CLINICAL STUDY

A dose titration model for recombinant GH substitution aiming at normal plasma concentrations of IGF-I in hypopituitary adults

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Abstract

Objective: To evaluate a dose titration model for recombinant human GH substitution in adult patients with GH deficiency, aiming at normal plasma levels of IGF-I.

Design and methods: Eighteen patients participated and a start dose of 0.17 mg GH/day was used except by two men who started with 0.33 mg/day. To demonstrate a clear GH effect the patients were first titrated, with steps of 0.17 mg GH/day every 6–8 weeks, to IGF-I levels in the upper range of age-adjusted reference values. The GH dose was then reduced 1 dose step and kept for a further 6 months. For comparison we investigated 17 healthy control subjects.

Results: Plasma IGF-I was increased after 2 weeks on the start dose and did not increase further for up to 8 weeks. Women had significantly lower GH sensitivity than men measured as net increment of IGF-I on the start dose of GH. GH sensitivity was not changed by age. The plasma IGF-I levels increased from 76.3 ± 47.0 (S.D.) to 237 ± 97 $\mu\text{g/l}$ at the end of the study ($P < 0.001$), and similar IGF-I levels were obtained in both sexes. The maintenance median GH dose was 0.33 mg/day in males and 0.83 mg/day in females ($P = 0.017$). The GH dose correlated negatively with age in both sexes. Body weight, very low density triglycerides, lipoprotein(a) (Lp(a)), and fasting insulin increased, whereas insulin sensitivity index (QUICKI) decreased significantly. In comparison with the controls, the patients had lower fasting blood glucose, fasting insulin and Lp(a) levels at baseline, but these differences disappeared after GH substitution. The two groups had equal insulin sensitivity (QUICKI), but 2 h oral glucose tolerance test values of blood glucose and insulin were significantly higher in the patients at the end of the study.

Conclusions: In conclusion our data suggest that the starting dose of GH substitution and the dose titration steps should be individualised according to GH sensitivity (gender) and the IGF-I level aimed for (age). The reduced insulin sensitivity induced by GH substitution could be viewed as a normalisation if compared with control subjects.

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Introduction

Retrospective studies have shown an increased mortality, mainly due to cardiovascular diseases, in patients with hypopituitarism not substituted with growth hormone (GH) (1). On the other hand GH excess in acromegaly is associated with diabetes mellitus, hypertension and premature death in malignant and cardiovascular disease (2, 3). Thus, both GH deficiency and excess of GH may be harmful. The GH-deficiency syndrome in adulthood is now well established and treatment with recombinant human GH has beneficial effects (4–6). These studies used high doses of GH without attention to gender and age and side-effects were common. The anabolic effects of GH on muscle,

skeleton and other tissues in adult men are mediated by insulin-like growth factor-I (IGF-I) (7). Moreover, circulating IGF-I levels seem to be the best marker for evaluating the GH effect during GH treatment (8). Therefore, titrating the GH dose according to population-based reference values of IGF-I (9, 10) might be a way to obtain a fairly physiological substitution dose of GH. According to population-based reference materials there are no or little gender differences in IGF-I levels (9, 10). Few attempts of individualised dose titration of GH in GH-deficient adults with close monitoring of IGF-I levels have been made. Johannsson *et al.* (11) dose titrated GH in GH-deficient adults according to both IGF-I levels and body composition, which resulted in many patients with supraphysiological

IGF-I levels and lower IGF-I levels in women compared with men. Also Drake *et al.* (12) failed to obtain equal IGF-I levels in both genders after dose titration of GH using IGF-I as a marker of a GH effect.

Since excess of GH is harmful, we designed and evaluated a dose titration model for GH substitution for GH-deficient adults, aiming at normal levels of IGF-I according to previously established population-based reference levels (9). We also investigated the effects of the titrated GH dose on anthropometric variables, lipids, carbohydrate metabolism and the frequency of adverse events.

