/l, respectively ($p<0.05$ in both instances). The highest individual glucose levels measured at any time during the study amounted to 9.0 and 8.1 mmol/l.

The mean plasma cholesterol level (pretreatment 4.75 ± 0.34) decreased after 1 (4.21 ± 0.22) and 6 months (4.11 ± 0.19) of GH treatment ($p<0.05$ in both instances). This decrease was due to a 10% decrease in plasma LDL-cholesterol. The suppressive effect of GH on total cholesterol was only seen in the 6 patients with a baseline cholesterol level above 4 mmol/l (fig 7). In those 6 patients the change in circulating cholesterol concentrations during GH treatment was negatively correlated with the change in IGF-I levels, at 6 months ($p<0.05$). Small changes in LPL activity and TG levels did not reach significance, nor were changes observed in HDL-cholesterol levels and HTL activity (data not shown).

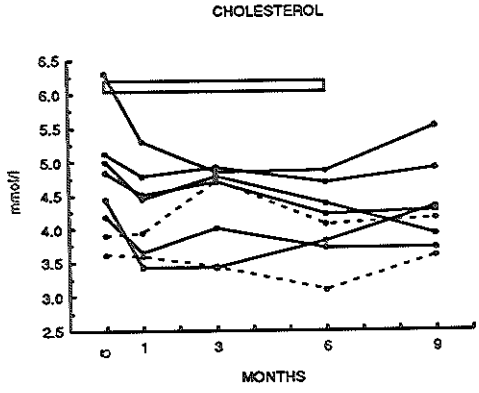

Figure 7: Individual cholesterol levels in the 8 patients. - - - patients with a baseline serum cholesterol of <4.0 mmol, — patients with a baseline cholesterol of >4.0 mmol/l.

As is depicted in Fig 8a-d levels of T3 increased during GH treatment, in parallel with a simultaneous decrease of reversed T3 levels. Total and free T4 tended to decrease during GH treatment. Three months after stopping GH therapy all thyroid hormone parameters had returned to baseline. These changes were noted both in the 5 patients who received thyroxine substitution as well as in the 3 patients with isolated GH deficiency.

DISCUSSION

GH deficient adults have lower scores on quality of life assessment compared with healthy adults, while this improves during GH substitution (21). Another report suggested a beneficial effect of GH treatment of GH deficient adults with regard to their cognitive functions (22). Our study lacks a placebo-controlled period. Therefore the marked subjective improvement in well being during GH treatment in nearly all patients, which was corroborated by the changes in the score of the psychological test, can only be interpreted with great caution. We want to stress especially that we do not yet have a definitive validation of the quality of life questionnaire used (van Duivenvoorden et al, in preparation). However, 7 of the 8 patients reported that, if given the opportunity, they would resumed GH injections because of the increase they had felt in daily energy and initiative.

The side effects of transient water retention which occurred in most of our patients, have been noted previously by others using comparable dosages of GH (4,5). These side effects were such that they forced us to reduce the GH dosage in 3 patients. Since the administered GH dose varied from 6 to 25 ug GH/kg BW no conclusion can be drawn from our study concerning an optimal GH dosage in GH-deficient adults.

The changes we observed in body composition during GH therapy need some comments. The observed increases in lean body mass (amounting to as much as 5% of total body weight) together with simultaneous decreases in fat mass of 2.4-3.0 kg are in agreement with earlier studies (4,5,23,24). Also the reversal of these changes after cessation of treatment was to be expected (25). However, it is not directly understandable why the maximal change in body composition was already observed 1 month after the start of GH treatment. The more positive nitrogen "balance" at 1 month of GH therapy, with the subsequent decline to baseline levels hereafter, supports our observation that the increase in LBM occurred during the first month of GH therapy. Another explanation might be that the initial rise in LBM at 1 month was at least partially due to an increase in ECW (edema), followed by an increase in ICW (cell mass) together with a simultaneous decrease in ECW. However, our results using ^{22}Na to measure ECW and calculate ICW do not support this.

