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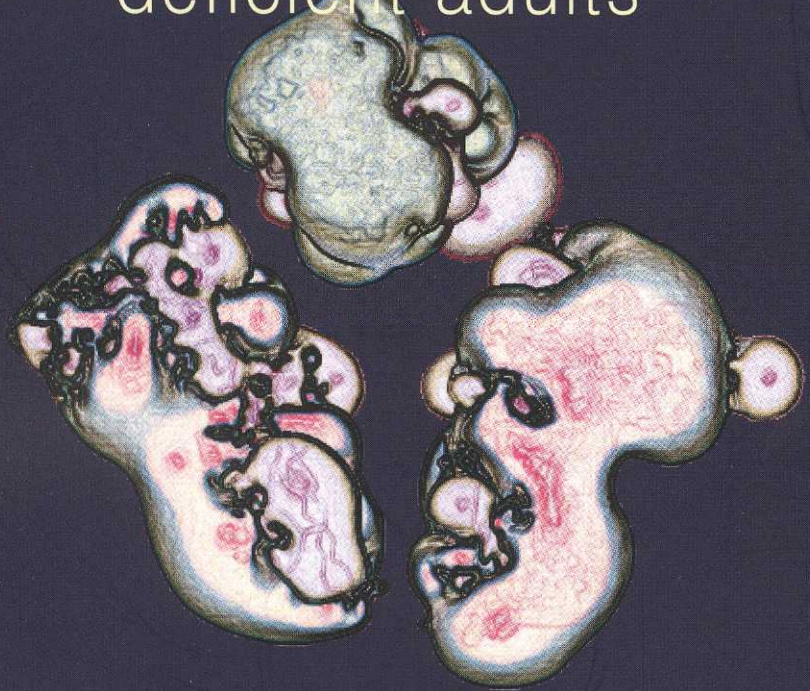
Metabolic and central nervous effects of Growth Hormone in Growth Hormone-deficient adults



by
Jan-Ove Johansson

GÖTEBORG
1996

Metabolic and central nervous effects of growth hormone in growth hormone-deficient adults

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen
vid Göteborgs Universitet
kommer att offentligen försvaras
i aulan, Sahlgrenska sjukhuset,
torsdagen den 23 maj 1996, kl 09.00

av

Jan-Ove Johansson

Avhandlingen baseras på följande delarbeten:

I. Johansson J-O, Fowelin J, Landin K, Lager I, Bengtsson B-Å. Growth hormone-deficient adults are insulin-resistant. Metabolism 1995; 44:1126-1129.

II. Johansson J-O, Landin K, Tengborn L, Rosén T, Bengtsson B-Å. High fibrinogen and plasminogen activator inhibitor activity in growth hormone-deficient adults. Arterioscler Thromb 1994;14:434-437.

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IV. Johansson J-O, Oscarsson J, Bjarnason R, Bengtsson B-Å. Two weeks of daily injections and continuous infusion of recombinant human growth hormone (GH) in GH-deficient adults: I. Effects on insulin-like growth factor 1 (IGF-1), GH and IGF binding proteins, and glucose homeostasis. Metabolism 1996;45:362-369.

V. Johansson J-O, Larson G, Andersson M, Elmgren A, Hynsjö L, Lindahl A, Lundberg P-A, Isaksson OGP, Lindstedt S, Bengtsson B-Å. Treatment of growth hormone-deficient adults with recombinant human growth hormone increases the concentration of growth hormone in the cerebrospinal fluid and affects neurotransmitters. Neuroendocrinology 1995;61:57-66.

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The aim of this study was to investigate adult GH deficiency in terms of metabolic and hemostatic factors associated with cardiovascular disease. Furthermore, the effects of long-term treatment with GH on hemostasis and the effects of GH on neurotransmitters and GH-dependent factors in the cerebrospinal fluid (CSF) were studied. We also compared the metabolic effects of different GH administration modes.

Insulin sensitivity was assessed using the hyperinsulinemic euglycemic clamp technique. Glucose infusion rate in GH-deficient subjects was less than half compared with controls ($p<0.001$), thus indicating that GH-deficient adults are insulin-resistant. Despite this, a normal fasting insulin level was found. Patients had similar or even lower fasting blood glucose levels ($p<0.05$) and lower fasting free fatty acid levels compared with controls ($p<0.01$). The waist over hip circumference ratio ($p<0.001$), plasminogen activator inhibitor (PAI-1) activity ($p<0.05$), fibrinogen ($p<0.001$) and serum triglyceride levels ($p<0.05$) were higher in the patients.

After 18-24 months of GH treatment, PAI-1 activity, PAI-1 antigen and tissue plasminogen activator (t-PA) antigen decreased ($p<0.05$). The rapid plasmin inhibitor, a_2-antiplasmin, ($p<0.05$) and the coagulation inhibitor, protein C ($p<0.05$), decreased. Blood glucose levels did not differ after two years of GH treatment, but fasting insulin levels ($p<0.01$) and lean body mass ($p<0.001$) increased .

In an administration study, serum insulin-like growth factor 1 (IGF-1) and IGF binding protein-3 (IGFBP-3) levels increased to a higher degree during two weeks of subcutaneous continuous infusion of GH compared with daily subcutaneous injections in the evening ($p<0.01$). Fasting free fatty acid levels only increased during treatment with daily injections of GH ($p<0.01$). The fasting blood glucose level and an oral glucose tolerance test indicated more impaired glucose tolerance after daily injections of GH compared with continuous infusion.

In another study, the mean CSF GH concentration increased tenfold compared with baseline ($p=0.002$) after one month of GH treatment. CSF IGF-1 ($p=0.005$), CSF IGFBP-3 concentrations ($p=0.002$) and CSF ß-endorphin immunoreactivity also increased ($p=0.002$) while dopamine metabolite homovanillic acid ($p=0.02$) and vasoactive intestinal peptide ($p=0.03$) decreased.

In conclusion, insulin resistance and abnormalities in fibrinolysis and coagulation can be added to the syndrome of GH deficiency in adults. During GH treatment, favourable changes in body composition and the fibrinolytic system were noted. Continuous infusion of GH resulted in higher serum IGF-1 and IGFBP-3 levels compared with daily injections. Moreover, our study indicates that GH passes the blood-CSF barrier and that GH affects the CSF levels of both IGF-1 and neurotransmitters. Neuroendocrine mechanisms might be involved in the improvement in psychological well-being observed during GH treatment.

Key words: Growth hormone, growth hormone deficiency, insulin-like growth factor 1, insulin resistance, free fatty acids, fibrinogen, plasminogen activator inhibitor-1, coagulation, fibrinolysis, waist over hip circumference ratio, cerebrospinal fluid, neuropeptides **ISBN 91-628-2024-9**

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by

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Göteborg 1996

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To Eva, Frida and Aron

Front page - Growth hormone and its receptor. After an original illustration kindly provided by Olle Nilsson.

List of papers

This thesis is based upon the following papers, which will be referred to in the text by their Roman numerals:

I. Johansson J-O, Fowelin J, Landin K, Lager I, Bengtsson B-Å. Growth hormone-deficient adults are insulin-resistant. Metabolism 1995; 44:1126-1129.

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Contents

Abbreviations

ACTH	Adrenocorticotropic hormone
BMI	Body mass index
CRF	Corticotropin-releasing factor
CNS	Central nervous system
CSF	Cerebrospinal fluid
FFA	Free fatty acid
GABA	γ-aminobutyric acid
GH	Growth hormone
GHBP	Growth hormone binding protein
GHD	Growth hormone deficiency
GIR	Glucose infusion rate
HDL	High-density lipoprotein
5-HIAA	5-hydroxyindoleacetic acid
HVA	Homovanillic acid (4-hydroxy-3-methoxyphenylacetic acid)
IGF-1	Insulin-like growth factor 1
IGFBP	Insulin-like growth factor binding protein
i.m.	Intramuscular
i.v.	Intravenous
LBM	Lean body mass
LDL	Low-density lipoprotein
MHPG	3-methoxy-4-hydroxyphenylethyleneglycol
MSH	Melanocyte stimulating hormone
PAI-1	Plasminogen activator inhibitor 1
rhGH	Recombinant human growth hormone
s.c.	Subcutaneous
t-PA	Tissue plasminogen activator
TSH	Thyroid-stimulating hormone (thyrotropin)
VIP	Vasoactive intestinal peptide
WHR	Waist over hip circumference ratio

1. Introduction

Historical background

Although it has been recognized for many years that growth hormone (GH) is also secreted in adult life, it has been claimed that GH exerts no effects in adults (205). However, early anecdotal evidence has hinted at the metabolic effects of GH in adults. In 1962, increased vitality following GH therapy in one adult patient with panhypopituitarism was reported (167). Furthermore, in his thesis in 1963, Thomas Falkheden described the pathophysiological effects of hypophysectomy in adult patients who were hypophysectomized because of metastatic mammary carcinoma, diabetes mellitus or acromegaly. Despite conventional hormone replacement therapy, there was a decrease in basal metabolism, renal function, blood volume and cardiac performance within one month postoperatively. It was postulated that the observed changes were due to the abolition of or reduction in GH secretion caused by the hypophysectomy (56).

Since GH deficiency (GHD) is an early event in the development of hypopituitarism, most patients with GHD acquired in adult life also have deficiencies in the regulation of the thyroid gland, adrenal cortex and gonads. Until recently, conventional hormone replacement treatment for these patients has been L-thyroxine, corticosteroids and sex hormones.

GHD in adults

In recent years, the clinical features of GHD in adults have been defined. In GH-deficient adults, characteristic changes in body composition with decreased LBM, reduced total body water, mainly extracellular, and increased body fat have been consistent findings (40, 95, 179, 185). In addition, a decrease in bone mineral content (106, 181), a reduction in glomerular filtration rate, renal plasma flow (95), sweating (162), thermoregulation (93), maximum oxygen uptake during exercise (36), cardiac function (7, 140), isometric muscle strength and exercise capacity have been demonstrated (100). Moreover, poor quality of life in terms of energy, social isolation and emotional distress has been recorded in GH-deficient adults (18, 133, 182).

An association with GHD and premature arteriosclerosis has been suggested by the observation of an almost twofold increase in cardiovascular mortality found in a retrospective study of 333 patients with hypopituitarism (178) and by the increased number of atheromatous plaques in the femoral and carotid arteries of patients with hypopituitarism (131). Increased concentrations of serum triglyceride, serum total cholesterol and LDL cholesterol, as well as decreased serum HDL cholesterol concentration, have been reported in GH-deficient adults (38, 122, 131, 139, 180). In one study by Rosén et al., the prevalence of treated hypertension was higher, whereas the prevalence of smoking was lower in the GH-deficient group compared with controls (180). The dyslipidemia may, at least in part, explain the increased prevalence of cardiovascular disease in GH-deficient adults. However, there are several other factors known to be associated with cardiovascular disease which have not been sufficiently evaluated in GH-deficient adults. These factors include fibrinogen levels, PAI-1 activity and insulin sensitivity.

From the late 1950s, GH-deficient children were treated with GH prepared from human cadaveric pituitaries. Due to the limited supply of GH, clinical trials of GH treatment in hypopituitary adults were not possible until biosynthetic human GH produced by recombinant DNA techniques became available in the mid-1980s.

