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# Pegvisomant-primed glucagon stimulation test in assessing GH reserve and GH/IGF kinetics in adults suspected of GH deficiency

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**Abstract**
*Purpose* The accuracy of the glucagon stimulation test (GST) in diagnosing adult GH deficiency (GHD) has recently been questioned. Because pegvisomant (PegV) increases endogenous GH secretion, we hypothesized that priming PegV to the GST (PegV-GST) 72 h beforehand would improve the diagnostic accuracy of this test. This pilot study aimed to prospectively compare PegV-GST to two other diagnostic tests for adult GHD.
*Methods* Adults suspected of GHD underwent PegV-GST, GST and insulin tolerance test (ITT) in random order. Growth hormone levels (measured by a PegV insensitive assay) during PegV-GST, GST and ITT were compared, and acute effects of PegV on GH/IGF kinetics were assessed.
*Results* Ten subjects with hypothalamic-pituitary disease and 1–4 pituitary hormone deficiencies were studied. Basal and peak GH levels with the PegV-GST were comparable to those of the GST and ITT. The five subjects that failed the GST and ITT were the same subjects that failed the PegV-GST, using the peak GH cutpoint of <3 ng/mL for this test. After PegV priming, basal GH and GH binding protein (GHBP) increased (both $P < 0.01$) and total IGF-I and bioactive IGF decreased (both $P < 0.05$), whereas IGF-II and IGFBPs −1, −2 and −3 were unchanged compared to pre-PegV priming. Serum PegV levels correlated positively with basal GH, peak GH, IGFBP-1 and IGFBP-2 levels, and negatively with ΔbioactiveIGF and ΔGHBP (all $P < 0.05$).
*Conclusion* Single dose PegV administration in adults suspected of GHD increased basal GH and GHBP, with concomitant rapid fall in IGF-I levels and bioactive IGF. PegV priming did not appear to improve the diagnostic accuracy of the GST. Further studies involving larger subject numbers are needed to verify the clinical utility of PegV-GST in evaluating adult GHD.

**Keywords** Pegvisomant · Glucagon stimulation test · Growth hormone deficiency · Insulin-like growth factor-I

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## Introduction

When GH replacement is considered in adults suspected of GH deficiency (GHD), the diagnosis requires confirmation by GH stimulation testing [1–3]. The insulin tolerance test (ITT) has been historically regarded as the gold standard test despite concerns about its practicality, safety, reproducibility, and specificity [4, 5]. Following the publication of several validation studies [6, 7] and recommendations from current consensus guidelines [1–3], the combination

of GH-releasing hormone (GHRH) plus arginine (GHRH-arginine) test has been accepted as the best alternative to the ITT. However, when Geref® (a GHRH analog) became unavailable in the United States in 2008, the need for an alternative to the ITT to replace the GHRH-arginine test became apparent.

In line with these developments, the glucagon stimulation test (GST) has been proposed as a safe and widely available test [8]. This test has been validated in the past in assessing GH reserve in both adults [9–11] and children [12, 13]. However, the standard GST passing peak GH cutpoint of <3 ng/mL was established by comparison with lean controls despite the fact that obesity is associated with decreased endogenous GH secretion [14], and that GHD itself is complicated by an increased risk of obesity [15]. By contrast, several studies have shown that the GST is capable of assessing the integrity of the hypothalamic–pituitary–adrenal (HPA) axis [16, 17], but the mechanism/s of the stimulatory effect of glucagon on endogenous ACTH and cortisol secretion remain unclear [18].

We previously described the experience of 515 GSTs from five tertiary centers in the United States, and found an inverse correlation between fasting, nadir and peak blood glucose levels and body mass index (BMI) with peak GH levels [19]. These data translate into a substantial proportion of patients with fasting hyperglycemia and high BMIs failing the GST, raising the question of overdiagnosing adult GHD in this population.

Pegvisomant (PegV) is a selective GH receptor antagonist that effectively decreases IGF-I levels in healthy adults and normalizes IGF-I levels in more than 95 % of patients with acromegaly [20]. Physiological studies by Veldhuis et al. [21, 22] have demonstrated that short-term lowering of systemic IGF-I levels induced by high dose PegV administration increased endogenous GH secretion, and that the increment of GH secretory activity is directly proportionate to the fall in IGF-I levels.