No nitrogen turnover studies in GH deficient patients receiving GH have been reported

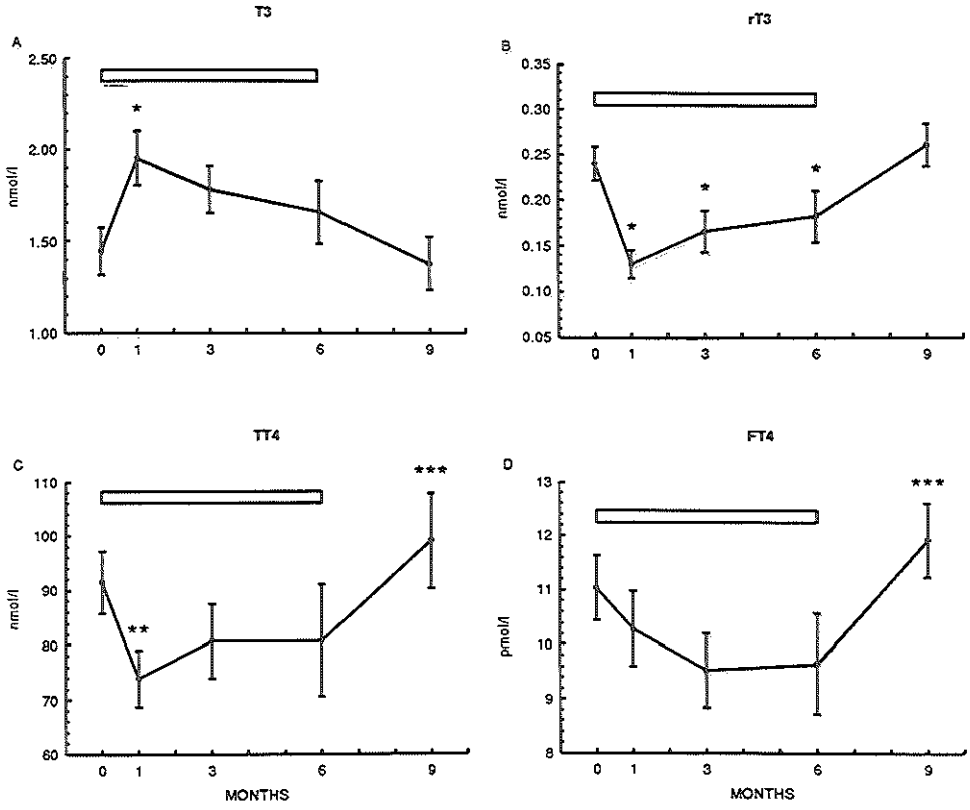

Figure 8: Thyroid hormone parameters: (a) Triiodothyronine (T3), (b) reversed T3, (c) total thyroxine (TT4) and (d) free T4 (FT4). * $p<0.05$ vs 0 and 9 months, ** $p<0.05$ vs baseline, *** $p<0.05$ vs 1,3 and 6 months. Mean $\pm$ SEM, N=8.

previously, to our knowledge. In postoperative patients receiving various nutritional regimens, short term (7 days) GH administration increased nitrogen turnover and synthesis while degradation either increased or remained unchanged (26-28). In obese patients, receiving hypocaloric nutrition, the nitrogen retaining effect of GH appeared to wear off after 3 to 5 weeks (29,30).

As expected a marked increase in IGF-I occurred during the 6 months of GH supplementation. The fact that supranormal circulating IGF-I levels were observed in two patients questions whether we have used a "physiological" substitution dose of GH throughout the study in all patients. Phosphorus levels increased in parallel with the increase in IGF-I. Recent studies (31,32) suggest that IGF-I and phosphorus metabolism are closely related, possibly via a direct effect of IGF-I on renal phosphorus handling (TmP/GFR). Indeed an increase in TmP/GFR was observed during GH administration in our patients.

