CLINICAL STUDY

Circulating IGF-I levels in monitoring and predicting efficacy during long-term GH treatment of GH-deficient adults

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Abstract

Objective: To investigate the effects of long-term GH in GH-deficient adults, as predicted by IGF-I levels.

Methods: Patients received GH, 5 μg/kg per day for 1 month and 10 μg/kg per day for another 12–30 months. Changes in body composition, cardiac structure/function, serum lipids and quality of life were measured.

Results: There was a significant increase in lean body mass (LBM) (2.21 kg; P < 0.0001) after 6 months, which was sustained throughout treatment. A larger increase occurred in males than females (2.97 vs 1.19 kg; P < 0.0001). Total fat mass was reduced (2.56 kg; P < 0.0001 (3.26 kg males, 1.63 kg females)). Responsiveness to GH varied greatly, but LBM changes correlated with IGF-I changes (P < 0.004). Furthermore, thinner patients experienced greater and progressive LBM increases. There was an increase in ejection fraction (3.85±9.95%; P = 0.0002) after 6 months, sustained to 18 months. These cardiac effects were equal for males and females, and did not correlate with IGF-I levels. Serum low-density lipoprotein/high-density lipoprotein ratios decreased within 6 months, and were sustained thereafter. Quality of life improved significantly after 6 months, an effect that was sustained/enhanced as treatment continued. No major adverse events were identified.

Conclusions: Improved body composition is both reflected by IGF-I changes and predicted inversely by baseline adiposity. Other effects of GH replacement on cardiac function, dyslipidaemia and quality of life, however, do not correlate with circulating IGF-I concentrations. Our findings validate the importance of sustained GH therapy, but caution on the interpretation of IGF-I levels in monitoring the long-term effects of GH treatment.

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Introduction

Growth hormone (GH) replacement therapy in adult GH-deficient (GHD) patients today is an established therapy. More than 100 published clinical trials have been conducted to characterize the GHD syndrome, to determine the effects of replacement therapy with recombinant human GH, and to provide the basis for optimization of treatment in order to maximize benefits and minimize potential risks. Numerous reviews have described the diversity of effects of GH replacement therapy (e.g. 1–4).

GH replacement aims to normalize the features of GHD, but the functional and long-term outcomes of therapy are relatively less well-documented. As a consequence, different clinical practices have developed in different countries on the rationale and approach to GH replacement therapy (3). GH replacement aims to either alleviate the symptoms of GHD and/or target GHD-associated abnormal markers, such as those for osteoporosis and cardiovascular disease. There is indirect evidence that GHD is related to the increased morbidity and mortality in cardiovascular disease demonstrated amongst hypopituitary patients who receive conventional hormonal replacement therapy without GH substitution (5–8). Higher frequencies of sick leave and disability pension amongst patients with a history of pituitary tumour have been reported compared with the general population (9).

Ample and robust evidence supports the remedial effects of GH replacement on body composition, including gradual normalization of lean body mass (LBM) and reduction in fat mass (10, 11). A number of studies have also demonstrated improvements in

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psychological well-being, with specific improvements in energy, emotional reactions and social isolation (12, 13). Moreover, healthcare consumption decreases in parallel with enhanced psychological well-being (14).

Improvements in muscle strength, exercise capacity and respiratory function have also been observed in some studies (15–17). Other characteristic features of longstanding GHD are cardiac structural and functional abnormalities (18), dyslipidemia, endothelial dysfunction, abnormal coagulation and abnormalities of the cardiac autonomic system (1, 3, 19).

The aim of the present study was to investigate the potentially therapeutic effects of low-dose (5–10 μg/kg per day) GH replacement during up to 3 years of treatment in a large group of GHD patients. We examined the role of circulating insulin-like growth factor (IGF)-I in monitoring the impact of GH on body composition, cardiac structure and function, lipid metabolism and quality of life at 6 month intervals.

Patients and methods

Clinical protocol

The study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice, with approval from individual human ethics review boards, and patient informed consent was obtained.