To test the hypothesis that priming PegV to the GST (PegV-GST) would improve its diagnostic accuracy in assessing for adult GHD, we conducted this pilot study to prospectively compare this test with the GST and ITT in adults suspected of GHD. Additionally, we sought to examine the utility of PegV-GST on evaluating endogenous cortisol secretion and the acute effects of PegV on the GH/IGF kinetics in this population.

## Materials and methods

### Subjects

Ten non-diabetic adults with a history of hypothalamic-pituitary disease and 1–3 pituitary hormone deficiencies attending the Oregon Health and Science University (OHSU) Endocrinology Out-Patient Clinic were recruited. Clinical characteristics of the subjects are displayed in Table 1. None of the subjects received GH therapy prior to study enrollment. The Institutional Review Board at OHSU approved the study, and written informed consent was obtained from each subject before study enrollment.

### Study design

In this prospective pilot study of within-patient comparison of 3 diagnostic tests for adult GHD, adults suspected of GHD underwent the PegV-GST, GST and ITT in random order with 3–6 weeks wash-out between the tests. In addition to GHD, subjects that had other pituitary hormone deficiencies were receiving stable doses of replacement therapy in the form of levothyroxine, hydrocortisone, desmopressin, and sex steroids (injectable and transdermal testosterone replacement in males; transdermal estrogen replacement in females) for at least 3 months before study entry. Subjects were excluded if they had underlying chronic systemic illnesses, diabetes mellitus, liver failure, renal disease, malnutrition, pregnancy, and those taking medications that could potentially affect the results.

### GH stimulation tests

#### *PegV-GST and GST*

For the PegV-GST, fasting blood samples were drawn between 0800 to 0900 h for assessment of serum GH, GH binding protein (GHBP), total insulin-like growth factor-I (IGF-I), bioactive IGF, IGF-II, IGFBP-1, IGFBP-2, and IGFBP-3 levels before 1 mg/kg of PegV was injected subcutaneously by the Endocrine Nurse (SAR). This dose of PegV was chosen based on previous studies in normal adults [21, 22] and in children with short stature [23]. The administration of PegV was timed 72 h before the GST to capture its maximal single dose effect on IGF-I levels given that initial Phase I trials in healthy adults demonstrated that peak PegV levels occurred at ~72 h [24]. For this part of the PegV-GST and GST, after a 10–12 h overnight fast, intramuscular glucagon was administered at a dose of 1 mg (1.5 mg if body weight >90 kg) (GlucaGen; NovoNordisk). Serum GH, GHBP, total IGF-I, bioactive IGF, IGF-II, IGFBP-1, IGFBP-2, IGFBP-3, total cortisol and free cortisol levels were measured at baseline, and serum GH, total cortisol and free cortisol levels were measured at 90, 120, 150 and 180 min.

#### *Insulin tolerance test*

After a 10–12 h overnight fast, blood glucose and serum GH, GHBP, total IGF-I, bioactive IGF, IGF-II, IGFBP-1,

**Table 1** Clinical characteristics of individual study subjects

| Subject number | Gender | Age (years) | Presenting diagnosis | IGF-I at presentation (ng/mL)[a] | IGF-I SDS at presentation | Other hormone replacement |
|---|---|---|---|---|---|---|
| 1 | Male | 44 | Traumatic brain injury | 125 | −0.7 | T |
| 2 | Female | 46 | Traumatic brain injury | 124 | −1.3 | E |
| 3 | Male | 51 | Non-functioning macroadenoma | 142 | −0.2 | T |
| 4 | Female | 41 | Traumatic brain injury | 143 | −2.0 | E, L-$T_4$ |
| 5 | Female | 44 | Non-functioning macroadenoma | 125 | −1.9 | L-$T_4$ |
| 6 | Male | 34 | Craniopharyngioma | 73 | −2.9 | L-$T_4$, HC, T, DDAVP |
| 7 | Female | 35 | Cushing's disease | 97 | −2.2 | L-$T_4$, HC, DDAVP |
| 8 | Male | 34 | Non-functioning macroadenoma | 82 | −2.6 | L-$T_4$, T |
| 9 | Female | 51 | Cushing's disease | 103 | −2.2 | L-$T_4$, HC |
| 10 | Male | 28 | Congenital hydrocephalus | 86 | −3.6 | L-$T_4$, HC, T |
| | | **40.8 (2.5)** | | **110.0 (8.0)** | **−2.0 (0.3)** | |

