Title: Predictors of Insulin Like Growth Factor-I responses to Growth Hormone replacement in young adults with Growth Hormone deficiency

Short Title: IGF-I responses in young adults

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Keywords: Growth hormone deficiency, IGF-I response, somatic maturation, transition, GH dose titration

Declaration of interest: The study was funded by an investigator-initiated research (IIR) grant from Pfizer Inc. Pfizer provided statistical support as well as advice as to logistical aspects of interrogating the KIMS database. D Capalbo and H Simpson have nothing to declare. A Thankamony received salary support from the IIR grant and speaker honoraria from Pfizer Inc. D.B Dunger was a member of the KIGS steering committee and received consultant and speaker honoraria from Pfizer Inc. P.J Jonsson is an employee of Pfizer Inc., Sweden and provided statistical support for the study.

Word Count: 3934; Tables-5, Figures- 1, supplementary figure-1

Abstract:

Background/Aims: Physiological growth hormone (GH) secretion and IGF-I levels are greater in young compared to older adults. We evaluated IGF-I levels and predictors of IGF-I responses in young adults on GH replacement.

Design: From KIMS database, 310 young adults (age, 15-26 years) with the severe GH deficiency related to childhood-onset disease, and commenced on 'adult GH replacement' were identified. 'IGF-I responses' were estimated from first-year increments in IGF-I SDS and adjusted for GH dose. Body composition was assessed by bioimpedance in 143 patients.

Results: IGF-I levels increased markedly from baseline to 1-year of replacement (-3.75±1.94 vs -1.36±1.86 SDS,$p<0.0001$), but remained low compared to normative data despite dose titration. In multivariate models, IGF-I responses were positively associated with age (B [SE] SDS/[mg/m^2]; 0.52 [0.15],$p=0.0007$) and BMI SDS (1.06[0.25],$p<0.0001$), and inversely associated with female gender (-4.45[0.79],$p<0.0001$) and baseline IGF-I SDS (-1.44[0.20],$p<0.0001$). IGF-I responses were positively associated with first-year increases in lean body mass ($r=0.19$,$p=0.003$) and HbA1c ($r=0.15$,$p=0.031$).

Conclusions: Low IGF-I levels in young adults on treatment may reflect suboptimal GH replacement. Identification of predictors for IGF-I responses could lead to a more appropriate replacement strategy. Association between IGF-I responses and lean body mass suggests that maintaining age-appropriate IGF-I levels is important during therapy.

Introduction:

Endogenous Growth Hormone (GH) secretion varies considerably with the developmental state [1]. It peaks in late puberty, decreases rapidly in the first half of the third decade, and gradually declines thereafter throughout adult life [2,3]. Circulating levels of insulin-like growth factor-I (IGF-I) follows a similar pattern [4]. The GH output estimated per unit surface area in young adults is as high as in pre-pubertal children, and almost two-fold greater than in middle-aged adults [2,3]. The post-pubertal state with the relatively high GH secretion and IGF-I levels corresponds to a period of somatic maturation which lasts until the middle of the third decade [5,6]. Reductions in lean body mass and bone mineral density (BMD), and increases in fat mass in patients with childhood-onset GH deficiency (GHD) who discontinued GH replacement, and physiological rates of accretion of lean body mass and bone mass on replacement support a critical role of GH in the continued physical development after the completion of linear growth [5-7]. However, the current recommendations for replacement in young adults with severe GHD at final height are similar to older adults, and include restarting therapy on a low 'adult dose' of GH (0.2-0.5mg) and titrating to achieve IGF-I levels in the upper half of age- and gender-appropriate normal ranges (0 to +2 standard deviation scores [SDS]) [8]. Yet, the majority of studies on GH replacement in young adults used considerably larger doses based on body size (10-25 μkg/kg/day), and the effects of the current strategy for replacement are not known.

Young women have 1.5 fold greater endogenous GH secretion compared to young men [9], however, the IGF-I levels are similar [4] and suggest lower IGF-I responses in women. Age, BMI and sex hormone replacement are linked to IGF-I responses to GH in older adults [10], however, their impact on IGF-I titrated GH treatment in young adults are not known. Although IGF-I levels are used to guide GH dose titration in adults, its relationship with clinical outcomes or adverse effects are poor [10]. In contrast, IGF-I levels are a sensitive marker of GHD in young adults compared to older adults [11], but, the relationship between IGF-I responses and other treatment outcomes in young adults have not been studied. The aim of

the study was to determine the IGF-I levels on GH replacement, the factors associated with IGF-I responses to GH, and the relationship between IGF-I responses and changes in body composition or glucose metabolism in young adults.