Longterm GH therapy in GH deficient children did not cause changes in 25(OH)-vit D and/or PTH levels, while the effects on circulating 1,25 $(OH)_2$-vit D concentrations were contradictory (33-35). The late increase of 1,25 $(OH)_2$-vit D during GH treatment might be the result of opposing effects of GH/IGF-I and increased phosphorus levels on the renal 1-α hydroxylase activity. During the follow-up period, however, phosphorus levels rapidly declined, whereas presumably renal cell mass remained at least initially increased. We want to hypothesize that this resulted in a net stimulation of 1-α hydroxylase activity and consequently in the late elevation of 1,25 $(OH)_2$-vit D levels.

Previous reports, confirmed by our results, show that GH treatment induces a rise in markers of bone formation like osteocalcin (36,37), type I procollagen (38,39) and alkaline phosphatase. In addition a marked increase in OH-proline excretion (40), caused by an increase in degradation of both bone and non-bone collagen (41), indicates a higher rate of bone resorption. This increase in markers of bone resorption and formation indicates that GH induces activation of bone remodelling. As could be expected the higher bone turnover, initiated by a higher bone resorption, results in an initial loss of axial bone mass. However, after treatment with GH has been ceased bone mass returns to its initial level, illustrating that new bone remodelling units, once initiated by GH, completed a full life cycle and thereby demonstrating normal coupling between bone resorption and formation.

As expected (42) an increase in blood glucose and insulin levels was observed after a normal breakfast during GH therapy. The significant negative correlation between the changes in cholesterol and IGF-I levels, as observed in the 6 patients with a baseline cholesterol level above 4 mmol/l, are in accordance with the slight decrease in cholesterol levels during GH therapy of GH-deficient adults as observed by other investigators (4,43,44), and supports the hypothesis that GH activity influences cholesterol levels (6,45). As in our study, TG levels are usually normal in GH deficiency (43,44), while they do not change during GH treatment (4,46).

The observed changes in thyroid hormone parameters during GH treatment are consistent with the observations in healthy adults given 125 ug GH/kg/day for four days (47) and in a GH treatment study in GH deficient adults given 2 U GH/m^2 for 4 months (4,48). They indicate a GH-induced increase in peripheral deiodination of T4 to T3. The increase in T3 levels might contribute to the observed changes in body composition and the decrease in fatigue. In normals fasting induces an increase in GH (49) and a decrease in IGF-I levels (50,51), while T3 levels decrease due to a reduction in peripheral conversion of T4 to T3 (54). Refeeding induces an increase in IGF-I (51-53) as well as an increase in T3 levels and a decrease in rT3 production (54). Therefore IGF-I and not GH itself might stimulate the peripheral conversion of T4 to T3 during GH treatment of GH deficient adults.
In conclusion, we think that based on the studies both in the literature and the study presented here GH suppletion in adults with GH deficiency is of value, both because of the subjective beneficial response reported by the patients, as well as because of some of the physical and biochemical changes observed in these patients. However, it is at present not clear which initial dose should be chosen and whether this dose might have to be increased after a certain 'wash-in' period in order to expand the improvements observed.

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CHAPTER 6

DISCUSSION OF THE MAJOR CONCLUSIONS

The observation (chapter 2) that in elderly catabolic adults the effects of a GH dose of 25 ug/kg/d on nitrogen retention is comparable to that of 50 and better than that of 12.5 ug/kg/d, while side effects in these short term studies were less with the 25 ug than with the 50 ug/kg/d dose, awaits confirmation by others. Recently a study was published in which elderly malnourished adults were treated for 21 days with 100 ug GH/kg/d in addition to a diet aimed at maintaining body weight (1). Basal glucose levels reportedly did not increase. However, no non-fasting glucose levels or insulin levels were studied. A weight gain of 2.3 kg was partially ascribed to a gain in muscle mass solely on anthropometric measurements and the observation of increased urinary nitrogen retention. No nitrogen and/or sodium balances were performed in this last study.