Data in bold are presented as mean (SE)

*DDAVP* desmopressin, *E* estrogen, *HC* hydrocortisone, *L-$T_4$* levothyroxine, *T* testosterone

[a] IGF-I measured using an Immulite 2000 Analyzer

IGFBP-2, IGFBP-3, total cortisol and free cortisol levels were measured at baseline. After these blood samples were collected, 0.10–0.15 U/kg regular human insulin was administered intravenously. When symptomatic hypoglycemia (determined by clinical findings and capillary blood glucose levels) was achieved, further blood samples were drawn to assess blood glucose and serum GH, total cortisol and free cortisol levels at 20, 40, 60 and 80 min. Only subjects with confirmed biochemical hypoglycemia (laboratory blood glucose levels of ≤40 mg/dL) were included in the data analysis.

### Assays and IGF-I SDS calculations

Glucose was assayed by a glucose oxidase and peroxidase enzymatic method (Glucose enzymatique PAP 7500; BioMérieux SA, Marcy-L'étoile, France) using a Beckman analyzer (Beckman Glucose Analyzer; Beckman, Fullerton, CA, USA). The coefficient of variation (CV) of this reference method was <3 %. GH was assayed using an automated assay that excludes PegV interference. The assay is based on a monoclonal antibody that does not cross-react with PegV up to 50 mg/mL and a monoclonal antibody specific for 22-kDa GH [25]. The standard was 22-kDa recombinant human GH International Reference Preparation 98/574. All samples were analyzed in one run. Median intra- and inter-assay CVs were 6.5 and 8.7 %, respectively, at the GH levels measured. GHBP was assayed by an in-house assay designed to be free of PegV interference. Combining a polyclonal goat anti-human GHBP antiserum as capture antibody with the same antiserum in a biotinylated form as the detection antibody in a sequential assay, a linear dose-relationship for GHBP with a lower detection limit of 20 pmol/L and an upper end of the linear working range at 4000 pmol/L for 25 µL samples was established. Recombinant non-glycosylated GHBP (Diagnostic Systems Laboratories, Inc., Webster, TX, USA) was used as the calibrator. Within-assay CVs was 9.4 % at 115 pmol/L and 6.1 % at 1550 pmol/L. At the same concentrations, between-assay CVs were 8.5 and 10.9 %, respectively. When the subjects initially presented to the OHSU Endocrinology Out-Patient Clinic, baseline IGF-I measurements were performed using an Immulite 2000 Analyzer calibrated to the 2nd International Standard, National Institute for Biological Standards and Controls (NIBSC) 98/574, with a detection level of 25 µg/L. Comparison studies were undertaken via Esoterix Inc., Laboratory Services (Esoterix) using a double antibody radioimmunoassay after ethanol extraction, with a detection level of 10 µg/L; CV of 5.4 %. After study enrollment, total IGF-I and IGFBP-3 were measured by commercial assays (IS-3900 and IS-4400) using the automated iSYS platform (IDS iSYS) [26]. The IGF-I assay was performed according to recently published guidelines [27] and calibrated against the international IGF-I reference preparation WHO 02/254. The IGF-I SDS calculations were made using normative data for this analytical method, as previously described by Bidlingmaier et al. [28]. Bioactive IGF was measured by an in-house IGF-I receptor kinase receptor activation (KIRA) assay, as previously described [29] with recent modifications [30]. The KIRA assay is a cell-based bioassay based on HEK-293 cells over-expressing the human IGF-I receptor. The assay primarily detects IGF-I activation of the IGF-I receptor in vitro [31]. However, IGF-II and pro-IGF-II activation of the IGF-I receptor may also be detected by this assay as these