Subjects & Methods:

Database

The analyses were performed in the KIMS (Pfizer International Metabolic Database), an international pharmaco-epidemiological registry established in 1994 for monitoring the long-term clinical and safety outcomes of GH replacement (Genotropin®) in adult patients with GHD, and run until 2013, the details of which have been described elsewhere [12]. Briefly, the KIMS is based on 14,000 adult GHD patients from 31 countries and has the data on background characteristics, details of GH therapy, clinical measurements, quality of life (QoL), adverse events and centrally measured IGF-I levels. The IGF-I levels and the derived IGF-I SDS values were promptly fed back to the investigators to assist titration of GH dose. However, the study did not specify or provide any guidance on the treatment, and the starting dose of GH and the dose increments were decided by the investigators according to the local practice.

Subjects

Young adults aged 15-26 years with childhood-onset disease and evidence of severe GHD on re-evaluation after attainment of final height, and had IGF-I measurements prior to (baseline) and after 1 year of starting adult GH replacement were selected from the KIMS (n=310). GH deficiency was defined by peak GH levels <5µg/L on a provocation test (n=246), and when the data were not available, by the presence of ≥2 additional pituitary hormonal deficiencies (n=54) or IGF-I levels ≤-2.0 SDS while off GH therapy in patients with an organic cause for pituitary dysfunction (n=10) [8,13]. Among patients who underwent GH provocation tests, a variety of stimuli were used including insulin (58.1% [n=143]), arginine (15.9% [n=39]), glucagon (8.5% [n=21], GHRH-Arginine (1.6% [n=4]) and others (4.1% [n=10]) whereas the type of test was not documented in 11.8% [n=29]. The IGF-I levels in patients

evaluated using the commonly recommended tests for adults [14] (insulin, glucagon or GHRH-Arginine, [n=168]) compared with other tests [n=78] were similar (baseline IGF-I SD: -3.84±1.89 vs -3.64±1.50, p=0.32; IGF-I SDS at 1 year: -1.36±1.93 vs -1.41±1.74, p=0.58). Although we used a higher threshold of peak GH levels (<5 µg/L) for the diagnosis, only 5.7% (14/246) patients had peak levels in the ≥3 µg/L. The patients included those who did not receive prior GH replacement (true naïve, n=61) or had previous GH therapy, but discontinued treatment for >6 months (semi naïve, n=249). The true naïve patients had childhood-onset pituitary disease (age of onset, 13.0±5.1 years), but GHD was diagnosed (age, 18.0±3.7 years) and GH replacement started (age, 21.0±2.5 years) during adulthood. Appropriate ethics approval was obtained in each country, the subjects or their parents provided written informed consent and the study was conducted according to the principles of the Declaration of Helsinki [12].

Assessments

The weight, height, and waist and hip circumferences were measured at baseline, and at 1 and 2 years after GH replacement. The body composition was estimated in a subgroup (n=143) using bioelectrical impedance analysis (BIA) employing a variety of different instruments in the participating centres. The QoL was evaluated using QoL-Assessment of GHD in Adults (QoL-AGHDA) questionnaire [14], which is based on a scale where a lower score represents a higher QoL. Circulating levels of IGF-I, lipids, fasting glucose and haemoglobin A1c (HbA1c) were also measured at these time points.

Assays

The serum IGF-I and lipids concentrations were measured centrally while blood glucose and HbA1c were analysed at the participating centres. Serum IGF-I measurements were performed at Kabi Pharmacia, Stockholm, Sweden during 1994-1997 and at Sahlgrenska University Hospital, Gothenburg, Sweden. Thereafter. The IGF-I assays used were a radioimmunoassay following acid/ethanol precipitation of IGF binding proteins (Nichols Institute Diagnostic, San Juan Capistrano, CA, USA) until November 2002 and a chemiluminescence immunoassay (Nichols advantage system) thereafter as previously reported [4,15].

Serum total cholesterol (TC), triglycerides and high-density lipoprotein cholesterol (HDL-C) were measured as previously described and low-density lipoprotein cholesterol (LDL-C) was calculated by the Friedewald's formula [16].