A total of 115 adult male and female patients with GHD were recruited and randomized to receive either recombinant human GH (Saizen; Serono), 5–10 μg/kg per day, or corresponding placebo for 6 months, followed by open-label GH treatment for all patients. Only a small minority (16%) of patients had childhood-onset GHD. Patients had severe GHD, as determined by peak GH response following a stimulation test; all patients had peak GH < 5 μg/l, 103 patients had peak GH < 3 μg/l and 95 patients had peak GH < 2.5 μg/l. One hundred and eleven patients continued from the double-blind, placebo-controlled phase of the study, and received active treatment in the open-label phase (only four placebo patients withdrew before reaching the open-label phase). Clinical and endocrine baseline characteristics are summarized in Table 1.

Inclusion criteria included: age between 20 and 70 years, acquired or idiopathic GHD present for at least 24 months, peak stimulated GH < 5 μg/l during an insulin tolerance test or a combined GH-releasing hormone and arginine test, adequate replacement therapy for associated thyroid-stimulating hormone, gonadotrophin or adrenocorticotropic hormone deficiencies (thyroid, gonadal steroid hormones and glucocorticoids) for at least 12 months, and no GH treatment for at least 2 years. Patients with a history of Cushing’s disease were included only after their disease was in remission for at least 1 year. Exclusion criteria included: tumor recurrence or an enlarging tumor remnant on imaging of the sella turcica within 6 months of study entry, chronic severe kidney or liver disease, diabetes mellitus, malignancy or unstable hypertension. Details of the double-blind phase of the study have been published previously by Ezzat et al. (20).

Following the 6 month double-blind, placebo-controlled phase, all patients received open-label GH for a period of 12–30 months (the duration of the open-label phase was different among participating centres). One hundred patients completed 6 months, 78 patients completed 12 months, 48 patients completed 18 months, 19 patients completed 30 months and 6 patients completed 36 months of GH treatment. Only 23 patients dropped out of active treatment. In order not to unblind the double-blind, placebo-controlled phase of the study, all patients started the open-label phase with the lower dose of 5 μg/kg per day for 1 month, followed by 10 μg/kg per day for the rest of the study.

Body composition methodology

Body composition was assessed by whole-body dual-energy X-ray absorptiometry (DXA) (Hologic model QDR-2000 (Hologic, Inc., Bedford, MA, USA), or Norland model XR26 (Norland Medical Systems, Inc., White Plains, NY, USA), calibrated using a calibration standard each day. DXA determinations provided data on LBM, fat mass, body cell mass and bone mineral content, and bone mineral density of the L2–L4 vertebrae, femoral neck. Ward’s triangle and trochanter major. Phantoms were used to monitor accuracy, precision, and trending variations for DXA measurements.
Echocardiography
Cardiovascular function was assessed by two-dimensional (2-D), M-mode and Doppler echocardiography at baseline and at each 6 month interval. Cardiac measures were performed following an overnight 12 h fast, usually following 30 min in the recumbent position. Patients were instructed to avoid alcohol- or caffeine-containing products. The echocardiographic analysis was performed with ultrasound systems equipped with 2.5, 3.5 or 5.0 MHz transducers, and the M-mode and 2-D tracings were obtained with the patients in the left lateral recumbent position according to the standardization of the American Society of Echocardiography (21).

Evaluation of left ventricular systolic function was made by determinations of ejection fraction (EF) and fractional shortening. EF is calculated from 2-D echocardiography measurements and is the ratio (expressed as a percentage) of the difference between the end-diastolic and end-systolic volumes (stroke volume) and the left ventricle end-diastolic volume. Fractional shortening is a rough measure of ventricular systolic function and is calculated from the determinations of the left ventricle internal dimensions, measured by M-mode echocardiography. Fractional shortening is the ratio (percentage) of the difference between the left ventricle end-diastolic and end-systolic diameters and the end-diastolic diameter. M-mode determinations also allowed calculations of left ventricle mass by the formula of Devereux & Reichek (22).