Subjects and methods

Patients

We studied 18 adult hypopituitary subjects (11 men and 7 women, mean age 43 years; range 22–64), recruited from our outpatient care unit. Fourteen patients had adult onset and four patients had childhood onset (one male and three females) of GH deficiency. The mean duration from the diagnosis of GH deficiency was 17 years (range 1–44). The patients had the following diagnoses: non-secreting pituitary adenoma (six), prolactinoma (five), idiopathic pituitary insufficiency (three), craniopharyngeoma (one), dysgerminoma (one), pituitary apoplexy (one) and pituitary stalk trauma (one). All patients, except one woman, had multiple hormone deficiencies and received additional hormonal replacement therapy if required: L-thyroxine 0.05–0.15 mg/day (males/females,

11/6) and cortisone acetate 18.75–37.5 mg/day (males/females, nine/five); i.m. injection of testosterone enanthate every second to fourth week (ten males) and testosterone undecane orally (one male); ethinyl oestradiol/levonorgestrel orally (three females), oestradiol/norethisterone orally (two females) and vaginal oestrone therapy (one female); desmopressin orally (one female) and dopamine agonists (three males).

Control subjects

Reference values of IGF-I were calculated from 101 men and 101 women, 20–70 years of age, randomly selected from the population registry (9). The participation rate was 67% of those invited. The control group was selected from the above reference population and was matched as closely as possible, for height, weight, age and gender, to the GH-treated group. For the two shortest women we could not find control subjects within the matching criteria for height (i.e. the controls were taller), and therefore body mass index (BMI) was calculated to be sure that these control subjects did not differ markedly from the body proportions of the patients. Seventeen control subjects were enrolled and 16 subjects fulfilled the study (Table 1). The primary aim of using an untreated control group was to control during the course of the study for systematic errors in measurements and secondarily for comparison with the GH-deficient group on GH-dependent efficacy variables.

Table 1 Subjects characteristics (means±s.d. or median and range) at baseline (T₀) and at the end of the study (T_{end}).

	GH-deficient (T ₀)	P	GH-deficient (T _{end})	Control (T ₀)	P	Control (T _{end})
Number	18	—	18	17	—	16
Males	11	—	11	11	—	10
Females	7	—	7	6	—	6
Age	42.8±11.6*	—	—	45.2±10.3*	—	—
Height (cm)	170.3±10.4*	—	—	173.1±9.0*	—	—
Weight (kg)	77.9±15.5	0.014	80.9±16.6	78±14.5	0.43	75.9±13.2
BMI	26.8±4.6	0.014	27.7±4.9	25.3±4.1	0.42	25.5±3.6
Waist/hip standing	0.93 (0.79–1.02)	0.078	0.92 (0.8–1.01)	0.88 (0.74–0.98)	0.19	0.89 (0.75–0.98)
Systolic BP (mmHg)	122.6±14.7	0.74	123.3±16.8	121.5±8.7	0.44	122.8±10.2
Diastolic Bp (mmHg)	77.9±10.9	0.24	80.7±9.9	78.5±7.6	0.78	77.1±5.6
Heart rate (beats/min)	64.1±10.7	0.029	68.3±10.1	62.8±6.5	0.11	66.6±10.8
Total cholesterol	5.3±0.85	0.11	5.1±1.08	5.0±0.74	0.60	5.0±0.88
VLDL-cholesterol	0.46±0.44	0.068	0.5±0.34	0.40±0.3	0.16	0.34±0.29
LDL-cholesterol	3.9±0.75	0.061	3.6±0.84	3.5±0.65	0.36	3.6±0.77
HDL-cholesterol	0.98±0.24	0.12	0.90±0.23	1.0±0.24	0.22	1.0±0.23
HDL ₂ -cholesterol	0.20±0.12	0.65	0.20±0.17	0.23±0.17	0.65	0.27±0.17
HDL ₃ -cholesterol	0.78±0.12	0.093	0.72±0.15	0.78±0.12	0.29	0.74±0.17
Triglycerides	1.19 (0.56–4.38)	0.071	1.36 (0.43–3.25)	0.93 (0.5–2.8)	0.65	0.96 (0.29–2.48)
VLDL-triglycerides	0.73 (0.21–3.71)	0.043	0.94 (0.21–2.62)	0.57 (0.19–2.14)	0.60	0.56 (0.08–2.16)
Lp(a)	32.5 (1–428)	0.006	46.5 (4–746)	156 (25–712)	0.014	114.5 (13–623)
Apolipoprotein A1	1.20±0.22	0.74	1.23±0.31	1.28±0.23	0.29	1.31±0.22
Apolipoprotein B	1.24±0.25	0.32	1.20±0.30	1.09±0.22	0.16	1.13±0.31

* $P < 0.05$ between groups.