Calculations

Physiological levels of IGF-I decline markedly from a peak at the end of puberty to the relatively stable adult levels by the middle of the third decade [4]. As the age of patients at the start of 'adult replacement' in the study ranged from 15 to 26 years, we used age and gender specific IGF-I SDS estimated using assay-specific normative data in the calculations [15]. In patients with GHD, an increase in GH dose results in a linear rise in IGF-I levels [17], however, the dose titration to achieve a target range of IGF-I levels results in a wide range of GH doses. Therefore, in line with the previous reports [18,19], we calculated 'IGF-I response' as a measure of responsiveness to GH, from the increments in IGF-I SDS from baseline (when patients were off replacement) to 1 year of replacement, and adjusted for the GH dose using the formula: IGF-I response = [IGF-I SDS at 1 year - IGF-I SDS at baseline] / [GH dose (mg/m^2) at 1 year]. GH doses were adjusted for body surface area estimated using the Mosteller formula. The BMI SDS was calculated using normative data [20].

Statistics

Spearman correlation was used to estimate univariate associations and the biserial correlations [21] between binary and continuous variables. The relationship between oestrogen treatment and IGF-I levels were adjusted for the number of hormonal deficiencies in a regression model. Prediction models were developed by multiple linear regression analysis, and a hierarchy of predictive factors was derived by the all possible regression approach using Mallow's C(p) criterion for ordering predictive factors as described by Weisberg [22]. Analyses were performed with SAS software (Statistical Analysis system, version 8.2, SAS Institute, Cary, NC, USA). Data are expressed as mean±standard deviation (SD) unless otherwise

specified. The associations between IGF-I responses and changes in body composition and HbA1c were adjusted for the baseline measurements in regression models.

Results:

Baseline data

The patients were recruited between 1994 and 2011 and the distribution of diagnoses and background details (n=310, 180 men) are shown in Table 1 & 2. The diagnosis of GHD was made during childhood at age 13.5±6.4 years, re-evaluated by GH provocation tests at age 20.3±2.6 years and the adult GH replacement was started (KIMS start) at age 21.2±2.5 years. Isolated GHD was seen in 23.2% (n=72) patients, whereas 32.2% (n=100) had 1-2 additional hormonal deficiencies and 43.9% (n=138) were deficient in ≥3 additional pituitary hormones. Deficiencies of TSH, ACTH, LH/FSH and antidiuretic hormone were seen in 64.5% (n=200), 51.0% (n=158), 62.9% (n=194) and 26.3% (n=81) patients respectively. Approximately half of the women (n=60, 46.1%) and men (n=82, 45.5%) were on oestrogens and testosterone treatment respectively. Among the women on oestrogens (n=60), 37 were treated with hormone replacement therapy (HRT), 21 with combined oral contraceptive pill, and 2 with oestrogen patches. There were no significant differences between semi naïve and true naïve patients in the age at start of 'adult replacement', GH doses, IGF-I levels at baseline or during treatment (data not shown). Three patients who were treated for brain tumour had diabetes mellitus. In 33 patients, the IGF-I levels were estimated by RIA at baseline, but by the chemiluminescence assay at 1 year. However, there was no significant difference in the IGF-I SDS estimated by the two assays at baseline or at 1 year (data not shown).

Baseline IGF-I levels

At baseline, the IGF-I levels were very low (-3.75±1.94 SDS) compared to the normative data and were lower in women compared with men (-4.14±1.86 vs -3.46±1.96 SDS, p=0.0018) (Table 2). The IGF-I levels were positively associated with peak GH levels on provocation tests (r=0.39, p<0.0001), and were

lower in patients with deficiencies of ACTH (r=-0.25, p<0.0001), TSH (r=-0.29, p<0.0001), LH/FSH (r=-0.31, p<0.0001) and in those with higher numbers of additional pituitary hormonal deficiencies (r=-0.30, p<0.0001) (Table 3). The IGF-I levels were positively correlated with lean body mass at baseline, but were not associated with age, age at diagnosis of GHD, BMI SDS or fat mass. Women on oestrogen treatment had lower IGF-I levels at baseline compared to other women (-4.74±1.56 SDS vs -3.62±-1.94 SDS, p=0.015).