Evaluation of the ventricular diastolic filling and function was made from Doppler echocardiography assessments. The isovolumetric relaxation time, which represents the time interval between aortic valve closure and mitral valve opening, was determined together with the peak filling rates during diastole E max (the early filling rate) and A max (the atrial filling rate). The E/A ratio and E/F slope were also determined, where the E/F slope is the early diastolic deceleration slope, and the E/A ratio is the ratio of peak mitral early diastolic and atrial contraction velocity.

Doppler echocardiography measurements were also used to calculate cardiac output (CO) and systemic vascular resistance (SVR). CO was calculated as the product of heart rate, aortic flow velocity and aortic cross-sectional area. SVR was calculated from mean arterial pressure (MAP), and CO as SVR = MAP × 80/CO, where MAP was calculated as the diastolic blood pressure + 1/3 of the pulse pressure.

Quality of life
The patient’s perceived well-being was evaluated with the use of the generic Nottingham Health Profile (NHP) (Part I) questionnaire (23). The NHP Part I comprises 38 ‘yes’ or ‘no’ questions that are combined into six domains: emotional reactions, energy, pain, physical mobility, sleep disturbances and social isolation. The results are interpreted as the proportion of ‘yes’ answers; the higher the value, the greater the distress experienced by the patient. As the participating patients had different native languages, the English and French versions of the NHP were used, and the results were not pooled but analyzed separately for each language version.

Growth factors
Serum levels of IGF-I, IGF-II, IGF-binding protein (IGFBP)-I and IGFBP-3 were followed during the study. Samples were assayed centrally for all study centres at the Serono Clinical Laboratories (Cambridge, UK), as previously described (20). IGF-I and IGFBP-3 were measured using Medignost RIAs (Medignost GmbH, Tübingen, Germany), IGF-II and IGFBP-1 using assays from Diagnostic Systems Laboratories (Webster, TX, USA) and Biogenesis (Kingston, NH, USA) respectively.

Laboratory analyses
Routine laboratory tests for hematology, blood chemistry, thyroid function tests and urinalysis were assessed throughout the study. Bone biochemistry (intact parathyroid hormone, bone-specific alkaline phosphatase, C-terminal propeptide of type I collagen, osteocalcin, 1,25-dihydroxyvitamin D3 and urinary deoxypyridinoline) was assayed centrally at the Serono Clinical Laboratories.

Statistical analysis
The primary analysis for each of the main outcome variables is a longitudinal analysis of all available GH-treated patients at 6 month intervals throughout 30 months of treatment. A linear mixed model was used (with a random intercept for patient, all other covariates being considered as fixed effects): \[ y = \alpha_0 + \alpha_1 + \beta x + \epsilon \]
where \( y \) is the outcome variable, \( \alpha_0 \) is the patient intercept, \( \alpha_1 \) is the average difference from active-treatment baseline after \( t \) months of treatment (constrained \( \alpha_1 = 0 \)), and \( x \) is a vector of explanatory variables with associated parameters \( \beta \) (including a time: covariate interaction term). All efficacy analyses are based on all available data for each analysis (including data from patients who had recently withdrawn from treatment).

A subsidiary analysis was made of changes after 6 months of active treatment within the former placebo group, offering confirmation of hypotheses generated by analyses of the double-blind phase. All patients who received a minimum of one injection of study drug were included in the safety analyses.

Results
Circulating IGF system
Baseline IGF-I levels (mean ± s.d., 98.0 ± 53.3 ng/l; \( n = 111 \)) and IGF standard deviation scores (SDSs)
Figure 1 Effect of GH treatment on the circulating IGF system, overall and by gender: changes are compared with baseline. (The error bars give 95% confidence intervals for the mean changes. The increasing width of the intervals over time is a reflection of decreasing sample size – see text – rather than increasing variation.)
Body composition