Study design and treatment

Patients were treated and assessed prospectively, and compared with an untreated healthy control group, using a defined protocol. The patients were on stable replacement therapy for other pituitary hormone deficiencies for at least 6 months before testing the GH reserve. Inclusion criteria for participation in the study were based upon maximal GH response to insulin-induced hypoglycaemia (blood glucose <2.2 mmol/l), and the mean GH peak value in the patients was 0.6 mU/l (range 0.05–2.4). The patients were instructed on self-injection of GH (Norditropin; Novo Nordisk A/S, Denmark) using a pen-injection device (Nordiject 24; Novo Nordisk), and taught to inject the GH dose between 2000 and 2200 h. The start dose of GH was defined according to body weight in the original protocol and the first two patients (two men) started with a daily dose of 0.33 mg GH/day (1.0 IU). However, due to symptomatic fluid retention the dose was reduced in these two patients to 0.17 mg GH/day after 2 weeks and 4 weeks respectively. Because of these adverse events an amendment was sent to the Swedish Medical Products Agency, and the start dose and the titration steps of GH were defined as a fixed dose of 0.17 mg GH/day (0.5 IU) in the other 16 patients. The two male patients starting with 0.33 mg followed the same protocol as the other 16 patients after the dose reduction (Fig. 1 shows the dose titration procedure in detail). The rationale for our dosing scheme was first to show a clear effect of the GH dose by titrating the dose until a high normal

IGF-I level was reached and then to slightly reduce the dose to achieve a maintenance GH dose giving IGF-I levels at or just below the mean.

Ethics

The study was approved by the local ethical committee and performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients and control subjects.

Measurements

Two specially trained research nurses did all anthropometric measurements and blood samplings. The same research nurse followed the individual patient and corresponding control subject during the study. Venous blood sampling was performed in the patients and the control subjects in the fasting state between 0730 and 0900 h. IGF-I was measured at all visits and analysed within 2 weeks for dose adjustments. All other efficacy variables were evaluated at baseline (T_0) and at the end of the study (T_{end}).

BMI and waist/hip ratio measurements

BMI was calculated according to the formula weight (kg) divided by height (m) squared. The circumference of the waist and hip was measured and the ratio was calculated. The measurement was made in duplicate and the mean of two values recorded.

Figure 1 Dose titration procedure. A = screening visit, B = insulin-induced hypoglycaemia, T_0 = start of GH substitution. The initial GH dose was maintained for 2 months and IGF-I was measured after 14 days (T_{14}), 1 month (T_1) and 2 months (T_2). If the IGF-I value was below age-related mean + 1 s.d. the GH dose was increased every 6–8 weeks in dose steps of 0.17 mg (T_3 , T_4 , T_n). The GH dose giving IGF-I levels around the mean + 1 s.d. (T_{max}) was maintained for another month and was then decreased by 0.17 mg ($T_{\text{maintenance dose}}$). The injection system (Nordiject) allowed steps of 0.17 mg GH and the lowest dose the patients could have was 0.17 mg/day. The decreased GH dose was the maintenance dose and was held for a further 6 months to T_{end} . The untreated non-hypopituitary control subjects did the corresponding visits A, T_0 and T_{end} .

Assessment of glucose tolerance (oral glucose tolerance test (OGTT)) and insulin sensitivity (QUICKI)

Glucose monohydrate (75 g) diluted in 500 ml water was given orally and blood glucose and serum insulin concentrations were measured before and 2 h after glucose intake. Insulin sensitivity was calculated from fasting insulin (I_0) and blood glucose (G_0) levels using a quantitative insulin sensitivity check index ($QUICKI = 1/(\log(I_0) + \log(G_0))$) (13). The units of blood glucose were converted from mmol/l to mg/dl and serum insulin from pmol/l to μ U/ml in this index to give the possibility for comparisons (13).