GH replacement

The GH doses at start were low, however, during the first year of replacement, the doses almost doubled (at start vs 1-year, 0.26±0.23 vs 0.51±0.28 mg, p<0.0001) (Table 2). The IGF-I levels increased markedly following GH replacement (baseline vs 1-year, -3.75±1.94 vs -1.36±1.86 SDS, p<0.0001). However, at 1 year of replacement, the levels were considerably lower than the recommended targets (0 to +2SDS) (Figure-1) and the majority of patients (n=233; 75.2%) had IGF-I SDS <0. Moreover, no further increases in IGF-I levels were observed at 2-years of replacement (IGF-I SDS: 1-year vs 2-years, -1.36±1.86 vs -1.46±2.03, p-NS). The starting doses of GH were similar in men and women, however, women were on higher GH doses (0.59±0.34 vs 0.45±0.21 mg, p<0.0001), but had lower IGF-I levels (-1.83±1.81 vs -1.03±1.82 SDS, p=0.0002) compared to men at 1 year. The IGF-I levels were positively associated with age (r=0.16, p=0.005), waist circumference (r=0.12, p=0.04), waist-hip ratio (r=0.13, p=0.025), and BMI SDS (r=0.11, p=0.052) (Table 3). In women, oestrogen treatment was not related to IGF-I levels at 1 year (IGF-I SDS on vs not on oestrogen: -2.02±1.67 vs -1.67±1.92, p=0.56).

IGF-I responses to GH

The IGF-I responses were lower in women compared with men (p=0.0002), and were inversely related to baseline IGF-I levels (r=-0.24, p<0.0001) and peak GH levels on provocation tests (r=-0.21, p=0.001) (Table-3). The responses were positively associated with age (r=0.16, p=0.005), BMI SDS (r=0.12, p=0.038), waist circumference (r=0.20, p=0.0015) and waist-hip ratio (r=0.15, p=0.019) (Table-3).

Multivariate regression models were constructed using age, gender, aetiology (idiopathic vs organic cause), deficiencies of ACTH, LH/FSH, TSH, ADH, number of pituitary hormonal deficiencies, cranial irradiation, BMI SDS, waist circumference, waist-hip ratio, baseline IGF-I SDS, oestrogen and testosterone treatment, and duration of treatment. Male gender, older age, greater BMI SDS and lower IGF-I SDS at baseline independently predicted higher IGF-I responses (R^2=0.22, p<0.0001) (Table-4). When the data were analysed separately by gender, significant measures of adiposity in the models were BMI SDS in men and waist circumference in women (Table-4). In women, oestrogen treatment was not associated with IGF-I responses.

Changes in body composition and metabolic parameters during GH replacement and the relationship with IGF-I response

During the first year of GH replacement, waist-hip ratio (p=0.010) and fat mass (p=0.034) decreased whereas lean body mass (p<0.0001) and BMI SDS (p=0.039) increased (Table-5). Total cholesterol (p=0.001) and LDL cholesterol (p=0.0003) decreased while HbA1c increased (p=0.001) without significant changes in fasting blood glucose levels. At 2 years of replacement, the changes in BMI SDS, lean body mass, total and LDL cholesterol remained significant compared with the baseline whereas alterations in fat mass were no longer significant. Furthermore, fasting blood glucose levels increased (p=0.019) and the HbA1c tended to be higher (p=0.08) at 2 years compared to the baseline. The QoL-AGHDA score was lower at 1 and 2 years compared with the baseline (both p<0.0001) suggesting an improvement in QoL on GH replacement.

The IGF-I responses were associated with increases in lean body mass (r=0.19, p=0.007) and HbA1c (r=0.15, p=0.033) during the first year and the first two years of treatment (r=0.28, p=0.003, and r=0.16, p=0.057 respectively) (Supporting Information, Figure-1). The responses were also positively associated with increases in BMI SDS during 2 years of replacement (r=0.16, p=0.013). However, IGF-I responses were not related to changes in fat mass, lipids, fasting blood glucose levels and QoL-AGHDA scores. The

IGF-I responses were also not associated with changes in body composition or metabolic parameters from 1 to 2 years (data not shown).

Discussion

We explored a large cohort of young adults with GHD and found that IGF-I levels on replacement were suboptimal. Age, gender, BMI and baseline IGF-I levels predicted IGF-I responses to GH. Furthermore, IGF-I responses were related to increases in lean body mass and HbA1c during replacement.