peptides exert a cross-reactivity of 12 and 2 %, respectively. Because both IGF-I and IGF-II participates in IGF-I receptor activation, we have designated the output of the assay as "bioactive IGF". The intra- and inter-assay CV of the KIRA assay was 10 and 15 %, respectively. IGF-II was measured by an in-house time-resolved monoclonal immunofluorometric assay (TR-IFMA) [32]. The assay has been calibrated against the international IGF-II reference preparation WHO 96/538. The intra- and inter-assay CV of the IGF-II assay was less than 5 and 10 %, respectively. IGFBP-1 was assayed by an in-house TR-IFMA, as previously described [30], with intra- and inter-assay CVs of <5 and <10 %, respectively. IGFBP-2 was measured by an in-house TR-IFMA, as previously described [30], with intra- and inter-assay CVs of <5 and <12 %, respectively. PegV was measured by a two-site competitive immunofluorometric assay, as previously described [33], where serum samples were initially diluted 100-fold to minimize GH interference. Total cortisol was measured from serum by a chemiluminescent immunometric assay on the automated immulite system from Siemens Healthcare Diagnostics. Free cortisol was measured by dialyzing serum overnight at 37 °C with a 96-well equilibrium dialyzer from Harvard Apparatus. During dialysis, the plate was kept in constant motion using a plate rotator. The sample dialysate was then used in the cortisol EIA from R and D Systems.

### Statistical analysis

All statistical analyses were performed using SPSS for Windows (version 20, Chicago, IL). Distributions of residuals were tested for normality by graphical methods. For normally distributed residuals, the Student's paired *t* test was used, whereas for not normally distributed residuals, the Wilcoxon signed-rank test was used. For qualitative variables, Chi squared test was performed. Correlations between variables were determined by Pearson's or Spearman's correlation coefficients for parametric and non-parametric variables, respectively. *P* values <0.05 defined statistical significance.

## Results

### Clinical characteristics of subjects

The clinical characteristics of subjects are displayed in Table 1. There was an equal distribution of males to females. All subjects had a history of hypothalamic-pituitary disease and IGF-I SDS < 0. Four subjects had one pituitary hormone deficit and six subjects had ≥2 pituitary hormone deficits.

### PegV-GST, GST and ITT results

Basal and peak GH, total cortisol and free cortisol (Supplementary Table) levels post-PegV priming to the GST did not differ significantly to those of the GST and ITT (Tables 2 and 3). When basal GH levels in non-obese were compared to obese subjects, there was a non-significant increase of post-PegV compared to pre-PegV priming of basal GH levels in non-obese subjects (Table 4). Figure 1 displays the individual changes of each subject after PegV priming for basal GH, GHBP, total IGF-I and bioactive IGF.

The majority of peak GH levels in the PegV-GST ($n = 8$) and GST ($n = 9$) were observed between 120 and 180 min. If the peak GH cutpoint of <3 and <5 ng/mL were used for the GST and ITT, respectively [1–3], the same five subjects deemed to have failed the GST and ITT were the same subjects that failed the PegV-GST if the peak GH cutpoint of <3 ng/mL was used for this test. When the subjects were asked which single test they preferred, 3 subjects stated that they preferred the PegV-GST, 4 to the ITT, and 3 to the GST.

### Side-effects of the GSTs

No subjects experienced clinically significant adverse events following PegV injections. One subject reported a transient stinging sensation at the site of PegV injection. The most commonly reported side-effects for PegV-GST and GST were nausea and vomiting, and these symptoms occurred during the GST. The rate of nausea and vomiting was similar between the PegV-GST (nausea: $n = 4$, vomiting: $n = 3$) and GST (nausea: $n = 3$, vomiting: $n = 3$). Nausea and vomiting were mainly reported between 90 and 150 min, and between 180 and 210 min for both tests, respectively. As the nausea was transient, subjects did not receive any anti-emetic treatment. For the ITT, eight subjects reported neuroglycopenic symptoms, and received rescue oral juice after all the blood samples for hormonal measurements were collected.