Replacement therapy with GH in GH-deficient adults alters body composition

through its lipolytic, protein anabolic and anti-natriuretic actions. An increase in lean body mass, a decrease in fat mass with a redistrubution of fat from visceral to peripheral depots, as well as an increase in extracellular water volume, have been consistent findings in many studies (12, 16, 43, 100, 185). GH replacement therapy increases the rate of bone turnover (193, 207) and increases both bone mineral density and bone mineral content (43, 89). Furthermore, a decrease in total serum cholesterol (87, 185) and LDL cholesterol levels, as well as an increase in HDL cholesterol levels, have been observed (87). In addition, GH replacement therapy in GH-deficient adults is associated with increased exercise capacity (95, 100, 207), improved cardiac function (37) and improved quality of life, mainly in terms of energy level and mood (133). However, the mechanisms underlying these beneficial effects on psychological well-being are unknown.

Coagulation and fibrinolysis

Hemostasis depends upon complex interactions between plasma coagulation, fibrinolysis, blood cells and vessel walls, as well as blood viscosity and blood flow. Fibrinogen (coagulation factor I) is a glycoprotein which is mainly produced in the liver. The cleavage of fibrinogen to fibrin by thrombin is the final step in the coagulation process. Fibrin and platelets then combine to form a clot. Previous population-based studies have shown that fibrinogen is an independent risk factor for stroke as well as myocardial infarction, at least as important as blood lipids and blood pressure (136, 208). Fibrinogen is also an acute-phase protein and the concentration increases as a result of inflammation, infection, injury and stress. In fibrinolysis, fibrin is proteolytically degraded in order to prevent thrombus formation. It is generally accepted that fibrinolytic activity influences the growth, size and dissolution of the thrombus. The fibrinolytic process is schematically outlined in **Fig. 1**.

t-PA converts plasminogen to the key enzyme plasmin, whereafter the degradation of the fibrin network can take place. PAI-1, the fast-acting t-PA inhibitor, is the major regulator of fibrinolytic activity in plasma. The mature PAI-1 molecule is a 379 amino acid long single-chain glycoprotein which is mainly synthesized in endothelial cells, vascular smooth-muscle cells, hepatocytes and platelets (109, 190, 192). In the circulation, PAI-1 antigen occurs in three main forms: as an active molecule, as a t-PA/PAI-1 complex and in a latent, inactive form. PAI-1 exerts its inhibitory action by forming an inactive complex with

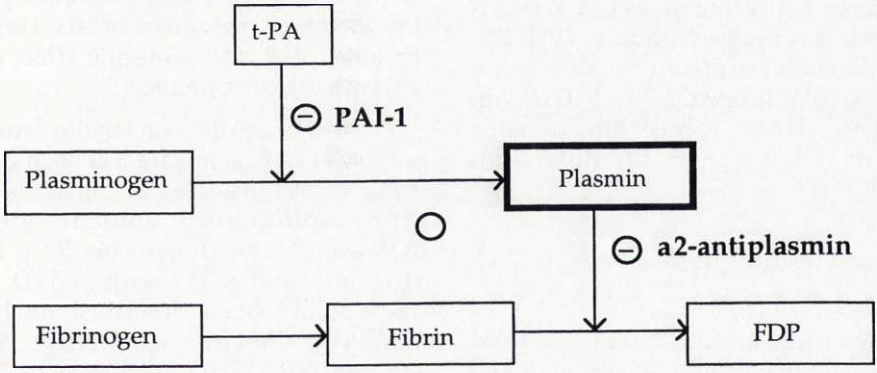

Fig. 1. Diagram of the fibrinolytic process. FDP=Fibrin degradation products.
Θ = inhibitory effect.

t-PA which is then cleared through the liver. The mechanisms underlying the changes in PAI-1 activity in plasma are largely unknown. However, PAI-1 synthesis in vitro is stimulated by a variety of factors including endothelial damage, inflammation, endotoxin, thrombin, transforming growth factor ß, basic fibroblast growth factor, epidermal growth factor and hormones such as glucocorticoids and insulin (109, 190, 192). Increased PAI-1 activity acts in a thrombogenic direction. Elevated PAI-1 activity has been associated with coronary artery disease (92, 158), increased risk of myocardial infarction in young patients (65), recurrent myocardial infarction (66) and deep vein thrombosis (153). In addition, high PAI-1 activity has been found in individuals with abdominal obesity, hypertension and type II diabetes (67, 83, 91, 114, 115, 144, 204). A positive correlation has been found between PAI-1 activity and VLDL triglyceride concentration, as well as with insulin concentration, BMI and WHR (10, 29, 52, 62, 67, 83, 114, 115, 144, 204). Other factors in hemostasis, such as factor VII and t-PA antigen, have also been associated with an increased risk of cardiovascular disease (84, 108, 136, 173).

In vivo studies involving GH administration for a few hours up to a week have suggested that GH plays a role in the regulation of von Willebrand factor which circulates in a complex with factor VIII (21, 80, 189). In contrast, no effect was seen on fibrinogen, t-PA antigen, factor VIII or von Willebrand factor levels during four months of GH treatment in adults with GHD (98).

Effects of GH on glucose homeostasis

GH exerts both insulin-like and insulin-antagonistic effects, although the physiological significance of the former is uncertain. A transient *insulin-like* effect is observed during the first three hours after GH administration (1, 17, 128, 201). The *insulin-antagonistic* or diabetogenic effect on glucose metabolism is obvious in acromegaly, a condition with chronic GH excess due to a GH-producing pituitary adenoma (39, 68). When high doses of GH are administered to normals, the insulin-antagonistic effect is observed after a lag of three to four hours (166, 183). In sustained, more physiological doses, GH appears to reduce the sensitivity to insulin in liver, muscle and fat tissue (25, 57, 128, 148, 149, 176). The impairment of peripheral insulin sensitivity is largely confined to skeletal muscle (25, 148). Several studies have indicated that GH effects insulin action at the post-receptor level (25, 68, 104, 124, 148, 176, 183). In addition, a decrease in glucose transporters has been noted during GH treatment (55, 200). GH increases lipolysis and elevates FFA levels in both normals (39, 213) and GH-deficient adults (94, 147). It has therefore been suggested that some of the insulin-antagonistic effect of GH may be secondary to the increased availability of FFA which competes with glucose as an oxidative substrate (Randle's glucose-fatty acid cycle) (39, 168). The lipolytic effect of GH may involve an increase in the activity of hormone-sensitive lipase which catalyses the breakdown of stored triglycerides into glycerol and fatty acids (47). Increased insulin levels are well-documented during GH treatment and may be secondary to the insulin-antagonistic effect of GH. However, the suggested insulinotropic effect of GH (151) may also contribute.

Most of the data on insulin sensitivity in the GH-deficient state has been derived from early studies of animals with panhypopituitarism without adequate replacement treatment (6, 77). Hypopituitary patients with GHD have traditionally been described as having increased insulin sensitivity. Young children with GHD are prone to fasting hypoglycemia (24, 60), possibly due to a decrease in hepatic glucose production, which is lower than glucose utilization (22).

Low glycogen stores (22), as well as decrease in the availability of ketone bodies (210), may contribute to this tendency towards fasting hypoglycemia. The availability of ketone bodies, in turn, depends upon an adequate supply of FFA and their synthesis into ketone bodies by the liver.

In GH-deficient *adults*, fasting blood glucose concentrations are normal (131) and hypoglycemia is rare. However, prolonged fasting (four days) leads to decreased glucose levels compared with controls. Associated with this profound decrease in glucose is a higher than normal decrease in plasma insulin levels and concomitantly higher levels of FFA and ketone bodies (138). Hypoglycemic responsiveness to i.v. insulin is normal in GH-deficient adults, but the return of the blood glucose towards basal concentrations is delayed (59, 116). The cause of this delay may be a combination of decreased glycogen stores and the lack of GH which is of importance to the subsequent phase of glucose counter-regulation after hypoglycemia (57).

Under oral glucose loads, glucose tolerance was impaired, but the majority of the patients displayed hypoinsulinemia, in both GH-deficient adults (137) and children (124). A greater prevalence of abnormal glucose tolerance in GH-deficient adults has been reported (131). Fasting plasma insulin levels were normal in lean GH-deficient adults (131) but increased in obese GH-deficient subjects compared with controls (186). However, information about insulin sensitivity in the untreated GH-deficient state has been limited.

Secretory pattern and different modes of administration of GH

The normal secretion pattern of GH is pulsatile, in both man and animals. In male rats, GH is secreted in a pulsatile fashion with low or undetectable levels between bursts, whereas, in female rats, secretion is more continuous with higher baseline levels than in males (50). Several differences in hepatic metabolism between male and female rats are explained by the sexually dimorphic secretory pattern of GH (50, 141). The intermittent administration of GH to hypophysectomized rats has been more effective than continuous administration in terms of body weight gain, longitudinal bone growth (34, 85), increase in plasma IGF-1 level (129) and increase in IGF-1 mRNA levels in various tissues (81, 130). The effect of different GH administration modes differs in some ways in the rat and in humans.

In humans, the secretory pattern of GH does not differ between men and women to the same extent as in the rat. However, the mean 24-hour integrated GH secretion is higher in women than in men, as are the mean baseline GH levels (197, 209). During pregnancy, the GH secretory pattern becomes more continuous, with higher basal levels from the beginning of the second trimester (54). An increase in basal GH levels has also been observed during fasting (69) and in poorly controlled diabetes mellitus (9). Thus, changes in basal GH concentration may produce various metabolic adjustments during physiological and pathophysiological states.

At present, rhGH is routinely administered as a daily s.c. injection in the evening. The serum GH profile following an evening s.c. GH injection differs from the physiological secretory pattern and can be characterised as an intermediate pattern between a pulsatile and a continuous pattern (32). Long-term replacement therapy with GH in GH-deficient subjects should theoretically mimic the complex physiological pattern of GH secretion. However, a physiological pattern of GH release is best simulated using the i.v route, which is difficult to achieve over prolonged periods. As an alternative to daily s.c. injections of GH, a sustained-release preparation has been proposed. The use of

such a GH preparation would presumably result in the continuous delivery of GH.

In GH-deficient children, an improved long-term linear growth response is obtained when a fixed weekly GH dose is administered as daily s.c. injections compared with i.m. injections two or three times weekly (105). The higher plasma IGF-1 level obtained with the former administration mode may contribute to the improved growth (33). Most studies designed to compare different modes of GH administration in GH-deficient adults have involved a treatment period of 24 hours only. In these studies, the increase in serum IGF-1 level was less pronounced following two i.v. GH pulses per day than following eight pulses or the continuous i.v. infusion. However, the effects on glucose homeostasis, lipid intermediates and IGFBP-3 were similar (97, 99). The metabolic effects of prolonged treatment with continuous GH administration in GH-deficient adults have not been fully elucidated.