The GH doses at the start of replacement were consistent with the low 'adult doses' currently recommended in young adults[14], but the GH dose titration resulted in a two-fold increase by 1 year. Despite the availability of IGF-I SDS measurements to the investigators in the KIMS study, the dose titration was inadequate as the IGF-I levels on replacement were >1SDS below the mean for healthy individuals during 2 years of treatment. The reasons for insufficient dose titration are not clear in this study, however, there are several plausible explanations. Lack of robust evidence to define optimal GH doses in young adults and a low starting dose may hinder titrating to a considerably higher age-appropriate doses compared to older adults. Low IGF-I levels with no patients achieving 50^{th} centile of normative data in a small prospective study of young adults treated by dose titration (median GH doses ~11µg/kg) [23] and studies using fixed doses showing that the recommended IGF-I levels are achieved only with doses as high as 25µg/kg suggest that young adults [24,25] require considerably larger doses than currently used. Treatment concordance was not evaluated in the current study, however satisfactory IGF-I levels in middle-aged adults from the KIMS (current study vs middle-aged adults, -1.36 SDS vs +0.8 SDS) on lower GH doses (0.51mg vs 0.43mg) [26] suggest that lower IGF-I responses substantially contribute to the suboptimal levels on replacement in young adults. Furthermore, a randomised double-blind study reported dose-dependent effects in young adults with the 'paediatric dose' (25µg/kg) resulting in greater effects on fat mass, BMD and IGF-I levels (+1 vs '0' SDS) compared with an 'adult dose' (12.5µg/kg) [24]. Although these findings were not reproduced in an open-label study [25], many studies

which observed beneficial effects of GH replacement in older adults maintained a mean IGF-I level above '0' SDS [27]. Whereas GHD is associated with adverse cardiovascular and metabolic outcomes [28], low IGF-I levels in the general population have also been linked to increased risks for type 2 diabetes and cardiovascular disease [29]. Therefore, suboptimal GH treatment and relative IGF-I deficiency during a period of somatic development could adversely affect the long-term outcomes. While alternate strategies using larger starting doses (e.g. continuing the same or half of the dose at final height) [30] and subsequent down-titration may assist in achieving IGF-I levels within recommended ranges, future studies should compare the effects of different replacement regimens on body composition, BMD, cardiovascular health and glucose metabolism.

Similar to older adults [26], we also observed marked inter-individual differences in GH doses consistent with variations in IGF-I response in young adults. The findings that IGF-I levels are unrelated to GH doses is consistent with dose titration according to IGF-I levels as previously reported [26]. Among the several inter-related factors associated with IGF-I responses to GH, we identified age, gender, BMI SDS and baseline IGF-ISDS as the independent predictors. Although the prediction model accounted for a lower proportion of the variance in IGF-I responses compared to similar models for the first-year growth response in children with idiopathic GHD (22% vs 61%) [22], the patients in our study were heterogeneous in the underlying diagnoses and possibly the comorbidities. Nevertheless, identification of the key predictors of IGF-I responses is useful in deciding the starting dose of GH and the incremental dose changes.

The findings of increased IGF-I responses with age suggest that physiological age-related declines in GH secretion are clinically relevant in young adults [2,31] and support a strategy for down-titrating from the dose at final height. Our cohort was older at the start of adult GH treatment compared to the patients who undergo the currently recommended seamless childhood-adult transition of GH treatment (20 vs 17 years) [32]. We speculate that the lower starting dose of GH may have an even greater adverse impact on the

IGF-I levels in the younger patients. While low IGF-I responses to GH in adolescent girls compared to boys with GHD have been inferred previously [33,34], our observations suggesting a direct effect of gender on GH replacement support a gender-appropriate replacement in young adults [18]. Although gender differences in IGF-I responses are thought to be mediated by oestrogen [35], we did not find an association with oral oestrogen treatment. Nevertheless, it showed negative associations or trends with baseline and 1- year IGF-I levels respectively possibly indicating the lack of power of the study or underreporting of the oral contraceptive use. Observations of increased IGF-I responses linked to measures of adiposity is particularly relevant to this population with high prevalence of hypothalamic obesity and are mediated through the effects of hyperinsulinaemia on hepatic IGF-I generation [14]. The association between lower baseline IGF-I levels and greater IGF-I responses may reflect the severity of GHD and is supported by a similar relationship with increased growth responses in children [22]. Although we used clinically relevant parameters, evaluation of other variables related to GH action such as GH binding protein and insulin levels, and GH receptor gene polymorphisms may provide further insights [19] into the variations in IGF-I responses to GH.