Before the routine use of GH in catabolic disease can be justified as an adjunct to nutritional treatment (either enteral or parenteral), larger studies of longer duration are needed demonstrating reduced morbidity and/or mortality and preferably accelerated recuperation. It is likely that the degree of catabolism, as well as the age of the patient are of influence on the effect of a standard GH dose. Therefore it might well be that while some malnourished/catabolic individuals optimally benefit from 25 ug GH/kg/d, others need a higher dose in order to achieve a maximal anabolic effect. The question of which dosage is optimal regarding desired and undesired effects is not only of medical interest but also a financial question, as presently 1.5 mg of GH (the daily dose of 25 ug/kg/d for a person of 60 kg) costs about 150,- Dfl.

Our results in rats (chapter 3) show no GH-induced stimulation of tumor growth, while cancer induced cachexia could be prevented. In humans, however, the potential beneficial effects of GH administration on tumor cachexia will be difficult to investigate, as the theoretical mitogenic and tumor growth-stimulatory effects of GH are at present difficult to refute. Few medical ethical committees will therefore approve GH trials in cachectic cancer patients. The use of GH for several weeks during a catabolic phase after trauma or severe disease is probably too short to induce tumors, while long-term GH replacement in GH deficient adults should pose no increased risk, provided the GH substitution doses are 'physiological'. However, in this regard the long-term use of pharmacological amounts of GH in healthy elderly people to reverse and/or prevent the consequences of normal aging (2), the use of GH in short but not GH-deficient children (3) and the long-term abuse of even higher amounts of GH as an anabolic agent in sports (4), should also looked at from the perspective of the potential mitogenic and tumor-growth promoting effects of the hormone.

Body composition assessment in GH deficient adults (chapter 4) showed decreased fat free mass and increased fat mass when compared with healthy age, sex, length and weight matched control subjects. In addition, in GH deficient subjects an altered distribution of intra- and extra-cellular water was found, which might limit the use of impedance measurements to assess changes in body composition in GH deficiency. However, the effects of GH substitution in GH deficient adults on body composition as measured by deuterium dilution were similar to the changes observed with impedance measurements (chapter 5). Body composition changed towards normal: fat mass decreased and fat-free mass increased. These observations and the report by Jorgensen et al that exercise capacity increased during GH replacement therapy (5), might explain the impressive improvement in subjective well being reported by virtually all patients. The effects of GH substitution on bone parameters demonstrated both increased bone formation and degradation. No increase in bone density was observed after 6 months of GH administration, despite the fact that bone densities in these patients at baseline was decreased when compared with controls. The GH-induced activation of bone remodelling might be of possible use in a combined treatment regimen for osteoporosis: while GH stimulates bone formation, bone degradation can be simultaneously reduced by biphosphanates. This combined approach might therefore lead to a net increase in bone density.

The increased peripheral conversion of T4 to T3 possibly contributes to some of the effects of GH replacement (for example the changes in body composition and the decrease in fatigue). No conclusion can be drawn from our study, nor from the other studies published so far (5,6) regarding an optimal GH replacement dose. Perhaps an initially low GH dose should be gradually increased towards an individual optimum, which could be found on the basis of the subjective reactions by the patient, as well as the course of the circulating IGF-I levels. Aside from the GH dose, other factors, like frequency, route and time of the administration also influence the results of GH replacement therapy (7).

At present, it can be concluded that GH treatment of catabolic adult patients is still in the research phase as to whether it indeed achieves clinically important effects, and if this is proven, at what optimal dose. However, GH replacement therapy in GH deficient adults has been shown to be of considerable benefit, both objectively and subjectively. It is recommended therefore, that the use of GH replacement in this kind of patients will be accepted by the authorities in the Netherlands shortly.