Assessment of plasma lipids and lipoproteins

Blood was drawn in vacutainer tubes containing EDTA. The tubes were kept in iced water for about 1 h and then centrifuged at 2500 *g* for 10 min for separation of plasma and blood cells. Plasma was stored at 4 °C no longer than 96 h. Very low density (VLDL) lipoproteins were separated from low (LDL) and high density lipoproteins (HDL) by ultracentrifugation in a Centrikon T-2060 ultracentrifuge (Kontron TFT 45.6, Switzerland) at $d = 1.006$ kg/l as previously described (14). In the infranantant LDL was precipitated with phosphotungstic acid/magnesium chloride, leaving HDL in solution. Total and lipoprotein cholesterol and triglyceride concentrations were determined by enzymatic calorimetric methods (Monotest cholesterol CHOD-PAP and triglyceride GPO-PAP; Boehringer-Mannheim GmbH, Mannheim, Germany). LDL concentrations were obtained by the difference between concentrations in infranantant before and after precipitation. The HDL₃ fraction was separated from the HDL₂ fraction by ultracentrifugation of plasma at $d = 1.125$ kg/l as previously described. The infranantant HDL₃ was removed for lipid analysis. The cholesterol concentration of HDL₂ was calculated as the difference between HDL and HDL₃ concentrations. Lipoprotein recoveries ((VLDL + LDL + HDL lipid)/total lipid concentration $\times$ 100) outside 90–110% for cholesterol and triglycerides were not accepted. Plasma total apolipoprotein A1 and B were determined by electroimmunoassay as previously described (15). Lipoprotein(a) (Lp(a)) was determined with an ELISA method (Biopool, Umeå, Sweden).

Blood pressure and heart rate

Blood pressure was measured in the supine position after 5 min rest. A mercury sphygmomanometer was used and the mean of two measurements in the right arm was recorded.

Measurements on control subjects

The control subjects did the same measurements as the patients at corresponding visits: a screening visit, at baseline (T_0) and at the end of the study (T_{end}).

Assays

Serum IGF-I was measured with a commercial kit (Nichols Institute, San Juan Capistrano, CA, USA) by RIA after acid-ethanol extraction of IGF-I from its binding proteins. The assay was performed according to the manufacturer's protocol. Inter-assays coefficient of variation for serum IGF-I was 10%. No difference between gender was found in our reference population regarding IGF-I levels (9) and predicted values of IGF-I were calculated from the entire population: $375 - (3.4 \times \text{age})$. The standard deviation score for IGF-I (IGF-I S.D. score) was then calculated: observed IGF-I value – predicted value/83.

GH was measured with a solid phase, two-site fluoroimmunoassay (Wallac Oy, Turku, Finland). Serum insulin concentration was measured with an enzyme immunoassay from Dako Diagnostics Ltd, Cambs, UK. Capillary blood glucose was measured at the department of Clinical Chemistry with a glucose dehydrogenase method.

Statistical analysis

Statistical calculations were made using a personal computer and SPSS version 10 software. Baseline clinical characteristics are presented as means $\pm$ S.D. or median and range. Differences within and between groups were tested with a paired two tailed *t*-test or a Wilcoxon signed rank sum test. Differences between gender were tested with an unpaired two tailed *t*-test or a Mann–Whitney U test. Multiple regression analysis was used to test possible explanatory variables affecting the maintenance GH dose. Differences of $P < 0.05$ were considered significant.