Central nervous effects of GH

During GH replacement therapy in GH-deficient adults, quality of life improves in terms of energy level and mood (133). In many patients, this improvement is dramatic. These beneficial effects of GH may be explained in part by the normalisation of body composition and the subsequent improvement in exercise capacity. However, it is also possible that GH may have direct neuroendocrine effects on the CNS. In fact, neural actions by GH were first documented by Zamenhof in experiments on tadpoles in the early 1940s (211). A possible direct effect by GH on the CNS is supported by the finding that GH-like material appears in the rat brain before its ontogenic appearance in the adenohypophysis (75). GH may thus play an important role in brain development, maturation and function. In studies of rodents and primates, it has been shown that the greatest content of immunoreactive GH is in the amygdala, hippocampus and hypothalamus. However, the concentrations are less than 1% of those in the anterior pituitary gland (74). The long-term presence of immunoreactive GH in the brain following hypophysectomy also suggests a central site of synthesis, as does the continuous release of immunoreactive GH from dispersed CNS cells from the rat grown in tissue culture (74). Moreover, the presence of GH mRNA in several areas of the brain supports the hypothesis of local synthesis of GH in the brain (61). It is also well established that GH stimulates neuronal and glial proliferation, myelination (154, 163) and RNA synthesis in the rat brain (14).

Acromegalics have higher CSF GH levels compared with controls (123). However, the question of the permeability of the blood-CSF barrier to GH has been controversial. The infusion of large amounts of human GH to rhesus monkeys was not followed by a parallel increase in CSF GH levels (11). On the other hand, intra-peritoneal injections of ^{125}I-labelled GH to rats resulted in an accumulation of radioactivity in several brain areas, thereby suggesting that GH crosses the blood-brain barrier in this species (196). It is also known that the systemic GH treatment of both normal and hypophysectomized rats influences the cerebral concentrations of monoamines. Monoamine changes were found as early as 15 min. after an injection of GH, thus suggesting that GH is rapidly taken up by brain tissue and quickly affects ongoing monoamine metabolism (195). GH also produced a rapid reduction in dopamine and noradrenaline concentrations in the median eminence (8). The widespread presence of GH receptors in the CNS of several species has been reported (46, 125), whereas the information about GH receptors in the human brain has been scarce (46).

2. Aims of the studies

- to investigate insulin sensitivity in untreated adult GH-deficient patients (Paper I)
- to study fibrinogen concentrations and PAI-1 activity in adult GH-deficient patients (Papers II and III)
- to evaluate the effects of long-term treatment with GH on coagulation and fibrinolysis (Paper III)
- to compare the metabolic effects of different GH administration modes (Paper IV)
- to study the effects of GH on neurotransmitters and GH-dependent factors in the CSF (Paper V)

Table 1. Characteristics of the subjects in Papers I-V

Characteristic	Paper I		Papers II and V		Paper III	Paper IV
Subgroup	Patients	Controls	Patients	Controls (II)	Patients	Patients
Number	15	15	20	20	17	9
Sex, M/F	11/4	11/4	10/10	10/10	9/8	9/0
Mean age, years	47.7±12.4	51.9±5.2	50.5±10.8	52.2±3.7	52.5±10.6	47.2±6.7
BMI, kg/m^2	27.5±5.7	27.3±3.6	26.4±3.0	26.1±3.0	27.2±2.9	27.7±2.9
Duration of hypopituitarism, years	12.7±12.7	-	11.8±10.6	-	12.6±11.3	5.3±3.7
Original diagnosis						
Non-secreting adenoma	5	-	14	-	12	7
Prolactinoma	6	-	2	-	1	1
Craniopharyngioma	-	-	2	-	2	-
Meningioma	1	-	-	-	-	-
Sheehan´s syndrome	1	-	1	-	1	-
Idiopathic hypopituitarism*	-	-	1	-	1	1
GHD since childhood	2	-	-	-	-	-
Surgery and radiation						
Transsphenoidal operation	2	-	8	-	8	7
Transcranial operation	9	-	10	-	7	2
Radiotherapy	10	-	9	-	7	5
Replacement therapy						
Corticosteroid	13	-	12	-	9	2
Thyroxine	13	-	16	-	13	6
Gonadal steroids	10	-	8	-	6	5
Desmopressin	3	-	4	-	3	1

*Enlarged and thick fibrous dura mater. Values are mean ± SD

3. Subjects

Patients

All the patients in Papers I-V came from the catchment area of the Endocrine Unit which is the city of Göteborg and the western part of Sweden, comprising about one and a half million inhabitants. The total number of patients included in the different papers were 34 (23 men and 11 women). The characteristics of the patients are shown in **Table 1**. The 20 patients in Papers II and V were the same and 17 of these patients were included in Paper III.

All the patients had been investigated as in-patients at the Endocrine Unit because of pituitary disorders. The majority of the patients had received surgical treatment for pituitary tumors and had multiple pituitary deficiencies. The patients were on stable replacement therapy with glucocorticoids (cortisone acetate, 12.5 mg twice a day), L-thyroxine (0.10-0.15 mg/day) and sex hormones. Two patients in Paper I had had isolated GHD since childhood and three patients in Papers II, III and V had isolated GHD acquired in adult life. Two of these three patients were included in Paper IV.

In Papers II, III and V, one patient was being treated with a calcium blocker, one with a ß-blocker and one with an angiotensin-converting enzyme inhibitor because of hypertension. Four men in Paper I, two men in Papers II and V and one man in Papers III and IV were being treated with bromocriptin. Four patients were current smokers, nine former smokers and 21 non-smokers.

Diagnosis of GHD

GHD was defined as a peak serum GH concentration of below 5 mU/L during an i.v. insulin tolerance test (0.1 IU insulin/kg body weight) (73). All the patients showed symptoms of hypoglycemia and had a blood glucose of < 2.2 mmol/L during the test.

Controls

The healthy control subjects in Papers I and II were recruited by advertisements in the local newspaper. The criteria for being healthy, in addition to subjective well-being, were a history of no hospital visits, no diabetes mellitus or hypertension and no medical treatment for any disease during the past two years. Of 255 answers in the 40-60 year age group, 207 fulfilled the criteria for healthy controls. The controls were matched groupwise against the patients for age, sex and BMI.

Ethical aspects

All the studies were approved by the Ethics Committee at the Medical Faculty at Göteborg University and all the patients gave their informed consent.

4. Methodological considerations

In this section, some methods are described in greater detail than in the separate papers. For methods not addressed here, the reader is referred to Papers I-V.

Glucose clamp technique

To evaluate insulin sensitivity, a hyperinsulinemic, euglycemic clamp was performed, essentially as described by DeFronzo et al. (42). All the clamps were started in the morning after an overnight fast and were performed in the recumbent position. Patients waited with their normal hormone replacement and medical treatment until after the clamp had been performed. A catheter (Venflon, Ohmeda, Helsingborg, Sweden) was inserted into a cubital vein for glucose, insulin and potassium infusions. Blood samples were collected from a second catheter (Venflon) placed in a hand vein on the contralateral

arm. Both arms were warmed with electric heating pads in order to arterialize the venous blood and increase the blood flow for repeated blood sampling. The plasma insulin concentration was acutely increased by an infusion of a priming dose of insulin (Actrapid Human, Novo Nordisk, Copenhagen, Denmark) for 10 min. followed by a constant infusion of insulin dissolved in isotonic saline (40 mU/m^2/min). The blood glucose concentration was kept constant close to 4.5 mmol/L by a variable 20% glucose infusion. Potassium chloride was infused at a rate of 7 mmol/h to prevent hypokalemia. Venous blood samples were drawn every 5 min to rapidly measure blood glucose with glucose test strips (BM-test glycemie 1-44, Boehringer Mannheim, Germany) and a reflectometer (Reflolux II, Boehringer). Blood samples for subsequent chemical determinations were also drawn every 20 min during the clamps. The glucose levels in the results are from these samples and were measured using the glucose-6-phosphatase dehydrogenase technique (Beckman, Fullerton, CA, USA).

The clamp was performed for two hours and the rate of insulin-mediated glucose uptake was calculated from the steady-state glucose infusion rate over the last 30 min. In these steady-state conditions, all the glucose infused is taken up by the peripheral tissues and thus serves as a measure of the sensitivity of the tissue to the infused insulin. The glucose disposal rate during the clamp was expressed as both the amount of glucose infused per kg of body weight and per kg of LBM. It is important to express glucose uptake as a function of muscle mass since the skeletal muscles are the major determinants of glucose elimination in response to insulin. With the present plasma insulin levels (approximately 80-90 mU/L), an almost total suppression of hepatic glucose production is obtained during the clamps (58).

Oral glucose tolerance test

In order to evaluate the effect of GH treatment on glucose tolerance, an oral glucose tolerance test with 100 g of glucose dissolved in water was performed after an overnight fast. The test started at 8-9 am and venous blood samples were taken for glucose, insulin and C-peptide determinations at 0, 30, 60, 90 and 120 min.

Measurements of fibrinolytic variables and fibrinogen levels

All the venous blood samples were drawn in the morning (8-9.30 am) after an overnight fast. To determine PAI-1 activity, t-PA antigen and fibrinogen, blood samples were drawn in 5-mL vacuum tubes containing 0.5 mL of 0.13 mol/L sodium citrate buffer (Venoject, Terumo Europe N.V., Leuven, Belgium). To determine PAI-1 antigen, blood samples were drawn in 5-mL vacuum tubes containing 0.5 mL of 0.45 mol/L sodium citrate buffer (Biopool Stabilyte, Biopool, Umeå, Sweden). All the samples were immediately centrifuged at 2000 *g* for 20 minutes.

PAI-1 activity was measured using a Spectrolyse (pL) PAI kit (Biopool). The total coefficient of variation (CV) was 12.0% (at 11.9 U/mL) and 6.3% (at 43.6 U/mL). The method is an indirect two-stage enzymatic assay. In the first stage, a fixed amount of t-PA is added to the plasma and allowed to react with the PAI-1 present. In the second stage, the residual t-PA activity catalyses the conversion of added plasminogen to plasmin, which in turn hydrolyses a chromogenic substrate. The amount of colour which develops is directly proportional to the amount of t-PA in the sample. As a result, the PAI-1 activity is equivalent to the inhibited amount of t-PA activity.

PAI-1 antigen was measured using a Tint Elize PAI-1 kit (Biopool) which is a double antibody enzyme-linked immuno-

sorbent assay (ELISA). The total CV was 10.0% (at 7.3 ng/mL) and 6.5% (at 12.9 ng/mL).

t-PA antigen was measured using a Coaliza t-PA kit (Chromogenix, Mölndal, Sweden) which is a double antibody ELISA. The total CV was 9.5% (at 5.9 ng/mL) and 8.1% (at 52.3 ng/mL).

Fibrinogen was measured according to a syneresis method (152). The total CV was 4.1% (at 2.3 g/L).

Measurements of GH, IGF-1 and IGFBP-3 in serum

GH concentrations in serum were determined using a polyclonal immunoradiometric assay (IRMA) method (Pharmacia hGH RIA, Pharmacia, Uppsala, Sweden). The method had a detection limit of 0.3 mU/L (zero standard ± 2SD) and the total CV for the method was 5.3% (at 19.8 mU/L) and 6.1% (at 84.9 mU/L).

IGF-1 concentrations in serum were determined using a hydrochloric acid-ethanol extraction radioimmunoassay (RIA) using authentic IGF-1 for labelling (Nichols Institute Diagnostic, San Juan Capistrano, CA, USA). The method had a detection limit of 13.5 µg/L with a total CV of 8% (at 67 µg/L) and 6% (at 332 µg/L).

IGFBP-3 concentrations in serum were determined using an RIA (Nichols Institute). The method had a detection limit of 0.06 mg/L with a total CV of 6.2% (at 2.05 mg/L) and 5.7% (at 3.49 mg/L).