Assessment of body composition by DXA demonstrated highly significant increases in LBM and decreases in fat mass. The estimated treatment difference in LBM increase during the first 6 months of treatment was 2.21 ± 2.17 kg (P < 0.0001), and further increases were small. LBM was maintained throughout the treatment period at levels that were consistently higher (P < 0.0001) than pre-treatment values. The corresponding difference in fat mass decrease was 2.56 ± 2.80 kg (P < 0.0001). A strong gender effect was observed for changes in both LBM and fat mass. Figure 3 shows the changes from baseline in LBM and fat mass as a group and by gender. Covariate analysis showed that higher baseline body mass index (BMI) predicted smaller changes in LBM (P < 0.0001) and in fat mass (P = 0.002). Conversely, patients with a BMI below the lower quartile of the sample (24.4 kg/m²) experienced greater increases in LBM. This differential response was maintained (with a trend towards further increase) throughout treatment, with an average 1 kg additional increase in LBM by month 6, rising to 1.9 kg by month 18 (P = 0.005). The changes in LBM were also associated with increases in IGF-I levels (P = 0.004) and IGF-I SDS (P = 0.009). No similar IGF-I associations were found with the decrease in fat mass.

Bone metabolism and density

GH treatment stimulated both bone formation and bone resorption, as judged from bone metabolism markers. Serum osteocalcin and the C-terminal propeptide of collagen type I, markers of bone formation, increased from 5.4 ± 2.8 µg/l (n = 111) to 10.7 ± 6.0 µg/l (n = 104; P < 0.001) and from 85.6 ± 40.3 µg/l (n = 111) to 136.0 ± 50.6 µg/l (n = 104; P < 0.001) respectively after 6 months, an effect that was sustained throughout the treatment period. Bone-specific alkaline phosphatase and urinary deoxypyridoline also increased, from 14.4 ± 7.3 U/l (n = 111) to 24.2 ± 13.9 U/l (n = 103; P < 0.001) and from 5.6 ± 2.6 nmol/l (n = 105) to 9.8 ± 4.7 nmol/l (n = 97; P < 0.001) respectively after 6 months; these effects were also sustained throughout the 3 years of treatment. Serum levels of calcium were unchanged whereas phosphate levels tended to increase, although this was not statistically significant.

DXA assessments of total body mineral content and bone mineral density at the lumbar spine (L2–L4), femoral neck, trochanter major and Ward’s triangle revealed a clear trend (although rarely individually statistically significant) towards an increase in bone mineral density in all these locations, as depicted in Fig. 3.

Figure 2 Effect of GH treatment on IGF-I SDS, overall and by gender: changes are compared with baseline. (The error bars give 95% confidence intervals for the mean changes.)
Cardiac changes

Echocardiographic determinations revealed that the internal dimensions of the left atrium, left ventricle, and left ventricular mass were unchanged following GH treatment. The single exception was the left ventricular posterior wall (LVPW) thickness, which tended to increase from $9.0 \pm 2.0$ mm at baseline ($n = 104$) to $9.4 \pm 2.2$ mm after 6 months ($n = 94$; $P = 0.051$). Such a change was already identified during the double-blind, placebo-controlled phase of the study (20). Similar findings were also observed in

Figure 3 Effect of GH treatment on body composition, overall and by gender: changes are compared with baseline. (The error bars give 95% confidence intervals for the mean changes.)
the former placebo group during their first 6 months of GH treatment.

A significant increase in EF from 56.3±10.3% (n = 101) at baseline to 60.6±9.4% after 6 months of treatment (n = 85; P < 0.001) was observed, and remained significantly greater than at baseline for at least 18 months of active treatment (after which the diminishing sample size makes interpretation difficult). Figure 4 depicts the changes in EF and LVPW thickness, both overall and by gender. The changes were not associated with increases in IGF-I levels. There were no statistically significant changes in fractional shortening or CO, nor were there any overall changes in heart rate or resting systolic or diastolic blood pressure. Indices of left ventricle diastolic filling and function, isovolumetric relaxation time, E max, A max, E/A ratio and E/F slope showed trends towards improvement. Similar effects were observed for males and females.