Results

Plasma IGF-I concentrations and titration of the GH dose

Mean serum IGF-1 concentration before GH substitution was significantly lower in the GH-deficient group (76.3 ± 47.0 μ g/l) compared with the reference population (9) (224 ± 83 μ g/l) ($P < 0.001$). Within the GH-deficient group there was a tendency to lower IGF-I levels in females at baseline (women 50 ± 40 μ g/l vs men 93 ± 45 μ g/l, $P = 0.052$). Mean IGF-I concentration at the end of the study (T_{end}) on the maintenance dose of GH was 237 ± 97 μ g/l for the whole group ($P < 0.001$ vs baseline), and no difference was seen between genders (255 ± 95 in males vs $209 \pm$

102 $\mu\text{g/l}$ in females, $P = 0.34$). The calculated mean IGF-I S.D. scores were at T_{end} : men 0.28 ± 1 and women -0.20 ± 1.2 ($P = 0.38$). Fourteen patients (seven men and seven women) who started with 0.17 mg (0.5 IU) had evaluable IGF-I measurements after 2 weeks, 1 and 2 months. The IGF-I levels were significantly increased after 2 weeks ($P = 0.021$) on the lowest GH dose (0.17 mg/day) and no further increase was found after 1 or 2 months on the same dose (Fig. 2). The net increment of plasma IGF-I on the start dose after 2 months, when all patients were treated with 0.17 mg GH/day, were $95 \pm 66 \mu\text{g/l}$ for men and $36 \pm 24 \mu\text{g/l}$ for women ($P < 0.05$) (Fig. 3). The mean increment of all the following dose steps did not differ significantly from the first step: men $58 \pm 24 \mu\text{g/l}$ and women $43 \pm 20 \mu\text{g/l}$. The individual IGF-I values in the GH-deficient group, in relation to population-based reference values before GH substitution (T_0), at the highest dose (T_{max}) and at the end of the study on the maintenance dose (T_{end}) are shown in Fig. 4. To be noted was a very low IGF-I value in one female patient, probably caused by poor compliance, as her IGF-I value 3 months before the study end was well within our goals. In addition one man had an IGF-I level above 2 S.D. at the last visit and this could have been due to an analytical error during the titration period (i.e. inappropriately low IGF-I levels).

Maintenance dose of GH

The maintenance GH dose was the same as the next highest dose in the dose titration period. If the net number of dose steps (of 0.17 mg/day) were calculated according to this dose, the median number of dose steps to reach the maintenance dose were 2 (range 1–7) for the 11 males and 5 steps (range 2–8) for the seven females. The median GH dose was significantly affected by gender (males 0.33 mg/day (0.17–1.17) vs females 0.83 mg/day (0.33–1.33), $P = 0.017$), and negatively correlated with age in both genders (Fig. 5). The three women with childhood onset of GH deficiency

Figure 2 Mean IGF-I levels in 14 patients (seven males and seven females) at baseline and after 2, 4 and 8 weeks on the start dose of 0.17 mg GH/day. Data shown with 95% confidence intervals.

Figure 3 Net increment of circulating IGF-I after 2 months on the start dose in relation to age. All 18 patients had a GH dose of 0.17 mg/day.

had a higher median maintenance GH dose, 1.0 mg (0.83–1.33), than the four women with adulthood onset, 0.6 mg (0.33–1.0) ($P = 0.15$). In a multivariate model with the maintenance GH dose as dependent

Figure 4 IGF-I levels in the GH-deficient group in comparison with age-adjusted reference values. (A) Before treatment (T_0), (B) on the highest GH dose (T_{max}), (C) after 6 months on the maintenance GH (T_{end}).

Figure 5 Maintenance doses of GH in women and men in relation to age.

factor, gender ($P = 0.003$), age ($P < 0.001$) and body weight ($P = 0.004$) were significant factors influencing the maintenance GH dose. Height or pre-treatment IGF-I levels did not predict the maintenance dose.

Anthropometric characteristics and blood pressure

Body weight, BMI and heart rate increased significantly in the GH-deficient group. Waist/hip ratio and systolic and diastolic blood pressure were unchanged during the study (Table 1).