Measurements of GH, IGF-1, IGFBP-3, ß-endorphin immunoreactivity and monoamine metabolites in CSF

The patients were put on a fast and confined to bed from midnight until the lumbar puncture was performed between 8 and 9 am on the following day. Only hormone-replacement medication was taken in the morning. From the first mixed 12 mL of CSF, 1-mL aliquots were frozen after centrifugation for the subsequent analysis of monoamine metabolites and IGFBP-3. The 15th to 25th mL of CSF were collected in 2-mL portions directly in prechilled polypropylene tubes (Cryotubes, Nunc, Roskilde, Denmark) containing peptidase inhibitors and frozen for the subsequent analysis of GH, IGF-1, opioid peptides and neuropeptides. Serum was collected simultaneously with the CSF and frozen in aliquots.

GH levels in CSF were measured using an IRMA (BioMérieux, Lyon, France). The method had a detection limit of 1.3 µU/L of GH and the intra-assay CV for the method was 3.5% (at 41.8 µU/L) and 3.3% (at 79.0 µU/L). The assay was calibrated against the first international standard from the WHO (80:505; 2.6 U/mg). The linearity of the method was tested with a serial dilution procedure of CSF samples. The dilution curves were parallel to the standard curve, thus excluding unspecific cross-reactivity.

IGF-I levels in CSF were determined using an RIA (Nichols Institute, Wijehen, the Netherlands). The method had a detection limit of 0.1 µg/L and the intra-assay CV for the method was 6.2% (at 0.28 µg/L) and 5.2% (at 1.42 µg/L). IGF-1 levels in CSF were measured after a C18 Sep-Pak column (Waters Assoc., Milford, MA, USA) extraction according to the manufacturer.

IGFBP-3 levels in CSF were determined using an RIA (Nichols Institute). The method had a detection limit of 1.0 µg/L and the intra-assay CV for the method was 3.3% (at 32.6 µg/L) and 3.6% (at 54.7 µg/L).

For ß-endorphin RIA, the anti-ß-endorphin rabbit antibody N 1621 (Amersham, Aylesbury, UK) was used. This antibody reacts 100% with human ß-endorphin (ß-lipotropin 61-91), ß-endorphin (1-5) + (16-31), ß-endorphin (6-31) and ß-endorphin (18-31). It has 1-2% cross-reactivity with ß-lipotropin and < 1% cross-reactivity with g-endorphin, met- and

leu-enkephalin. The analyses were run directly on CSF. The method had a detection limit of 4.0 pmo/L and the intra-assay CV for the method was 4.9% (at 40 pmol/L).

The dopamine metabolite HVA, the norepinephrine metabolite MHPG and the serotonin metabolite 5-HIAA in CSF were separated using a high-performance liquid chromatography (HPLC) system (Kontron Instruments, Zurich, Switzerland). The separation was carried out in a reverse-phase mode on an Ultrasphere ODS column (Beckman Instruments, San Ramon, CA, USA). A Coulochem II electrochemical detector (ESA Inc., Bedford, MA, USA) with a conditioning cell (Model 5021) and a high-sensitivity analytical cell (Model 5011) was used. The intra-assay CV for the methods was 4.2% (at 153.7 nmol/L) for HVA, 4.2% (at 25.5 nmol/L) for MHPG and 8.9% (at 318.4 nmol/L) for 5-HIAA.

Statistical methods

Conventional statistical methods were used to calculate means, standard deviations and standard errors of the means. Correlations were sought by calculating Pearson's linear correlation coefficient (Papers I-IV) or Spearman's rank correlation coefficient (Paper V).

In Paper I, differences between patients and controls were tested using Wilcoxon's test for paired differences and, in Paper II, using the exact permutation test (groupwise comparison).

In Paper III, the intraindividual differences between the initial level and the levels at various time points of each variable were tested using Wilcoxon's test for paired differences.

In Paper IV, a one-way analysis of variance (ANOVA) with the complete block design followed by the Student-Newman-Keul multiple-range test was used to test the effects of GH treatment.

In Paper V, comparisons between the treatment groups were performed using Fisher's permutation test. The intra-individual differences between the initial level and the level after one month of treatment were analysed using the exact permutation test.

Values of $p < 0.05$ were considered statistically significant. All the tests were two-sided. Descriptive values are given as the mean ± SEM unless otherwise specified.

5. Study design and results

Paper I

Study design: In a cross-sectional study, 15 GH-deficient adults were compared with healthy controls matched groupwise for sex, age and BMI. Insulin sensitivity was studied using the hyperinsulinemic euglycemic clamp technique.

Results: Fasting blood glucose, 4.4 ± 0.1 v 4.7 ± 0.2 mmol/L, and fasting plasma insulin, 9.5 ± 1.4 v 8.8 ± 1.1 mU/L, were similar in patients and controls respectively. Blood glucose during the steady state of the clamp was also similar in patients and controls, 4.6 ± 0.1 v 4.9 ± 0.1 mmol/L, as were plasma insulin levels, 81 ± 4 v 93 ± 4 mU/L.

A decrease in glucose infusion rate (GIR) was seen during the clamp in the GH-deficient patients, 3.9 ± 0.5 v 9.9 ± 0.7 mg/kg body weight/min, as compared with controls ($p = 0.001$, **Fig. 2a**). Even if corrections were made for body fat, GIR in the patients was less than half that in controls, 5.8 ± 0.8 v 13.9 ± 0.9 mg/kg LBM/min ($p<0.001$, **Fig. 2b**). Since insulin levels during the clamp tended to be lower in the patients ($p = 0.1$), the insulin sensitivity index (GIR/plasma insulin) was evaluated and found to be about half that of controls ($p<0.01$, data not shown).

Fasting FFA levels were lower in the patients, 444 ± 35 v 796 ± 94 µmol/L ($p<0.01$). At the end of the clamp, the FFA levels were lower in the patients, 53 ± 10 v 121 ± 18 µmol/L, as compared with controls ($p=0.001$, **Fig. 3**). However, the decrease from the fasting level was similar in the two groups, 87% ± 2.6% and 84% ± 1.7% respectively.

GIR per LBM among the patients correlated negatively with BMI ($r = -0.56$, $p<0.05$), fasting blood glucose ($r = -0.58$, $p<0.05$) and fasting plasma insulin concentrations ($r = -0.60$, $p<0.05$).

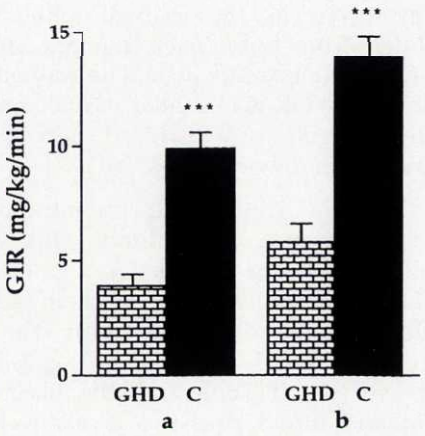

Fig. 2. GIR in adults with GHD compared with controls (C) (n=15). a) GIR per body weight b) GIR per LBM. ***p<0.001 v GHD

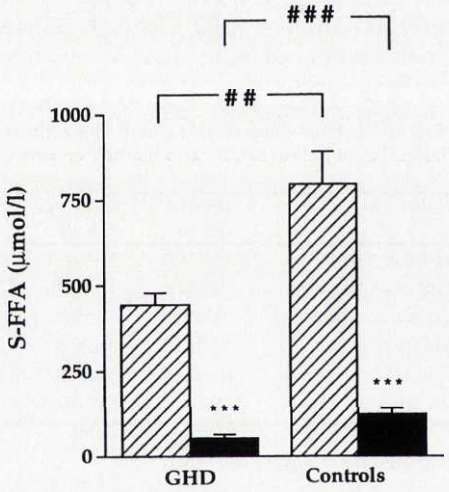

Fig. 3. Serum FFA concentrations before (lined) and at the end of the clamps (filled) in patients with GHD compared with controls (n=15). ***p<0.001 v baseline levels, ##p<0.01 and ###p=0.001 v controls

Paper II

Study design: In a cross-sectional study, 20 GH-deficient adults were compared with healthy controls matched groupwise for sex, age and BMI. Fibrinogen levels, PAI-1 activity, metabolic and anthropometric data were studied

Results: WHR was higher in the patients than in the control subjects calculated from both sexes together and was most pronounced in female patients (**Table 2-3**). WHR was similar in male and female patients but differed between healthy men and women ($p<0.001$).

The serum triglyceride concentration was higher and the blood glucose concentration lower in patients calculated from both sexes together, as well as in GH-deficient men compared with their respective control group (**Tables 2-3**). Serum cholesterol (**Table 2**), fasting plasma insulin and blood pressure levels were similar in both patients and controls including both sexes. However, the cholesterol concentration was higher in GH-deficient men (**Table 3**) and the systolic blood pressure was higher, 142 ± 21 v 123 ± 9 mm HG (mean ± SD), in GH-deficient women compared with their sex-matched controls ($p<0.05$).

Table 2. Metabolic data, fibrinogen, PAI-1 activity and WHR in GH-deficient adults and healthy controls.

Variable	Patients (n=20)	Controls (n=20)	p
fB-glucose, mmol/L	4.0±0.5	4.4±0.7	0.042
Cholesterol, mmol/L	6.3±1.2	5.8±1.2	0.266
Triglycerides, mmol/L	1.5±0.5	1.1±0.5	0.019
Fibrinogen, g/L	3.2±0.7	2.4±0.6	0.0001
PAI-1 activity, U/mL	13.2±10.6	6.8±4.3	0.013
WHR	0.97±0.03	0.87±0.09	0.0001

fB=fasting blood. Values are mean ± SD.

The fibrinogen concentration was higher in patients than in controls (**Table 2**) irrespective of sex (**Fig. 4**). PAI-1 activity was also higher among the patients including both sexes than in the control subjects (**Table 2**), whereas the differences were less pronounced when men and women were studied separately (**Table 3**).

PAI-1 activity among the patients correlated with the triglyceride concentration ($r = 0.40$, $p<0.05$), the insulin concentration ($r = 0.51$, $p < 0.001$) and the blood glucose concentration ($r = 0.51$, $p < 0.001$).

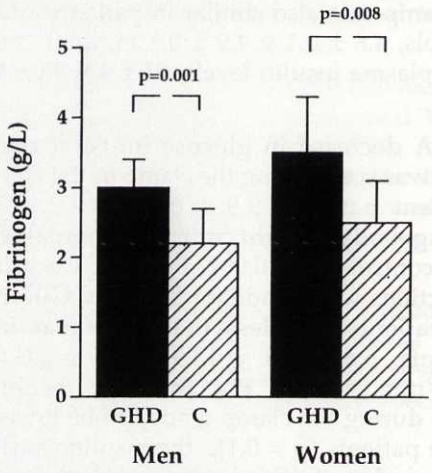

Fig. 4. Fibrinogen concentrations in adults with GHD compared with controls (C). n=10 in each of the four groups. Values are mean ± SD.

Table 3. Metabolic data, PAI-1 activity and WHR for men and women separately in GH-deficient adults and healthy controls.