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SUMMARY

The development of recombinant DNA technology has expanded the amount of human GH available for clinical use. GH treatment regimens in GH deficient children have been improved, cq dosages have been increased to maximize growth and treatment is now without the risk of transferral of viral infections.
For the use of GH in adults questions have arisen like: in which clinical conditions, at what age and in which dose GH administration can be of possible benefit, and what are the possible (dose-related) side effects. The useful indications of GH treatment in adults can be divided in a) GH treatment, as an adjunct to the normal (nutritional) care of catabolic patients who, when healthy, secrete(d) normal amounts of GH and in b) GH replacement, in adult patients who secrete no or a lowered amount of GH.
However, possible adverse effects of GH on sodium and fluid homeostasis, carbohydrate metabolism as well as possible mitogenic effects (chapter 1.5) raise questions concerning the safest (lowest) effective GH dose.
As described in chapter 2, in elderly catabolic adults who consume an optimal enteral diet, a GH dose of 25 ug/kg/d for 4 days was shown to be equal to 50 and superior to 12.5 ug/kg/d in inducing nitrogen retention. The 25 ug/kg/d dose seemed to cause less -though not completely absent- side-effects: even at this dose (sodium and) fluid retention occurred.
The use of GH in the treatment of cancer-induced cachexia seems troublesome at best, considering the possible mitogenic and tumor growth-stimulating effects of GH and IGF-I (chapter 1.5). In chapter 3 it was shown, however, that in vivo in a rat model GH administration (in a pharmacological dose of 1 mg/rat/day) was found to prevent cancer induced cachexia (and to increase lowered IGF-I levels) without stimulating tumor growth, in spite of the observation that in vitro IGF-I stimulated the growth of cultured tumor cells prepared from this transplantable rat pituitary tumor.
As discussed in chapter 1.2 and 1.3 physiologic GH secretion continues well into old age and GH affects many tissues, either directly or indirectly through IGF-I. Therefore, adults with GH deficiency (either congenital or iatrogenic) might benefit from GH replacement. As demonstrated in chapter 4 GH deficient adults have an abnormal body composition when compared with age, height, weight and sex matched controls: they have more fat and less fat-free mass. In chapter 5 GH substitution treatment for 6 months in 8 GH deficient adults had impressive effects on this abnormal body composition, as well as on bone, carbohydrate and protein metabolism. Body composition changed towards normal: fat mass decreased and fat free mass increased. In parallel a great improvement in subjective well-being was observed. Markers of bone resorption and bone formation increased both, indicating an increase

in bone remodelling. However, during this 6 month study period bone density had not increased under the influence of GH administration. Glucose levels increased slightly without increasing insulin levels. Nitrogen turnover studies showed a transient increase in nitrogen balance due to a slightly greater increase in protein synthesis than in protein degradation. Finally changes of thyroid hormone parameters indicated increased peripheral conversion of T4 to T3. The impressive subjective benefit of GH replacement in adults with GH deficiency warrants the consideration of accepting such therapy in the routine treatment of this type of patients.