Blood glucose, serum insulin and insulin sensitivity

All patients had blood glucose levels in the fasting state within the reference range at baseline. There was no significant change in fasting blood glucose levels (4.1 ± 0.4 mmol/l before substitution vs 4.3 ± 0.4 mmol/l at the end of the study, $P = 0.38$). Fasting insulin levels increased significantly during the study (35.9 ± 25.7 pmol/l vs 52.5 ± 25.6 pmol/l, $P = 0.02$). Prior to GH substitution one man and one woman had impaired glucose tolerance according to an OGTT, which in the man normalised during the study. No patient developed diabetes mellitus, but in addition two more patients developed impaired glucose tolerance at the end of the study. There was a tendency to increased 2 h concentrations of blood glucose (6.0 ± 1.3 vs 6.5 ± 1.5 mmol/l, $P = 0.13$), and in serum insulin (228.6 ± 53.9 vs 440.9 ± 103.9 pmol/l, $P = 0.054$) during GH substitution. Insulin sensitivity index (QUICKI) decreased after substitution (0.41 ± 0.07 vs 0.36 ± 0.03 , $P < 0.02$).

Lipids and lipoproteins

VLDL-triglycerides and Lp(a) increased significantly ($P = 0.043$ and $P = 0.006$). Both HDL-cholesterol and LDL-cholesterol tended to decrease but the changes did not reach statistical significance. No changes were seen in total cholesterol, apolipoprotein A1 and B or

the ratio between apolipoprotein B and LDL-cholesterol (Table 1).

Adverse events

In total 64 events were reported in the patients and most were graded as transient and mild. The most frequently reported adverse events were: complaints about the musculoskeletal system (ten patients), increased weight (seven patients), oedema (four patients), increased blood pressure (three patients) and decreased libido (three patients). The two men who started with a daily dose of 0.33 mg both experienced early oedema, which disappeared after dose reduction, and two female subjects experienced transient oedema after 6 and 9 months respectively; both subjects were at that time treated with 1.0 mg/day. Three patients were hospitalised during GH treatment: a 64-year-old female for exacerbation of pains due to known osteoarthritis, and as a consequence a previously planned arthroplasty was done; a 47-year-old man due to viral infection and signs of adrenal insufficiency; and a 24-year-old male previously operated for a cystic craniopharyngeoma who was treated with stereotactic instillation of radioactive yttrium in a known cyst, which had increased in size.

Comparisons between the GH-deficient group and the control group before GH substitution

Table 1 shows demographic data. A significant difference in IGF-I levels at baseline was found (76.3 ± 47.0 µg/l in the GH-deficient group and 181 ± 49.2 µg/l in the control group, $P < 0.001$). There were slight significant differences in age and height: GH-deficient group 43 ± 12 years and 171 ± 11 cm vs control group 45 ± 10 years and 173 ± 9 cm ($P = 0.034$ and $P = 0.020$). Fasting blood glucose was significantly lower in the GH-deficient group before substitution compared with the control group (4.1 ± 0.4 vs 4.5 ± 0.51 , $P = 0.021$), but there was no difference in the 2 h OGTT blood glucose levels between the two groups (6.0 ± 1.3 vs 5.8 ± 0.9 mmol/l, $P = 0.94$). Fasting insulin levels in the GH-deficient group before treatment tended to be lower compared with levels in the control group (32.8 ± 22.7 vs 60.2 ± 53.7 pmol/l, $P = 0.089$). No difference was found in 2 h insulin levels (256 ± 229 vs 99 ± 118 pmol/l, $P = 0.329$). No significant differences were found in baseline plasma levels of lipids (Table 1).

Comparisons between the GH-deficient group and the control group after GH substitution

Within the control group no significant changes occurred during the study in bodyweight, BMI, waist/hip ratio, blood pressure, heart rate, blood glucose, serum insulin levels in the fasting state or after OGTT,

or in insulin sensitivity (QUICKI). No changes in the lipid concentrations were seen besides a significant decrease in Lp(a) ($P = 0.014$) (Table 1). The difference in fasting blood glucose between the GH-deficient group and the control group disappeared (4.3 ± 0.4 vs 4.5 ± 0.51 mmol/l, $P = 0.17$). The 2 h value of blood glucose during OGTT increased in the patients compared with the controls (6.5 ± 1.5 vs 5.7 ± 1.2 mmol/l, $P = 0.028$). The fasting insulin levels tended to increase in the GH group and were equal to the control group (52.4 ± 26.2 vs 63.9 ± 57.5 pmol/l, $P = 0.483$). The 2 h value of serum insulin during OGTT increased in the patients compared with the controls (489 ± 460 vs 154.5 ± 94 pmol/l, $P = 0.018$). No difference in insulin sensitivity (QUICKI) was found (0.36 ± 0.03 in the patients and 0.36 ± 0.04 in the control subjects, $P = 0.16$).