Variable	Men			Women		
	Patients (n=10)	Controls (n=10)	p	Patients (n=10)	Controls (n=10)	p
fB-glucose, mmol/L	4.1±0.5	4.8±0.6	0.01	3.8±0.4	3.9±0.5	0.707
Cholesterol, mmol/L	6.7±0.7	5.0±0.6	0.0001	5.9±1.4	6.7±1.1	0.154
Triglycerides, mmol/L	1.8±0.5	1.0±0.5	0.004	1.3±0.4	1.3±0.4	0.959
PAI-1 activity, U/mL	15.4±13.5	8.0±2.7	0.135	11.1±6.6	5.7±5.3	0.063
WHR	0.97±0.04	0.93±0.05	0.062	0.97±0.02	0.81±0.07	0.0001

fB=fasting blood. Values are mean ± SD.

Paper III

Study design: In an open prospective study, fibrinolysis and coagulation were studied in 17 of the patients in Paper II during two years of GH treatment. The impact of the contemporary changes in metabolic variables and body composition on coagulation and fibrinolysis was studied. The primary objective was to evaluate the changes after two years.

Results - Fibrinolytic variables: PAI-1 activity decreased after one and half years and after two years of GH treatment ($p<0.05$). PAI-1 antigen decreased after one and half years ($p<0.01$) and tended to decrease after two years ($p = 0.06$). t-PA antigen also decreased ($p<0.05$) (**Fig. 1 in Paper III**).

The change in PAI-1 activity after two years of GH treatment was negatively correlated with PAI-1 activity at baseline, i.e. the decrease in PAI-1 activity was more pronounced in patients with initially high PAI-1 activity ($r = -0.90$, $p<0.0001$). Similar patterns were seen for PAI-1 antigen and t-PA antigen (**Figs. 2a-c in Paper III**).

a_2-antiplasmin decreased from 119.3% ± 5.0% to 102.1% ± 4.5% after two years of GH treatment ($p<0.05$).

Coagulation variables: Fibrinogen concentrations tended to decrease from 3.3 ± 0.2 g/L to 3.0 ± 0.1 g/L after two years of GH treatment ($p = 0.06$). The change in fibrinogen was negatively correlated with fibrinogen at baseline, i.e. the decrease in fibrinogen was more pronounced in patients with initially high fibrinogen levels ($r = -0.72$, $p<0.01$). The decrease in fibrinogen was positively correlated with the decrease in WHR after two years of GH treatment ($r = 0.52$, $p<0.05$).

Protein C decreased from 1.25 ± 0.06 IU/mL to 1.11 ± 0.05 IU/mL after two years of GH treatment ($p<0.05$).

Other fibrinolytic and coagulation variables measured in the study were unchanged after two years of GH treatment. The effects on all fibrinolytic and coagulation variables were similar in both sexes when studied separately.

Metabolic data and body composition: Fasting plasma insulin concentrations increased from 8.3 ± 1.0 mU/L to 11.5 ± 1.6 mU/L ($p<0.01$), but blood glucose concentrations did not differ after two years of GH treatment. Serum triglycerides and total cholesterol concentrations were unaltered, while HDL cholesterol concentrations increased during the first year ($p<0.05$) but then levelled off ($p = 0.09$ after two years). Body fat decreased during the initial GH treatment but was unaltered after two years, while LBM increased from 53.5 ± 3.2 kg to 56.5 ± 3.2 kg ($p<0.001$) and WHR tended to decrease ($p = 0.06$).

Paper IV

Study design: The effect of conventional evening s.c. injections was compared with a continuous s.c. infusion of GH (0.25 IU/kg body weight/week resulting in an average daily GH dose of 3.4 ± 0.2 IU) for 14 days in nine GH-deficient men. All the patients received treatment with daily injections during the first treatment period and a continuous infusion during the second period. There was one month of wash-out between the treatment periods.

Results: The two modes of administration resulted in similar total 24-hour urine GH excretion and similar morning serum GH concentrations. Daily injections and a continuous infusion of GH exerted similar effects in terms of body weight and body composition **(Fig. 2 in Paper IV)**.

Serum IGF-1 (**Fig. 5**) and IGFBP-3 concentrations increased during both modes of treatment but more markedly during 14 days of continuous GH infusion.

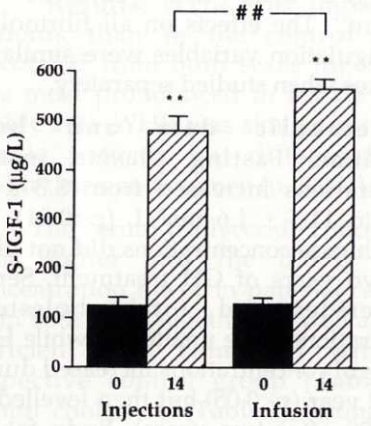

Fig. 5. Effects of 14 days of one evening injection and continuous infusion of GH on serum IGF-1 in nine GH-deficient men. **p<0.01 v day 0 corresponding treatment, ##p<0.01 v the effect of treatment with one evening injection

The molar IGF-1/IGFBP-3 ratio was similar for the two administration modes after 14 days (data not shown). IGFBP-1 concentrations decreased during daily injections of GH (p<0.05). During the continuous infusion of GH, a marked decrease was noted, although it was not statistically significant. Serum GHBP concentrations were unaltered during both modes of GH treatment.

Fasting blood glucose concentrations increased during treatment with daily GH injections, but they were not affected by the continuous infusion. The increase in fasting blood glucose concentrations was thus more marked after 14 days of daily GH injections compared with the continuous infusion (**Fig. 6a**). Fasting serum FFA concentrations increased during treatment with one daily injection of GH but did not change during the continuous infusion (**Fig. 6b**). Fasting plasma insulin and C-peptide concentrations increased in a similar way during both modes of treatment.

During the oral glucose tolerance test, blood glucose, plasma insulin and C-peptide concentrations at 120 min. increased more markedly during daily injections of GH (p<0.01, p<0.05 and p<0.01 respectively). The sum (values at 0, 30, 60, 90 and 120 min.) of blood glucose increased more markedly during daily injections of GH (p<0.01), while the sum of plasma insulin and the sum of plasma C-peptide concentrations increased to a similar degree during the two modes of GH treatment.

There were no differences between the two modes of treatment in terms of thyroid hormones or TSH concentrations.

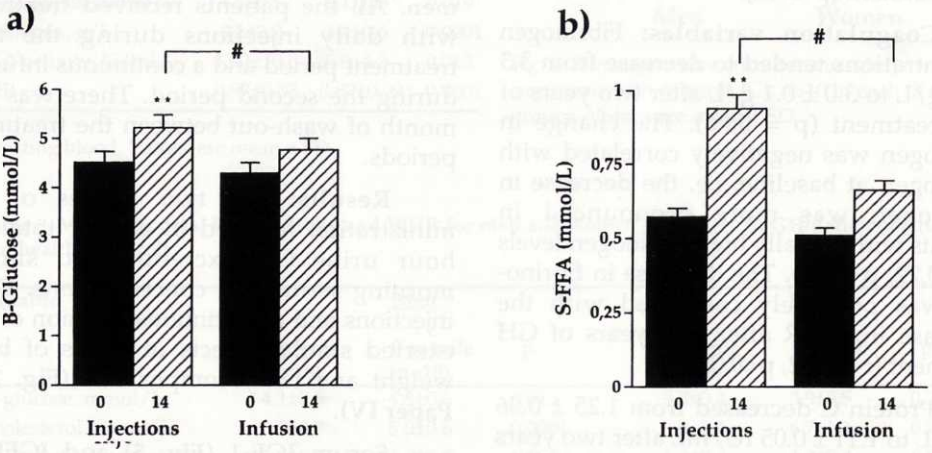

Fig. 6. Effects of 14 days of one evening injection and continuous infusion of GH on a) blood glucose and b) serum FFA concentrations in nine GH-deficient men. **p<0.01 v day 0 corresponding treatment, #p<0.05 v the effect of treatment with one evening injection

Paper V

Study design: In a randomized, placebo-controlled study, 10 patients in each of two groups were treated for one month with GH (0.25 IU/kg body weight/week) or placebo. Changes in neurotransmitters and GH-dependent factors in the CSF were studied.

Results: In the GH-treated group, the mean CSF GH concentration increased tenfold compared with baseline ($p = 0.002$, **Fig. 7**). No correlation was found between the CSF and serum GH concentrations or between CSF GH concentrations and the GH dose given.

The plasma IGF-1 concentration increased by 413.8% ± 106.2% ($p = 0.002$), while the CSF IGF-1 concentration increased by 47.1% ± 10.4% during GH treatment ($p = 0.005$, **Fig. 8a**). Similarly, CSF IGFBP-3 concentrations increased in the group allocated to GH ($p = 0.002$, **Fig. 8b**). The increase in CSF IGF-1 concentration correlated positively with the increase in CSF IGFBP-3 concentration ($r = 0.81$, $p<0.05$). However, no correlations were found between the increase in CSF and plasma IGF-1 or between the increase in CSF GH and CSF IGF-1 concentrations.

CSF ß-endorphin immunoreactivity increased in all patients during GH treatment ($p = 0.002$, **Fig. 9a**), while the dopamine metabolite HVA decreased ($p = 0.02$, **Fig. 9b**), as did VIP, 4.1 ± 0.6 pmol/L to 3.7 ± 0.4 pmol/L ($p = 0.03$). CSF concentrations of HVA correlated with the serotonin metabolite 5-HIAA both before ($r = 0.91$, $p<0.001$) and after GH treatment ($r = 0.89$, $p<0.001$). A similar positive correlation was noted in the placebo-treated group (data not shown).

CSF concentrations of enkephalin, dynorphin A, the norepinephrine metabolite MHPG, 5-HIAA, GABA, somatostatin and CRF were unchanged after one month of GH treatment. All the CSF variables were unchanged in the placebo treated group.

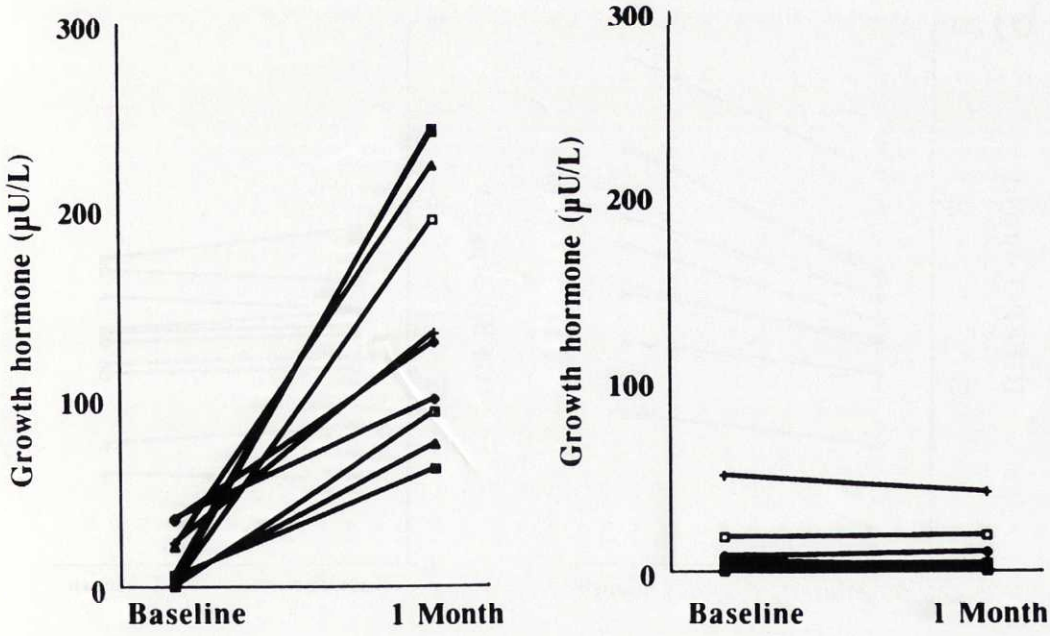

Fig. 7. Effects of GH (left) and placebo (right) on the CSF GH concentration in 10 GH-deficient adults. With the kind permission of *Neuroendocrinology*, Karger, Basel.