Other less commonly reported side-effects (≤2 subjects) included hunger, headaches, sleepiness, and lightheadedness; most of these events were rated as "mild" or "moderate" in severity. All side-effects resolved by the end of the test.

### Effects of PegV on GH/IGF kinetics

PegV levels averaged 3734 ± 1060 ng/mL at 72 h after drug injection. Compared to pre-PegV priming, PegV priming significantly increased basal GH by 241 % and GHBP by 85 %, with mean basal GH and GHBP increases of 0.28 ± 0.13 (range 0–0.49) ng/mL and 446.8 ± 64.2

**Table 2** GH, IGF and IGFBP components with PegV-GST, GST and ITT

| | PegV-GST | | GST | ITT |
|---|---|---|---|---|
| | Pre-PegV | Post-PegV | | |
| Basal GH (ng/mL) | 0.13 (0.04) | **0.41 (0.15)**[a] | 0.60 (0.28) | 0.71 (0.41) |
| Peak GH (ng/mL) | N/A | 3.05 (0.89) | 5.92 (2.20) | 5.59 (2.92) |
| GHBP (pmol/L) | 626.2 (113.6) | **1073.0 (174.2)**[b,c,d] | 530.6 (89.8) | 726.6 (142.6) |
| Total IGF-I (μg/L) | 85.1 (8.3) | **64.8 (10.2)**[c,e] | 107.6 (7.9) | 93.1 (12.0) |
| Bioactive IGF (μg/L) | 1.09 (0.08) | **0.93 (0.08)**[e] | 1.12 (0.11) | 1.03 (0.08) |
| IGF-II (μg/L) | 476.0 (32.6) | 481.0 (27.6) | 471.2 (26.0) | 474.5 (23.6) |
| IGFBP-1 (μg/L) | 46.1 (15.0) | 58.1 (14.7) | 67.8 (17.7) | 62.0 (13.2) |
| IGFBP-2 (μg/L) | 205.0 (51.3) | 209.5 (52.9) | 215.8 (45.7) | 203.3 (43.8) |
| IGFBP-3 (μg/L) | 3939 (263) | 3801 (273) | 3654 (135) | 3767 (216) |

Data are presented as mean (SE). N/A: not analyzed

Data with significant *P* values are in bold

[a] $P < 0.01$ versus Pre-PegV, [b] $P < 0.0001$ versus Pre-PegV, [c] $P < 0.01$ versus GST, [d] $P < 0.01$ versus ITT, and [e] $P < 0.05$ versus Pre-PegV

**Table 3** Individual basal and peak GH levels (ng/mL) of each subject with PegV-GST, GST and ITT

| Subject number | Body mass index (kg/m$^2$) | PegV-GST | | GST | | ITT | |
|---|---|---|---|---|---|---|---|
| | | Basal[b] | Peak | Basal | Peak | Basal | Peak |
| *Non-obese subjects* | | | | | | | |
| 1 | 26.1 | 0.58 | 7.34 | 0.24 | 18.49 | 0.34 | 31.03 |
| 2 | 23.2 | 0.13 | 5.01 | 0.12 | 17.16 | 0.05 | 6.54 |
| 3[a] | 27.9 | 0.29 | 2.45 | 0.16 | 2.16 | 0.20 | 1.58 |
| 8 | 22.4 | 1.66 | 4.92 | 1.37 | 8.19 | 4.06 | 5.67 |
| 9 | 21.0 | 0.62 | 6.38 | 2.70 | 5.35 | 0.36 | 2.79 |
| 10[a] | 22.5 | 0.11 | 0.18 | 0.05 | 0.07 | 0.05 | 0.05 |
| | **23.9 (1.1)** | **0.57 (0.24)** | **4.38 (1.08)** | **0.77 (0.44)** | **8.57 (3.14)** | **0.84 (0.65)** | **7.94 (4.72)** |
| *Obese subjects* | | | | | | | |
| 4 | 33.5 | 0.39 | 3.79 | 1.05 | 7.07 | 1.86 | 5.10 |
| 5[a] | 38.8 | 0.08 | 0.16 | 0.05 | 0.33 | 0.05 | 3.11 |
| 6[a] | 37.1 | 0.05 | 0.08 | 0.05 | 0.05 | 0.05 | 0.05 |
| 7[a] | 43.4 | 0.17 | 0.17 | 0.19 | 0.40 | 0.13 | 0.15 |
| | **38.2 (2.1)** | **0.17 (0.08)** | **1.05 (0.91)** | **0.34 (0.24)** | **1.96 (1.70)** | **0.52 (0.45)** | **2.10 (1.23)** |