SAMENVATTING

De ontwikkeling van recombinant DNA technieken heeft geleid tot een toename van de voor de kliniek beschikbare hoeveelheid humaan GH. GH behandeling in GH deficiente kinderen is verbeterd cq de geadviseerde doseringen teneinde een maximale lengte te bereiken zijn gestegen en de behandeling is nu zonder het risico van de overdracht van virale infecties.
Eventueel gebruik van GH bij volwassenen leidt tot vragen als: bij welke aandoeningen, op welke leeftijd en in welke doseringen kan GH behandeling wellicht nuttig zijn en wat zijn de mogelijke (dosis afhankelijke) bijwerkingen. De indicaties voor GH behandeling bij volwassenen zijn te verdelen in a) GH toediening naast de normale (voedings) behandeling van katabole patiënten die, indien gezond normale GH productie hebben en in b) GH substitutie therapie bij volwassenen die geen of te weinig GH produceren.
In verband met eventueel nadelige effecten van GH op natrium en vocht balans, koolhydraat stofwisseling en mogelijke mitogene effecten (hfdst 1.5) rijst de vraag wat de veiligste (laagste) effectieve GH doseringen zijn.
In hfdst 2 wordt beschreven dat in oudere katabole volwassenen die een optimaal enteraal dieet gebruiken een GH dosis van 25 ug/kg/d gedurende 4 dagen net zo effectief als 50 en effectiever dan 12.5 ug/kg/d aanleiding geeft tot stikstof retentie. De 25 ug/kg/d dosis leek minder doch niet geheel zonder bijwerkingen te zijn: ook deze dosis veroorzaakte (natrium en) vocht retentie.
Het gebruik van GH bij de behandeling van cachexie veroorzaakt door maligniteiten is op zijn minst dubieus gezien de mogelijke mitogene en tumor groei bevorderende effecten van GH en IGF-I (hfdst 1.5). In hfdst 3 wordt beschreven dat in vivo, in een ratten model, GH toediening (in een farmacologische dosering van 1 mg/rat/dag) cachexie voorkomt (en verlaagde IGF-I spiegels deed stijgen) zonder tumor groei te stimuleren, ondanks het feit dat in vitro IGF-I de tumorcel groei van deze ratten-hypophyse tumor stimuleert.
In hfdst 1.2 en 1.3 wordt beschreven dat de fysiologische GH productie aanwezig blijft tot op hoge leeftijd en dat GH effecten heeft op vele weefsels, hetzij direct of indirect via IGF-I. Derhalve kunnen volwassenen met GH deficiëntie (hetzij aangeboren of verkregen op latere leeftijd) mogelijk baat hebben bij GH behandeling. In hfdst 4 wordt beschreven dat GH deficiente volwassenen een afwijkende lichaams samenstelling hebben. In vergelijking met leeftijd, lengte en gewicht gematchte controles hebben zij meer vet en minder vet-vrije massa. In hfdst 5 worden indrukwekkende effecten beschreven van GH substitutie gedurende 6 maanden bij 8 GH deficiente volwassenen op de abnormale lichaams samenstelling, alsmede op bot, koolhydraat en eiwit metabolisme. Lichaams samenstelling normaliseerde: vet massa nam af en vet vrije

massa nam toe. Tegelijkertijd trad er een forse verbetering op van het subjectief welbevinden. Zowel de parameters voor bot resorptie als voor bot vorming stegen ten teken van een toegenomen bot ombouw. Gedurende de 6 maanden durende GH behandeling trad er echter geen stijging van de bot densiteit op. Bloedsuiker spiegels stegen iets, zonder stijging van de insuline spiegels. Onderzoek van de stikstof turnover liet een tijdelijke stijging van de stikstof balans zien ten gevolge van een iets grotere toename van de eiwit synthese in vergelijking met de eiwit afbraak. Tenslotte toonden de veranderingen in schildklier hormoon parameters een toegenomen perifere conversie van T4 naar T3. De indrukwekkende subjectieve verbetering van GH toediening aan volwassenen met GH deficiëntie rechtvaardigt de overweging om deze behandeling bij deze patiënten routinematig toe te gaan passen.

NAWOORD

Zoals altijd is ook dit boekje tot stand gekomen met hulp van velen. Zonder de ongenoemden tekort te willen doen wil ik enkelen met name bedanken:

Mijn promotor, Steven Lamberts, heeft mij, met zijn aanstekelijke optimisme, in voor- en tegenspoed de juiste weg gewezen. Het was een voorrecht om samen met hem onderzoek te kunnen doen.

Cis Baarschens heeft samen met de andere verpleegkundigen van de "Balans afdeling", waar al het patientgebonden onderzoek plaats vond, zorg gedragen voor de nauwkeurige uitvoering van de diverse protocollen. Vooral bij de balans studies was de hulp van Cootje van Aller en van de afdeling Dietiek onontbeerlijk.

Bob Zietse was oa. bij vele PC-problemen mijn steun en toeverlaat, tevens heeft hij de lay-out van dit boekje snel en zeer goed verzorgd.