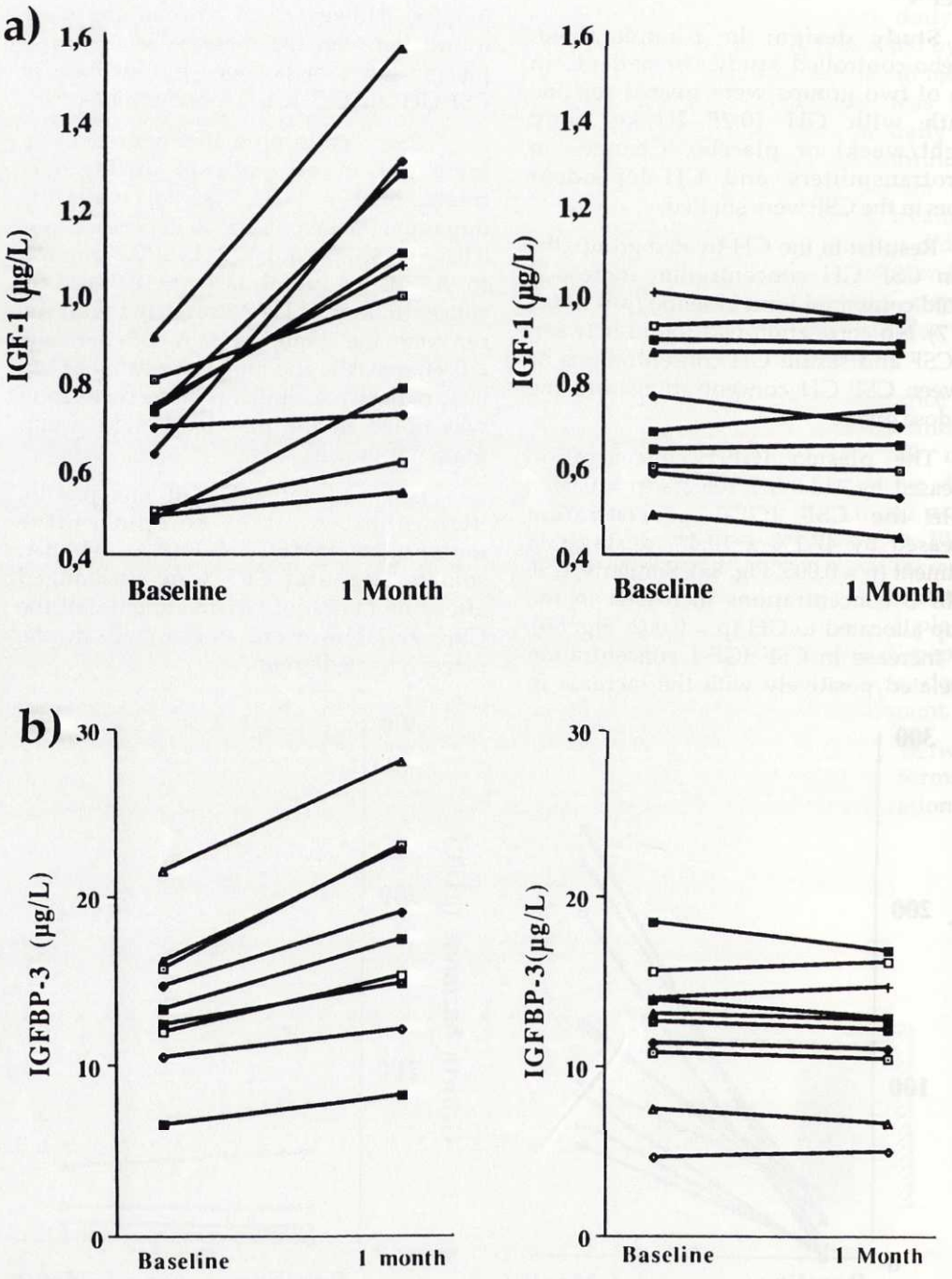

Fig. 8. Effects of GH (left top and bottom) and placebo (right top and bottom) on a) the CSF IGF-1 concentration and b) the CSF IGFBP-3 concentration in 10 GH-deficient adults. With the kind permission of *Neuroendocrinology*, Karger, Basel.

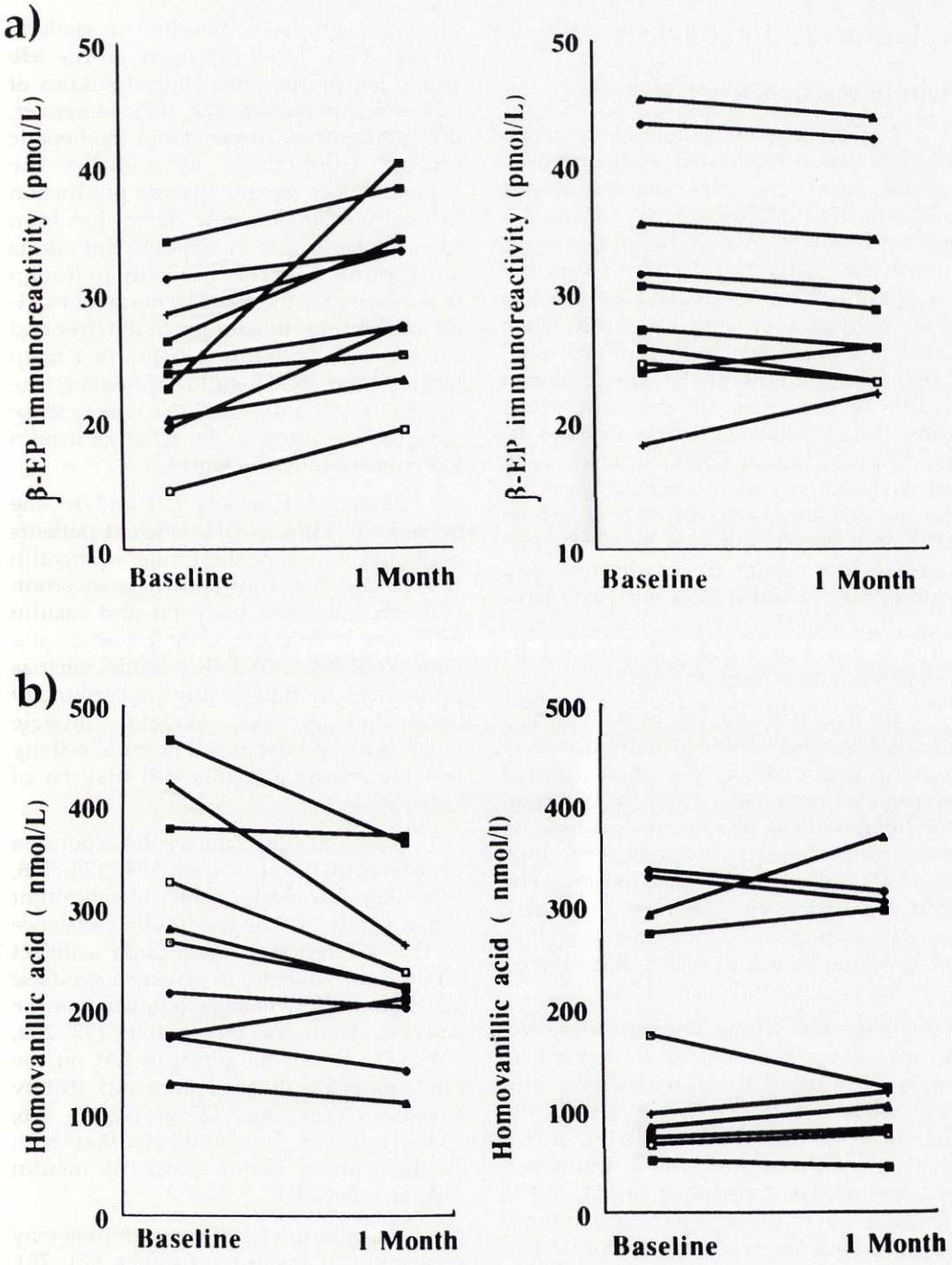

Fig. 9. Effects of GH (left top and bottom) and placebo (right top and bottom) on a) the CSF ß-endorphin immunoreactivity and b) the CSF HVA concentration in 10 GH-deficient adults. With the kind permission of *Neuroendocrinology*, Karger, Basel.

6. General discussion

Insulin resistance in GHD

GHD has traditionally been described as a condition associated with increased insulin sensitivity. However, our results indicate that GH-deficient adults are insulin-resistant in peripheral tissues as measured with the hyperinsulinemic euglycemic clamp technique. GIR was less than half that of controls, both when calculated according to body weight and when corrected for body fat. The finding of insulin resistance in GH-deficient adults adds further metabolic abnormalities to the syndrome of adult GHD. A decrease in insulin sensitivity when estimated from i.v. glucose tolerance tests (156) or from insulin tolerance tests (71) supports the finding that GH-deficient adults are insulin-resistant. Furthermore, studies in recent years have suggested a greater prevalence of abnormal glucose tolerance in GH-deficient adults compared with controls (15, 131).

Insulin resistance can be defined as a situation in which a given concentration of insulin produces a less than normal biological response. It is a common condition and can be seen, for example, in non-insulin-dependent diabetes mellitus (type II), obesity and hypertension. The inter-relationship between insulin resistance and these conditions, as well as the exact mechanisms for insulin resistance, have not yet been fully clarified.

In studies with a single insulin level during clamp, it is difficult to distinguish between insulin sensitivity and responsiveness (102). In our study, the insulin level was about 90 mU/L which elicits a near maximum insulin-stimulated glucose uptake in peripheral tissues. It is therefore likely that the insulin resistance seen in GH-deficient adults is mainly due to a decrease in responsiveness (104). It has recently been suggested that the reduction in glucose disposal is mainly due to a decrease in glucose storage rate and glycogen synthase activity in skeletal muscle (72). Low glycogen stores are supported by previous clinical studies of GH-deficient subjects (22, 187). Moreover, the basal glucose disposal and the hepatic glucose production, as well as the suppression of hepatic glucose production during hyperinsulinemic clamp, has been found to be similar in GH-deficient adults and controls (72). The sensitivity to insulin in the liver may therefore be normal in GH-deficient adults. In addition to the liver and skeletal muscle, adipose tissue is a main target tissue for insulin. However, the degree to which the adipose tissue in these patients is sensitive to the action of insulin has not been fully elucidated.

Abdominal obesity (II) and/or the decrease in LBM in GH-deficient patients may play an important role in insulin resistance. The well-known association between increased body fat and insulin resistance is stronger in the presence of a high WHR (64, 113). Other factors such as differences in muscle fibre morphology (reviewed in 41), skeletal muscle metabolism and decreased physical activity in GH-deficient patients (18) may be of importance.