Data in bold are presented as mean (SE)

[a] Subjects defined as GHD based on the following peak GH cutpoints: PV-GST and GST < 3 ng/mL and ITT < 5 ng/mL

[b] Basal: basal GH levels 72 h after PegV priming

(range 147–718) pmol/L, respectively (Tables 2 and 3). In addition, GHBP levels after PegV priming was significantly higher compared to those of the GST and ITT (both $P < 0.01$). By contrast, compared to pre-PegV priming, PegV priming significantly decreased total IGF-I by 23 % and bioactive IGF by 14 %, with mean total IGF-I and bioactive IGF decreases of $-20.3 \pm 7.9$ (range: $-85$ to $-1$) μg/L and $-0.16 \pm 0.06$ (range: $-0.47$ to 0.12) μg/L (both $P < 0.05$), respectively (Table 2). When IGF-I SDS in non-obese were compared to obese subjects, there was a non-significant decrease of post-PegV compared to pre-PegV priming of IGF-I SDS in non-obese subjects (Table 4). Additionally, total IGF-I levels after PegV priming was significantly lower compared to those of the GST ($P < 0.01$) (Table 2). In contrast, after PegV priming, IGF-II, IGFBP-1, IGFBP-2 and IGFBP-3 levels were unchanged compared to pre-PegV priming.

## Correlations

After PegV priming, serum PegV levels measured during the PegV-GST correlated with basal GH ($r = 0.831$,

**Table 4** Individual IGF-I SDS and basal GH levels pre- and post-PegV administration of each non-obese and obese subject

| | Non-obese subjects | | | | Obese subjects | | | |
|---|---|---|---|---|---|---|---|---|
| | Subject number | Pre-PegV | Post-PegV | ΔPost-PegV to pre-PegV | Subject number | Pre-PegV | Post-PegV | ΔPost-PegV to pre-PegV |
| IGF-I SDS | 1 | −0.13 | −0.45 | −0.32 | 4 | −1.57 | −1.61 | −0.04 |
| | 2 | −0.16 | −4.75 | −4.14 | 5 | −1.49 | −2.12 | −0.63 |
| | 3 | −0.97 | −1.00 | −0.03 | 6 | −1.99 | −2.42 | −0.43 |
| | 8 | −1.71 | −2.78 | −1.07 | 7 | −0.68 | −1.15 | −0.47 |
| | 9 | −1.56 | −2.87 | −1.31 | | | | |
| | 10 | −4.37 | −4.48 | −0.11 | | | | |
| | | **−1.56 (0.61)** | **−2.72 (0.72)** | **−1.16 (0.63)** | | **−1.43 (0.27)** | **−1.83 (0.28)** | **−0.39 (0.13)** |
| Basal GH (ng/mL) | 1 | 0.08 | 0.58 | 0.49 | 4 | 0.05 | 0.39 | 0.34 |
| | 2 | 0.12 | 0.13 | 0.01 | 5 | 0.05 | 0.08 | 0.03 |
| | 3 | 0.05 | 0.29 | 0.24 | 6 | 0.05 | 0.05 | 0 |
| | 8 | 0.36 | 1.66 | 1.30 | 7 | 0.17 | 0.17 | 0 |
| | 9 | 0.28 | 0.62 | 0.34 | | | | |
| | 10 | 0.05 | 0.11 | 0.06 | | | | |
| | | **0.16 (0.05)** | **0.56 (0.24)** | **0.41 (0.19)** | | **0.08 (0.03)** | **0.17 (0.08)** | **0.09 (0.08)** |