**Lipid levels**

Serum triglycerides and free fatty acids exhibited small, although not statistically significant, changes during GH treatment. High-density lipoprotein (HDL) cholesterol increased significantly, while total cholesterol and low-density lipoprotein (LDL) cholesterol decreased significantly. The ratio between LDL and HDL cholesterol decreased gradually and significantly from 3.37±1.39 (n = 109) at baseline to 2.98 (decrease by 0.39±0.90; n = 98; P = 0.017) at 6 months. This suppression was sustained or further improved throughout the period of GH treatment. These lipid changes were not associated with increases in IGF-I levels, and similar effects were observed for males and females.

**Psychological well-being**

The results from the English version (n = 88) of the NHP questionnaire demonstrated significant improvements between baseline and month 6 in four of the six domains: emotional reactions (P < 0.0001), social isolation (P = 0.0003), energy (P < 0.0001) and sleep disturbance (P = 0.022). Figure 5 shows the results for baseline and for changes from baseline at 6 month intervals. As noted, there was considerable distress at baseline in several of the domains, particularly for energy, emotional reactions and sleep disturbances. The improvements after 6 months of treatment were between 6 and 14%, and were sustained throughout
Figure 5 Effect of GH treatment, as measured using the NHP, on psychological well-being, overall and by gender: changes are compared with baseline. (The error bars give 95% confidence intervals for the mean changes.)
assess and predict the efficacy and safety of GH. In particular, we focused on the ability of circulating IGF-I levels to monitor GH treatment, we specifically report here our findings on the long-term follow-up of a large cohort of GHD adults demonstrated beneficial effects on cardiac function and body composition (20). We previously reported (20, 24–26). Most significantly, the increases in LBM correlated with increases in IGF-I levels, in marked contrast to the reduction in fat mass, which did not correlate with IGF-I changes. The apparent greater benefit in LBM achieved in males was in part accounted for by differences in IGF-I levels achieved. At baseline, females had significantly lower IGF-I levels than males (79.4±43.5 vs 111.6±56.1 μg/l respectively). Nevertheless, the magnitude of the IGF-I increase and LBM gains was similar in females and males, as determined by relative or SDS changes. Our findings indicate that the dose of GH may need to be adjusted against IGF-I levels in order to achieve optimal favorable effects on body composition. Moreover, female patients may require higher GH doses to achieve comparable IGF-I levels and LBM gains. The fact that nearly three-quarters of female patients in our study were receiving oestrogen is consistent with the attenuating effect of this hormone on IGF-I responsiveness to GH administration (26). An additional key finding from our study was the prediction of gains in LBM based on the degree of baseline adiposity. Covariate analysis demonstrated here for the first time that patients with the highest BMI at baseline experienced the smallest increases in LBM and also sustained the least amount of loss of fat mass. Conversely, patients with the lowest baseline BMI (below the lower quartile: 24.4 kg/m²) benefited the most in terms of progressive and significant increases in LBM. The mechanism for this observation is unclear and may require further prospective validation. The present study demonstrates sustained increases in cardiac function. GH treatment resulted in

Adverse reactions

The most common fluid balance-related adverse events (AEs) were arthralgia (30.6% of patients), myalgia (13.5%), peripheral oedema (12.6%), paraesthesia (9.0%), carpal tunnel syndrome (7.2%), generalized oedema (7.2%) and hypoaesthesia (5.4%). The majority of these were mild or moderate in severity. Interestingly, the incidence of AEs was neither associated nor predicted by increases in IGF-I levels. In fact, there was no increase in the incidence of AEs among patients with abnormally high IGF-I levels compared with those with normal adult levels. Thirteen patients withdrew from GH treatment due to AEs. These included an expansion of a pituitary tumour, an enlargement of a glioma, expansion of a cystic component of a residual pituitary tumour, headache, carpal tunnel syndrome, arthralgia, joint swelling and pain, basal cell carcinoma, and myalgia with increased alanine aminotransferase levels.

It is noteworthy that in the double-blind, placebo-controlled phase, we observed an abnormal glucose tolerance test in one patient after 3 months of GH treatment. This patient, with a BMI of 32.7 kg/m², had already demonstrated abnormal glucose tolerance before the study, and consequently no change in GH dose was considered necessary following the abnormal glucose tolerance test in the study. The patient completed GH therapy without any further glucose metabolism-related AEs.