Since GH effects the insulin action at a post-receptor level (25, 68, 104, 124, 148, 176, 183), a post-receptor defect might consequently explain the insulin resistance in the GH-deficient state. Such a defect might be a reduction in glycogen synthase activity but also a change in function or the amount of glucose transporters (55, 200, 201). Studies of the effect of GH on the numbers of insulin receptors and affinity have been inconsistent (25, 39, 68, 124, 176, 183). However, it is unlikely that such changes are a major cause of insulin resistance in GHD.

A low serum IGF-1 concentration may contribute to insulin resistance (78, 79), since IGF-1 stimulates the glucose transport in the skeletal muscle in vitro (48, 127). Increased FFA levels due to abdominal

obesity appear to be an unlikely explanation of insulin resistance (86, 113, 168) since we and others have observed low fasting FFA levels in these patients (4, 22, 23), possibly due to decreased lipolysis in the absence of GH.

No compensatory increase in fasting plasma insulin was observed in the patients (I-II, 72, 131). It is possible that an increase in insulin concentration is not required in the fasting situation. The fasting blood glucose level was also normal (I, 72, 131) or even lower (II) in the patients compared with controls. In addition, low fasting FFA levels cause less competition with glucose as an oxidative fuel and may therefore lead to decreased insulin requirements (168). Moreover, the lack of GH may contribute to low fasting insulin concentrations since it has been suggested that GH is an insulinotropic hormone (151).

It has been questioned whether the metabolic changes in GH-deficient adults are due to the lack of GH or are the result of imperfect cortisol, thyroid and gonadal replacement therapy. Insulin resistance has been described in thyrotoxicosis (164, 194) and hypercortisolemia (134, 177). In addition, androgens (35, 64, 76, 146, 165) and female sex steroids influence insulin sensitivity (103, 119, 132, 165, 184). However, the data available on the change in insulin sensitivity induced by hormone replacement therapy in hypopituitarism is limited (3). It is unlikely that the replacement doses used in this study induce changes in insulin sensitivity of the magnitude observed in these patients. Furthermore, insulin resistance was found both in patients with isolated GHD and in patients with multiple pituitary hormone deficiencies (104) and, in our study, the GIR was also low in the two patients with isolated GHD (1.7 and 6.8 mg/kg body weight/min respectively).

The effect of long-term treatment with GH on insulin sensitivity in GH-deficient adults is not fully known. Six weeks of GH treatment in GH-deficient adults only induced a temporary decrease in insulin sensitivity which, after six months of treatment, was restored to baseline (58). An oral glucose tolerance test performed after three years of GH treatment disclosed no evidence of glucose intolerance (101). It remains to be seen whether insulin sensitivity further improves after longer GH treatment.

Fibrinolysis and coagulation in GH-deficient adults

The elevation of fibrinogen levels and PAI-1 activity adds a new dimension to the risk factor profile for GH-deficient adults. These findings, together with dyslipidemia and abdominal obesity, link thrombogenesis with atherogenesis and might explain the increased risk of cardiovascular disease in hypopituitarism (53, 131, 178).

In contrast to our findings, the fibrinogen levels did not differ between patients and controls in a previous study (131). However, in that study the fibrinogen levels were quite high in both patients and controls (about 3.5 and 3.2 g/L respectively). Obesity has been associated with both increased fibrinogen levels and PAI-1 activity (10, 49, 52, 108, 114, 144, 203). Although patients and controls were matched for BMI, we observed both higher fibrinogen levels and PAI-1 activity in the patients, suggesting that other factors in addition to obesity per se are of importance. Both the elevated fibrinogen levels and PAI-1 activity may be linked to the abdominal and visceral obesity, indicated by a high WHR in these patients (II). A higher WHR in GH-deficient adults has been found by others, in both sexes together (40, 185), as well as in men and women studied separately (15). In healthy women with a BMI similar to that of our female patients, the prevalence of a WHR of ≥ 0.80 was only 9% (112). In fact, such a high WHR as that observed among our patients was not seen even in grossly obese women

with a BMI of 36 kg/m^2. In normal subjects, the average WHR is 0.70-0.80 in women (117) and 0.90-0.95 in men (118).

Smoking has a powerful influence on fibrinogen levels (49, 108, 136, 208). However, the high fibrinogen levels among our patients cannot be explained by smoking habits since only one of these patients was a current smoker. Interestingly, a lower prevalence of smoking has previously been reported in GH-deficient adults compared with controls (180). High serum triglyceride concentrations have previously been noted in both GH-deficient men and women as compared with controls (180). Triglycerides correlated positively with PAI-1 activity in our patients as they did in healthy subjects (52, 65, 83, 115, 144, 198).

Short-term studies lasting between two and four months have failed to show any effect by GH on t-PA antigen or PAI-1 activity (98, 160). In contrast, we observed a decrease in PAI-1 activity and PAI-1 antigen after one and a half to two years of GH treatment. Furthermore, t-PA antigen, which forms a complex with and mainly measures PAI-1, decreased, thereby supporting a true decrease in PAI-1 during GH treatment. PAI-1 is the major regulator of fibrinolytic activity in plasma and a decrease in PAI-1 is therefore assumed to result in increased fibrinolysis.

The mechanism by which GH decreases PAI-1 activity, PAI-1 antigen and t-PA antigen is unclear. PAI-1 synthesis is influenced by a variety of factors including several growth factors (109, 190, 192). IGF-1 has been shown to stimulate the synthesis of PAI-1 in vitro (160) but not during short-term administration in vivo (160). The decrease in fibrinolytic variables might be secondary to the favourable changes in body composition and lipid metabolism (83, 144, 198) following GH treatment (87) or a direct effect of GH itself. The changes in body composition in our study were similar to what has previously been observed in response to GH treatment (12, 16, 43, 100, 185). In spite of increased insulin levels and unchanged triglyceride concentrations, the PAI-1 activity decreased which counter-indicates any significant influence by these two factors on PAI-1 activity in GH-deficient adults. It remains to be seen whether the decrease in PAI-1 activity, PAI-1 antigen and t-PA antigen during long-term GH treatment reduces the cardiovascular risk in GH-deficient adults.

α_2-antiplasmin is the rapid plasmin inhibitor which forms a complex with and inactivates plasmin. The observed decrease in α_2-antiplasmin may therefore be the result of an increase in plasmin activity caused by the decrease in PAI-1.

The fibrinogen levels were unaltered during the study period, although the levels tended to decrease after 24 months of GH treatment. In general, it has been difficult to influence fibrinogen levels during different treatments except for smoking cessation.

GH is of importance for vitamin K-dependent coagulation factors in the rat (150, 199). Despite an unaltered factor VII during GH treatment in the present study, an effect by GH on the biosynthesis of other vitamin K-dependent plasma coagulation factors in the liver is possible. The present study also confirms the previous observation of an unchanged factor VIII and von Willebrand factor antigen during prolonged GH treatment in GH-deficient adults (98).

Similarities between GHD in adults and The Metabolic Syndrome

The present study has extended our knowledge of the GHD syndrome in adults by adding insulin resistance and abnormalities in fibrinolysis and coagulation to the syndrome. Striking similarities thus exist between the so-called Metabolic Syndrome, also labelled Syndrome X or Primary Insulin Resistance

Syndrome, and the GHD syndrome in adults (13).

The most central findings in both these syndromes are abdominal/visceral obesity and insulin resistance (169, 170, I). Other features common to both conditions are high triglyceride and low HDL cholesterol concentrations (169, 180), elevations of fibrinogen levels and PAI-1 activity (170, II), an increased prevalence of hypertension (169, 180), premature atherosclerosis (131, 169) and increased mortality from cardiovascular diseases (169, 178). The similarities between the two syndromes suggest that GH plays a role in the metabolic syndrome.

Metabolic effects of GH related to mode of administration

Continuous infusion and daily injections of GH (Paper IV) had similar effects on GH-dependent factors, but in some cases the magnitude of the effects differed. No carry-over effects were noted after one month of wash-out. It was therefore concluded that the effects of the first treatment period did not influence the effects of the second regimen. The urinary excretion of GH was similar during the two different modes of GH administration, indicating that similar amounts of GH reached the circulation.

A diurnal variation in serum IGF-1 concentration with the highest values in the morning has been observed in adult GH-deficient patients receiving evening injections of GH (94). In control experiments, we observed a similar diurnal variation in serum IGF-1 concentration (88). Consequently, since all our measurements were made in the morning, we concluded that a continuous infusion of GH resulted in a significantly higher mean daily serum IGF-1 concentration. Similarly, frequent i.v. injections and the continuous administration of GH for 24 hours induced a greater increase in circulating IGF-1 compared with a few injections a day (97). In the rat, liver GH receptors are upregulated by the continuous administration of GH, but not by repeated injections (130). A similar difference in the upregulation of GH receptors in the human liver is possible and might explain our finding of a more marked increase in IGF-1 concentration during the continuous administration of GH. Since GHBP is believed to originate from the proteolytic cleavage of GH receptors, especially of hepatic origin, the lack of effects of GH treatment on GHBP concentrations in our study militates against this possibility. However, a continuous infusion of GH during a period of six months in GH-deficient children resulted in a more marked increase in GHBP concentrations compared with daily injections (202).

Like IGF-1, serum IGFBP-3 concentrations increased to a greater degree during the continuous infusion of GH than during daily injections of GH. Previous short-term (24 h) administration of GH did not reveal any difference in IGFBP-3 concentrations between intermittent and continuous administration (99, 120). However, in a four-week study, slightly higher IGF-1 and IGFBP-3 levels were noted during a continuous s.c. infusion of GH compared with daily single injections (121).

The observed increase in fasting glucose and insulin levels is in agreement with previous findings during the GH treatment of GH-deficient adults (58, 94, 185, III). The higher fasting glucose and FFA levels during treatment with daily injections in the evening could be explained by a higher concentration of GH during the night and at dawn compared with the continuous infusion (94, 96). A higher GH concentration would cause an enhanced insulin-antagonistic effect (57) and increased lipolysis (166). The more impaired oral glucose tolerance after daily injections of GH may thus be explained by a higher serum GH concentration at dawn, since all the measurements were made in

the morning. Since IGF-1 has insulin-like effects (48, 63, 127), the more marked increase in serum IGF-1 during the continuous infusion of GH could theoretically counteract the deterioration in glucose homeostasis (78, 79). Recently, four weeks of daily single injections of GH were shown to result in higher daytime insulin levels and a tendency towards higher insulin levels after a morning oral glucose tolerance test compared with continuous infusion. In addition, the mean 24-hour levels of FFA tended to be slightly higher after daily injections of GH (121).

A continuous s.c. infusion of GH for several weeks thus results in higher levels of IGF-1 and IGFBP-3 and less impaired glucose tolerance compared with daily injections. On the other hand, the continuous s.c. infusion of GH may have the disadvantages of a less physiological diurnal pattern of FFA (121), a more marked increase in lipoprotein (a) and a less pronounced decrease in LDL-cholesterol compared with daily injections (159). Additional studies of the longer term metabolic effects of continuous GH delivery versus daily administration are necessary before a sustained-release preparation of GH can be routinely used.

Central nervous effects of GH

Previous findings of impaired quality of life in GH-deficient adults (18, 133) have been confirmed by more recent studies (27, 44, 174, 182). Common complaints before GH treatment have related to tiredness, low energy, lack of concentration, memory difficulties and irritability (12, 44). Beneficial effects on certain cognitive functions, including memory, were reported at an early stage in the development of GH treatment in GH-deficient adults (5). Additional studies have subsequently reported improvements in energy level, mood, emotional reactions, vitality and social isolation during GH treatment (12, 27, 133, 145).