Data in bold are presented as mean (SE)

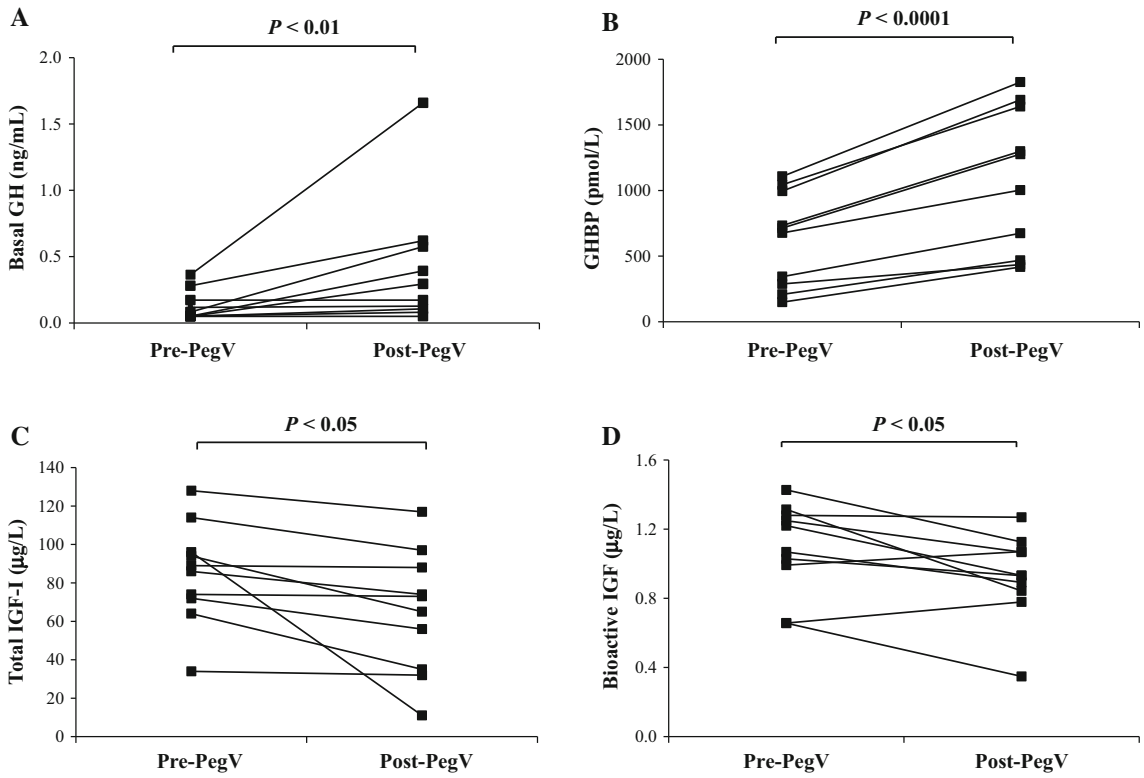


**Fig. 1** Individual changes of each subject after PegV priming for **a** basal GH, **b** GHBP, **c** total IGF-I, and **d** bioactive IGF

$P < 0.01$), peak GH during the PegV-GST ($r = 0.628$, $P < 0.05$), IGFBP-1 ($r = 0.690$, $P < 0.05$), IGFBP-2 ($r = 0.961$, $P < 0.00001$) and Δbasal GH post- versus pre-PegV priming ($r = 0.846$, $P < 0.01$). By contrast, serum PegV levels inversely correlated with ΔGHBP ($r = -0.626$, $P < 0.05$), Δbioactive IGF ($r = -0.601$, $P < 0.05$) and Δfasting glucose ($r = -0.654$, $P < 0.05$) post- versus pre-PegV priming. No correlations were

observed between serum PegV levels and total IGF-I, IGF-II, GHBP, IGFBP-3, total cortisol or free cortisol levels.