No other clinically significant changes or apparent trends were observed in any laboratory safety assessments during treatment.

Discussion

The results from the first 6 month double-blinded, placebo-controlled phase of GH treatment in this large cohort of GHD adults demonstrated beneficial effects on cardiac function and body composition (20). We report here our findings on the long-term follow-up of this cohort. As IGF-I measurements are commonly employed in monitoring GH treatment, we specifically focused on the ability of circulating IGF-I levels to assess and predict the efficacy and safety of GH replacement. Our data indicate that the same dose of GH achieves equally beneficial effects on cardiac function in male and female patients, despite significant differences in LBM gains and IGF-I levels.

Virtually all of the observed changes during the double-blinded, placebo-controlled phase of the study were sustained during continued longer-term GH treatment. In fact, the major improvements – changes in body composition, improvements in cardiac systolic function, as well as a perceived sense of well-being – occurred early during treatment and stabilized following 6 months of treatment. This was also true for most of the changes in circulating IGF system components, including an increase in IGF-I and IGFBP-3 levels. The decrease in IGFBP-1 levels, a sensitive inverse indicator of insulin resistance noted early during GH treatment, was an apparently transient change, that reversed over time, a pattern similar to clinically observed patterns in glucose homeostasis. IGF-II levels, however, increased gradually during 18 months of treatment, becoming stable thereafter.

Consistent with other reports (reviewed by Carroll et al. (1)), the increase in LBM and decrease in fat mass were sustained during long-term GH treatment. The responses were greater in males than females. As previously reported (20, 24–26). Most significantly, the increases in LBM correlated with increases in IGF-I levels, in marked contrast to the reduction in fat mass, which did not correlate with IGF-I changes. The apparent greater benefit in LBM achieved in males was in part accounted for by differences in IGF-I levels achieved. At baseline, females had significantly lower IGF-I levels than males (79.4±43.5 vs 111.6±56.1 μg/l respectively). Nevertheless, the magnitude of the IGF-I increase and LBM gains was similar in females and males, as determined by relative or SDS changes. Our findings indicate that the dose of GH may need to be adjusted against IGF-I levels in order to achieve optimal favorable effects on body composition. Moreover, female patients may require higher GH doses to achieve comparable IGF-I levels and LBM gains. The fact that nearly three-quarters of female patients in our study were receiving oestrogen is consistent with the attenuating effect of this hormone on IGF-I responsiveness to GH administration (26).
near-normalization of EF. In contrast to other studies, however, our patients did not exhibit appreciable changes in diastolic cardiac function. The E-velocity, A-velocity, E/A ratio and isovolumic relaxation time remained within reference values for healthy controls (27). Further, we noted no changes in heart rate or blood pressure, while systemic peripheral resistance demonstrated a trend of reduction. Unlike body composition responses, males and females exhibited similar changes in cardiac function despite different circulating IGF-I levels achieved. These findings are consistent with variable tissue sensitivity to circulating GH (28) and IGF-I concentrations, and the relative inability of circulating IGF-I levels to reflect cardiac function improvement.

An additional feature of patients suffering from GHD is their impaired sense of psychological well-being. Our study demonstrated sustained improvements in several specific domains, including energy, emotional reactions and social isolation. Again, these improvements did not correlate with nor were predictable by circulating IGF-I levels. Moreover, the incidence of AEs, such as arthropagias, did not correlate with nor were predictable by circulating IGF-I levels.

In conclusion, this study demonstrates that modest doses (5–10 μg/kg per day) of long-term GH treatment can result in sustained beneficial effects. Some of the favourable effects such as increases in LBM are reflected by circulating IGF-I levels. Other sustained effects including fat mass reduction, improvement in left ventricular function, hyperlipidaemia and enhanced sense of well-being do not correlate with IGF-I levels. Monitoring long-term GH treatment of hypopituitary patients will require even more refined measures to better predict the efficacy and tolerability of GH treatment.

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