Discussion

Since excess of GH is harmful we hypothesised that a GH dose giving circulating IGF-I levels at or slightly below the age-adjusted mean could be a safe and effective maintenance dose. To be sure of the GH effect and compliance, we first increased the dose stepwise to reach plasma levels of IGF-I well within the upper range of age-adjusted reference levels, whereafter the dose was reduced one step (0.17 mg/day). With this titration procedure we could achieve, in both males and females, a clear effect of GH substitution, with circulating IGF-I levels close to the goal of treatment.

Even on the start dose of 0.17 mg/day some male patients reached IGF-I levels in the upper range directly. In contrast, women tended to have lower IGF-I levels at baseline and significantly lower GH sensitivity measured as net increment of IGF-I on the start dose compared with the men (Fig. 3). In one dose titration study the patients started at 0.27 mg GH/day and a minority of the women but most of the men achieved age-adjusted IGF-I levels in the upper range directly on the start dose (12). Janssen *et al.* (16) studied doses of 0.2, 0.4 or 0.6 mg GH/day and found that a dose of 0.2 mg/day was sufficient to normalise IGF-I levels in the majority of the male patients but not in the female patients. Available data thus strongly suggest that the start dose of GH should be individualised according to gender.

Already 2 weeks after the start of GH substitution the IGF-I levels were significantly increased, but the levels did not increase further after 1 or 2 months (Fig. 2). This is in accord with Drake *et al.* (12), who found increased IGF-I levels 2 weeks after the start of treatment and no additional increase after 4 weeks. This indicates that a full response of circulating IGF-I on the start dose can be measured within 2 weeks or even earlier. Even if dose adjustments could be done after 2 weeks according to IGF-I levels, it seems more physiological to use longer dose intervals, as the

symptoms related to fluid retention seem to occur mainly during the first phase of GH treatment (5, 17). These early side-effects could be caused by increased GH sensitivity in the untreated state of GH deficiency (18). The intervals of 6–8 weeks between dose adjustments of GH used in our study therefore seem reasonable to recommend.

Similar IGF-I levels were obtained in men and women but the median maintenance GH dose was significantly lower in males compared with females. Several studies have found gender differences in the IGF-I-generating effect of GH treatment (19, 20), but according to population-based reference materials there are no or little gender differences in IGF-I levels (9, 10). Analysis of 24 h GH profiles showed a daily GH production 2–3 times greater in women than in men to maintain an equivalent IGF-I level, suggesting that women need higher doses of GH (21). Even in dose titration studies with stepwise increments of the GH dose and despite a higher maintenance dose, females had lower IGF-I levels compared with males (11, 12, 22). In contrast, our investigation showed that with systematic dose titration of GH an equal normalisation of IGF-I levels could be achieved in both males and females. However, our results could have been influenced by the fact that most women in the study used oral replacement of oestrogens, which could have decreased the IGF-I-generating effect of GH (23), and by the gender difference in the number of patients with childhood onset of GH deficiency (three females and one male) (24).