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Machteld, dank voor je steun die je me gaf ondanks je begrijpelijke ambivalentie ten opzichte van dit boekje. Ik was in tijden van tegenslag, blij met je bevrijdende, relativeringsvermogen. Het is nu af, vakantie zal weer vakantie zijn!

STELLINGEN

behorend bij het proefschrift van A. Binnerts

1. De minimale dosering GH nodig om de stikstof balans positief te beinvloeden bij oudere volwassenen tijdens optimale orale voeding bedraagt 25 µg/kgBW/dag.

2. In het ratten model van de transplanteerbare hypofysetumor 7315b voorkomt GH toediening het ontstaan van door kanker geinduceerde cachexie, zonder dat dit aanleiding geeft tot stimulatie van kankergroei.

3. Groeihormoon deficientie op de volwassen leeftijd geeft aanleiding tot een afwijkende lichaamssamenstelling.

4. Ook op de volwassen leeftijd dient GH deficientie behandeld te worden.

5. De term "groeihormoon" dekt de lading niet, het zou beter zijn dit hormoon "anaboline" te noemen.

6. Het nuttig effect van totale parenterale voeding is onbewezen.

7. Niet enzymatische glycosylering van hemoglobine wordt mede beinvloed door factoren anders dan glucose, oa. zuurstof spanning en 2,3-diphosphoglyceraat gehalte.
Smith RJ, Koenig RJ, Binnerts A, Soeldner JS, Aoki TT. J Clin Invest 1982;69:1164-9.

8. De verhoging van het risico op auto ongevallen bij diabetici is te gering om beperking van de afgifte van rijbewijzen te rechtvaardigen.
Hansotia P, Broste SK. N Engl J Med 1991;324:22-6.

9. Bij diabetici gaat de behandeling van hypertensie met diuretica gepaard met een viervoudige stijging van de sterfte.
Warram JH, Laffel LMB, Valsania P, Christlieb AR, Krolewski AS. Arch Intern Med 1991;151:1350-6.

10. Wettelijke maatregelen dienen genomen te worden ter beteugeling der kwakzalverij.
(stelling bij het proefschrift van A. Binnerts, Over lokalisatie van functies in het cerebellum; Amsterdam, 7 april 1908)

11. Een algeheel verbod van het gebruik van saccharine in voedings- en genotmiddelen behoort uitsluitend afhankelijk gesteld te worden van de beantwoording van de vraag, of deze stof al dan niet schadelijk is voor de gezondheid.
(stelling bij het proefschrift van A.J. Kluyver, Biochemische suikerbepalingen; Delft, 15 mei 1914)

12. Het is van belang het huidige honoreringssysteem voor artsen bij te stellen, opdat het gebruik van technologie niet hoger wordt beloond dan bijvoorbeeld het gesprek met een patient of het lichamelijk onderzoek.
(Kiezen en delen, advies in hoofdzaken van de commissie "Keuzen in de zorg").

13. Het feit dat per geinstitutionaliseerd geestelijk gehandicapt kind veel meer gemeenschapsgeld wordt uitgegeven dan er beschikbaar is voor ouders die met hulp van derden hun geestelijk gehandicapt kind thuis willen opvoeden is strijdig met de geest van het begrip "zorgzame samenleving".

14. Het gebruik van groeihormoon in de veehouderij dient gestimuleerd te worden, onder andere omdat hierdoor het stikstof en fosfaat gehalte van de mest, en derhalve de verzuring, verminderd kan worden.

Rotterdam 13 mei 1992

CURRICULUM VITAE

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12 maart 1959	:	Geboren te Dordrecht.
1971-1977	:	VWO, Gemeentelijk Lyceum Dordrecht.
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1984-1989	:	AGIO, afd Inwendige Geneeskunde III, Academisch Ziekenhuis Rotterdam-Dijkzigt, opleider Prof Dr J.C. Birkenhäger.
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