Recently, GH receptors have been found in many locations in the rodent and human brain (110, 111, 126, 143, 155). The highest density of GH receptors in the adult human brain have been noted in the choroid plexus, the hippocampus, the hypothalamus and the pituitary gland (110) (**Fig. 10**). While GH binding sites in the latter regions perform an obvious function in the regulation of pituitary GH secretion, the physiological relevance of GH binding sites in other brain regions is less clear. In

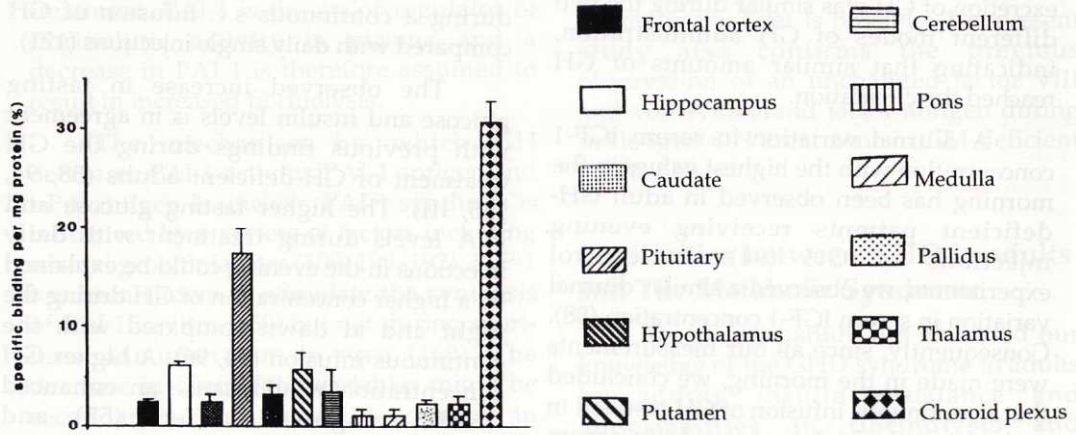

Fig. 10. Specific binding of [^{125} I]hGH to membranes from different parts of the human brain. The values are mean ± SD (n=6). With the kind permission of *Brain Research*, Elsevier Science - NL, Amsterdam and the authors (110).

most brain tissues examined, the number of GH receptors was higher in women and there was an age-related decrease in the number of GH receptors irrespective of sex (111).

The question of the permeability of the blood-CSF barrier to GH has been controversial (reviewed in 70). In our study, a mean ten-fold increase in GH in the CSF was observed during GH treatment, thus indicating that rhGH does pass the human blood-CSF barrier. The possible passage of GH over the blood-CSF barrier is also supported by the dose-related increase in the GH concentration in CSF reported in a recent study (28). The concentration of GH in the CSF is still low compared with serum (CSF:serum ratio about 5%). The increase in GH concentrations in the CSF was similar in patients with and without a damaged blood-CSF barrier.

One possible mechanism behind the increase in GH in CSF is ultrafiltration, since most of the proteins in CSF are derived by this process. Alternative explanations are a GH receptor-mediated transport in the choroid plexus (110), a carrier-mediated transport system or a diffusion via the incomplete blood-brain barrier in the median eminence of hypothalamus (70).

We observed that the mean increase in CSF IGF-1 concentrations was about 50%. IGF-1 may pass the blood-brain barrier in the rat (172), but it has not yet been demonstrated that a similar passage exists in humans (161). Previous clinical studies have indicated the existence of a blood-CSF barrier for IGFs and that IGF-1 present in the CSF is probably mainly derived from local CNS production (30). In line with these findings, we did not observe any correlation between the changes in plasma and CSF IGF-1 concentrations. The variant form of IGF-1 (truncated IGF-1) and IGF-1 receptors have been found in a wide distribution throughout the adult human brain (2, 31, 188, reviewed in 20). IGF-1 may play an important role in brain maturation, neural differentiation, survival (19), myelination (135) and energy metabolism (206). CSF IGFBP-3 concentrations also increased during GH treatment. It is likely that several of the IGFBPs which have been found in brain tissues modulate the action of IGF-1 (157).

The fall in the CSF HVA concentration in response to GH treatment indicates that GH affects the dopamine turnover in the CNS. A similar decline in CSF HVA concentration was also noted during nine months of GH treatment (28). The results relating to CSF monoamine metabolites in depression and during treatment with antidepressants are equivocal (171, 175, 212). Although GH-deficient adults often have symptoms similar to those seen in depressed patients, the mechanism behind these symptoms may not necessarily be the same. In animal studies, region-dependent changes in dopamine, noradrenaline, serotonin and 5-HIAA levels during GH treatment have previously been reported (8, 195). As a result, region-dependent changes in brain monoamines may be present but not mirrored in the CSF during GH treatment in GH-deficient patients.

A significant increase in CSF ß-endorphin immunoreactivity was noted during GH treatment. Although the mean increase was limited (24.6% ± 7%), an increase was observed in all patients. The antiserum used was specific to the carboxy-terminal half of the ß-endorphin peptide and had very low cross-reactivity with intact ß-lipotropin as well as with the amino-terminal parts of the ß-endorphin molecule.

ß-endorphin is an endogenous opioid peptide consisting of 31 amino acid residues which, together with ACTH and MSH, is derived from the precursor pro-opiomelanocortin (POMC). The post-translational processing of POMC is regionally selective in the brain and other tissues, which means that different brain

regions contain different sets of POMC-derived peptides. In addition to ß-endorphin production by corticotrophic cells in the pituitary, ß-endorphin nerve cells can mainly be found in specific areas in the hypothalamus and the brain stem (107). ß-endorphin immunoreactivity in the CSF decreases markedly after hypophysectomy (191) and hypopituitary patients may thus have a decreased concentration of CSF ß-endorphin immunoreactivity.

The mechanism behind the increase in CSF ß-endorphin immunoreactivity is unclear, but it may be a direct effect by GH on cells producing peptides derived from POMC or secondary to factors produced in the peripheral tissues that penetrate the blood-brain barrier. In contrast, no change in CSF ß-endorphin immunoreactivity was noted after nine months of GH treatment (28). The contrast in ß-endorphin results may reflect methodological differences. Recently, intracerebroventricular injections of GH to rats were found to increase the ß-endorphin level in the pituitary and the levels of another opioid peptide in the spinal cord. (90). Previously, it has been shown that opioid peptides are involved in the stimulation of GH release (26, 45). Increased immunoreactivity of ß-endorphin in plasma after exercise is well-documented and several studies support a role for ß-endorphin in the emotional well-being that follows exercise (82, 142). The possibility that the increase in CSF ß-endorphin immunoreactivity may be of importance for the improved psychological well-being noted during GH treatment in GH-deficient patients cannot be excluded.

Taken together, this data shows that there are changes in CSF levels of GH, GH-dependent factors and neurotransmitters during GH treatment in GH-deficient adults. Neuroendocrine mechanisms may very well be involved in the improvement in psychological well-being observed during GH treatment.

7. Summary and conclusions

In the present study, metabolic and hemostatic factors associated with cardiovascular disease were evaluated in hypopituitary adults with untreated GHD. Furthermore, the effects of long-term GH treatment on hemostasis and the effects of GH on neurotransmitters and GH-dependent factors in the CSF were studied. We also compared the metabolic effects of different GH administration modes.

Insulin sensitivity, as assessed with the hyperinsulinemic euglycemic clamp technique, was markedly reduced in GH-deficient subjects compared with matched controls, thus indicating that GH-deficient adults are insulin-resistant. Despite this, a normal fasting insulin level was found. GH-deficient subjects had a similar or even lower fasting blood glucose level and a lower fasting FFA level compared with controls.

The WHR, PAI-1 activity, fibrinogen and serum triglyceride levels were higher in GH-deficient patients compared with controls.

After 18-24 months of GH treatment, PAI-1 activity, PAI-1 antigen and t-PA antigen decreased significantly. The rapid plasmin inhibitor, a2-antiplasmin, and the coagulation inhibitor, protein C, decreased. Fasting insulin levels increased, but blood glucose did not differ after two years of GH treatment. Favourable changes in body composition in terms of increased lean body mass were observed. It remains to be seen whether the decrease in fibrinolytic variables following GH treatment reduces the cardiovascular risk in these patients.

In an administration study, daily s.c. injections in the evening and a continuous s.c. infusion of GH for 14 days had similar effects on several GH-dependent factors, but in some cases the magnitude of the effects differed. Serum IGF-1 and IGFBP-3 levels increased to a higher degree during the continuous infusion of GH. Fasting FFA levels only increased during treatment with daily injections of GH. The fasting blood glucose level and an oral glucose tolerance test indicated more impaired glucose tolerance after daily injections of GH compared with continuous infusion.

In another study, the mean CSF GH concentration increased tenfold compared with baseline after one month of GH treatment, thus indicating that GH does pass the human blood-CSF barrier. CSF IGF-1, CSF IGFBP-3 and CSF ß-endorphin immunoreactivity also increased during GH treatment, while the dopamine metabolite HVA and VIP decreased. The improved psychological well-being following GH treatment in GH-deficient adults might be related to neuroendocrine changes.

8. Acknowledgements

I wish to express my warm and sincere thanks to everyone who has helped and supported me during this work and especially to:

Bengt-Åke Bengtsson, my supervisor for guiding me into the world of science and GH, for his encouragement and concern and his outstanding leadership.

All the participating patients for making the actual study possible.

Kerstin Landin for her invaluable help, support and constructive criticism from the very first second of this study.

My co-authors Mats Andersson, Ragnar Bjarnason, Anders Elmgren, Jesper Fowelin, Lars Hynsjö, Gudmundur Johannsson, Ibe Lager, Göran Larson, Anders Lindahl, Sven Lindstedt, Per-Arne Lundberg, Jan Oscarsson, Thord Rosén and Lilian Tengborn for fruitful discussions and helpful advice.

Professor Olle Isaksson and Professor Ulf Smith for showing great interest in my work and providing excellent working conditions.

Lena Carlsson, Björn Carlsson, Jan Eriksson, Jörgen Isgaard, John-Olov Jansson and Fred Nyberg for stimulating discussions and valuable advice.

The staff at the Research Centre for Endocrinology and Metabolism, Ingrid Hansson, Sigrid Lindstrand, Annika Reibring, Anne Rosén and Lena Wirén, for their comradeship and excellent technical assistance.

Renate Bittner, Andrzej Grzegorczyk, Carola Gustafsson, Kurt-Verner Johansson, Maria Karlsson, Margareta Landén, Marie-Louise Norberg and the staff at the Coagulation Unit, Department of Clinical Chemistry, for their excellent technical assistance.

Ulla-Britt Liebera, Kaisa Torstensson, Birgitta Sandrén and the staff at the endocrine ward for their invaluable help and friendship.

My friends and colleagues at the Endocrine Division and Department of Medicine for their friendly support.

Anders Odén and George Lappas for their expert statistical aid.

Jeanette Kliger for revising the English language.

Frank Brundin for his long friendship, help with the graphic design and for the delicious Thai chicken in curry.

Finally, my wife Eva and our twins Frida and Aron for their love and for making life outside the hospital enjoyable and meaningful.

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