Like other investigators (25, 26), we found that some patients with GH deficiency had IGF-I levels within the lower part of the age-related reference range, and that this was more common in the elderly patients. Our dose titration model could be a solution to this problem. The GH dose is increased stepwise until a high normal age-adjusted IGF-I level (between 1 and 2 s.d.) has been achieved with a clear separation from baseline IGF-I levels, and the dose is then reduced to obtain IGF-I levels within defined goals. In analogy with the physiological decrease in GH secretion with increasing age (27) we found that the GH dose was lower in older age in both females and males in order to normalise the age-related IGF-I value (Fig. 5). This is in accord with the study by Toogood & Shalet (26) who showed that elderly patients (62–86 years) normalised their IGF-I levels or achieved levels above 2 s.d. for actual age with GH doses between 0.17 and 0.33 mg/day. Since we found that the GH sensitivity did not change significantly with increasing age (Fig. 3), the lower GH dose in the elderly patients was to a great extent explained by the fact that age-adjusted IGF-I levels decrease with age (9, 10). Taking the data together we found that the size of the starting dose and the maintenance dose of GH should be individualised according to gender (GH sensitivity) and age (the magnitude of IGF-I increment aimed for). With newer injection systems now available and dependent of age and

gender, starting GH doses of 0.1–0.2 mg/day for young and middle-aged women and 0.05–0.1 mg/day for men and old subjects of both genders seem appropriate. The size of the dose titration steps is then determined by the IGF-I-generating effect of this start dose and of the IGF-I levels aimed for. Moreover, the GH dose should be repeatedly revised in order to mimic the physiological fall in GH secretion rates and the correspondingly lower IGF-I levels by increasing age.

A key question was if the titrated GH dose giving physiological IGF-I levels had any effects on GH-dependent variables such as body composition, lipids and glucose tolerance previously described in studies using fixed high GH doses (4–6). With euglycaemic–hyperinsulinaemic clamp techniques increased insulin resistance in GH-deficient adults before GH treatment has been reported (28, 29). We used an index of insulin sensitivity (QUICKI) (13) to calculate insulin sensitivity in the fasting state and it was apparent that our GH-deficient patients were not insulin-resistant before substitution. After GH substitution there was an increase in insulin levels, a tendency towards higher fasting and 2 h glucose concentrations, and a significant decrease in insulin sensitivity index (QUICKI) indicating a decrease of insulin sensitivity. One possible explanation for this could be weight gain of the patients compared with the controls. The waist/hip ratio, however, remained unchanged indicating that the body composition had not changed unfavourably. Other studies using fixed high GH doses, with large proportions of the patients having supraphysiological IGF-I levels, have shown beneficial effects on body composition with increased lean body mass and decreased fat mass but despite that an impaired glucose tolerance (4, 6). Even though our patients had IGF-I levels well within the reference range for age, GH substitution did reduce insulin sensitivity. In agreement with our data, Rosenfalck *et al.* (30) also found a decrease in glucose tolerance in several patients despite IGF-I levels in the normal range during GH treatment. In contrast Bulow & Erfurth (31) found no change in glucose tolerance in young adults treated with GH. In our study the decreased insulin sensitivity caused by GH could be viewed as a normalisation in most patients if compared with the control subjects, but the physiological range seems narrow as two patients developed impaired glucose tolerance during the study.

We found no obvious dyslipidaemia at baseline in the GH-deficient group in contrast to other studies (32–34) or when compared with the control group. The controls were in general 2 years older, which could have masked a difference between the two groups, but according to the natural course of cholesterol concentrations during life it seems that the age difference was of minor importance (35). Our patients had normal waist/hip ratios and glucose tolerance, which in part could explain the differences from previous studies, as GH deficiency has been associated with central adiposity

(36) and insulin resistance (29). Although most of the changes in the lipoprotein concentrations during GH substitution did not reach statistical significance the changes taken together support the concept of decreased insulin sensitivity. Increase of plasma triglycerides or VLDL-triglyceride concentration and lowering of plasma HDL-cholesterol concentration are commonly found in insulin-resistant individuals with obesity or type 2 diabetes (37). As observed in several studies, Lp(a) increased during GH substitution (38, 39) but no difference between the GH-deficient group and the control group could be found at the end of the study, questioning the clinical importance.

In conclusion our data suggest that the starting dose of GH substitution and the dose titration steps should be individualised according to GH sensitivity (gender) and the IGF-I level aimed for (age). Even if the reduced insulin sensitivity by GH substitution could be viewed as a normalisation if compared with control subjects, it seems appropriate to monitor glucose tolerance repeatedly with an OGTT.

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