Whereas GH doses in the current study are lower than previous studies in young adults (~ 8µg/kg vs 10-25µg/kg), we observed lesser improvements in lean body mass (2.3kg vs 4-5kg) and reductions in fat mass (0.9kg vs 1.6-5.5kg) [24,36,37], which suggest a dose-dependent effect of GH on body composition. However, the improvements in lipid profile in the study are similar to other smaller studies [23,24]. Direct effects of short-term GH therapy on insulin sensitivity may explain our findings of small increases in HbA1c during replacement [37]. Long-term improvements in the body composition are reported to reverse the effects on insulin sensitivity, however, these findings are not universal [38,39]. Our findings of consistent improvements in the QoL-AGHDA scores at 1 and 2 years on replacement extend a previous report [16] and support the beneficial effects of GH replacement on QoL in young adults.

The findings that IGF-I responses are related to gains in lean body mass are consistent with the physiological role of IGF-I in mediating the anabolic actions of GH in skeletal muscles [40]. While GH signalling in liver influences the IGF-I levels, changes in lipid metabolism represents the direct actions in adipose tissue, and may explain the lack of associations between IGF-I responses and fat mass or lipid profile [40]. Although GH actions on glucose metabolism involve both direct and IGF-I mediated mechanisms, the direct effects on hepatic insulin sensitivity are predominant [41]. We speculate that common pathways for GH signalling for IGF-I generation and regulation of glucose metabolism in the liver underlie the association between IGF-I responses and increases in HbA1c [40]. Our findings suggest that IGF-I responses are a marker of outcomes of GH replacement in young adults, and are consistent with the high sensitivity of low IGF-I levels in the diagnosis of GHD in young compared to older adults [8,11].

This study based on KIMS database has several strengths such as a large cohort of relatively rare, young adults with GHD [42] which provided sufficient power for multivariate analysis, the data reflecting real-life patient management, and centrally analysed IGF-I levels with the use of appropriate normative data. However, there are a few limitations mainly related to passively collected data with no reliable measures of concordance with therapy. The subjects were evaluated by a variety of GH provocation tests, some of which are not currently recommended in adults, although, their performance in young adults with a relatively high endogenous GH secretion is not well-defined [14,39,43]. The details the GH assays performed locally or evaluations for GHD during childhood were also not available. Data on GH provocation tests were available in only 80% of the patients, and in the remaining cases, we applied well-established criteria for a high likelihood for severe GHD in young adults to increase the study size [6,8,14]. Despite these drawbacks, the proportion of idiopathic and isolated GHD in our study is similar to previous studies in young adults [7,24,36]. The different assays for IGF-I used in the study are not directly comparable, however, we used assay-specific normative data in the analysis. The heterogenicity in age and diagnoses, and possibly the associated comorbidities in our study population is a potential

drawback and may reduce the strength of associations we explored, however, they are unlikely to influence the direction of relationships in this large study. The correlations of individual predictors of IGF-I responses and the relationship between IGF-I responses and changes in HbA1c were small. However, our findings are consistent with observations in older adults, reflect plausible biological mechanisms, and are potentially useful in formulating treatment strategies. Furthermore, when combined, the effect of the predictors in the prediction model for IGF-I responses explained a clinically significant proportion of the variance. Although BIA has been validated against DXA [44], the measurements are limited by the heterogeneous study population and the use of varying instruments [45]. However, our analysis focussed on the changes in body composition rather than the absolute values.

In summary, we report novel data on the effects of the current strategy for GH replacement in young adults with GHD showing suboptimal IGF-I levels on treatment. The associations between the IGF-I responses to GH and increases in lean body mass suggest that it is a marker for the outcomes of GH replacement in young adults and that maintaining age appropriate IGF-I levels is important for optimising the benefits of replacement. The predictors for IGF-I responses that we describe could lead to a more appropriate GH replacement strategy in young adults.

Acknowledgements: We thank the patients who consented to have their data included in the database, the KIMS investigators and study nurses worldwide who provided the data on their patients. The KIMS database is sponsored by Pfizer. The project was supported by an Investigator-Initiated Research grant from Pfizer Inc. A.T received salary support from NIHR, Cambridge Biomedical Research Centre, and from Pfizer through the investigator-initiated research grant.

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Figure legends

Figure 1: IGF-I levels before and 1 year following GH treatment in females and males. Each dot represents one patient. Black dots represent the levels prior to replacement and gray dots levels at 1 year of treatment. The lines represent age and gender-specific normative data of IGF-I levels (-2, -1, 0, +1 and +2 SDS).