## Discussion

The diagnosis of GHD in adults is often challenging because of the lack of a single biological end-point, such as growth failure seen in children. Hence, accurate biochemical diagnosis of GHD is crucial in adults with hypothalamic-pituitary disease because the decision for implementing long-term GH replacement is based upon the demonstration of a subnormal peak GH response to a GH stimulation test. Previous studies that examined the diagnostic utility of the GST for adult GHD either did not take BMI into consideration [9, 10], or included only controls with normal BMIs [34, 35]. We recently reported that the GST tended to overdiagnose GHD in obese/overweight adults [19, 36], and in those with glucose intolerance [19]. One strategy of improving the diagnostic accuracy of the GST is by taking BMI into consideration and lowering the GH cutpoint, as demonstrated by Dichtel et al. [36]; another is by adding a priming agent with potent GH stimulatory properties.

This pilot study was undertaken to test the hypothesis that PegV priming would increase the diagnostic accuracy of the GST by exploring its effects in decreasing IGF-I levels, and thereby increase endogenous GH secretion by reducing the negative feedback of IGF-I on the pituitary and hypothalamus. We evaluated adults suspected of GHD that would, otherwise in routine clinical practice, be considered for GH stimulation testing to assess for the possibility of adult GHD. However, unlike the GHRH-arginine test where priming a GHRH analog to arginine improved its diagnostic accuracy, we were unable to demonstrate that PegV priming improved the diagnostic accuracy of the GST on endogenous GH secretory reserve. We also did not observe any effects of obesity on PegV-induced effects on basal GH and IGF-I changes.

However, we noted that subject 8 with a non-functioning macroadenoma, who was deemed to be GH-sufficient in all three tests (Table 3), was a non-obese male subject that had the highest basal GH level pre- and post-PegV administration compared to the other nine subjects (Table 4). We speculate that there might be a threshold basal GH level above which PegV can effectively increase endogenous GH secretion when adequate prevailing endogenous basal GH secretion is present, whereas in more severe GHD with very low endogenous basal GH secretion, PegV has little stimulatory impact. Additionally, it has been proposed that GH stimulation testing is unnecessary when the IGF-I SDS $< -2.0$ [1–3]. In this study, we found three subjects who were exceptions to this rule. Subject 4 (an obese female) with an IGF-I SDS of $-2.0$ passed the PegV-GST, GST and ITT, subject 8 (a non-obese male) with an IGF-I SDS of $-2.6$ passed the PegV-GST, GST and ITT, and subject 9 (a non-obese female) with an IGF-I SDS of $-2.2$ passed the PegV-GST and GST but failed the ITT. When the IGF-I was measured using the automated iSYS platform (IDS iSYS) [26], these three subjects were found to have IGF-I SDS between $-1.0$ and $-2.0$. This observation highlights the variability of using different IGF-I assays measured in different laboratories [37]. We also noted that peak GH levels for the PegV-GST mainly occurred between 120 to 180 min, comparable to the GST. By contrast, basal and peak total and free cortisol levels were unchanged with the PegV-GST indicating that PegV-GST was no more superior than the GST and ITT in evaluating adults for adrenal insufficiency.

Utilizing PegV priming to improve the reliability of a GH stimulation test is not a new concept, and was first tested by Radetti et al. [23] in 2008, where they primed PegV to L-DOPA, a weaker pharmacological stimuli of GH secretion than glucagon, in assessing GHD in short children. Using a PegV dose of 1 mg/kg, these investigators demonstrated improved reliability of the L-DOPA in diagnosing GHD, with 10 out of the 18 (56 %) children that initially failed the L-DOPA test but passed the test after PegV priming.

The limitation of the Peg-GST is that the measurement of GH during this test cannot be routinely analyzed by ordinary commercial GH assays as the presence of PegV can overestimate or underestimate GH levels [25]. Thus, an automated assay with specifically selected monoclonal antibodies with a high affinity for wild-type GH that does not cross-react with PegV is required. This is because PegV differs from wild-type GH by only nine amino acid substitutions, and therefore, the close structural homology together with almost 1000-fold higher concentrations required to achieve adequate antagonism causes interference in many conventional assays. In this study, we used a new automated GH assay that was recently reported by Manolopoulou et al. [25] (IDS-iSYS, Immunodiagnostic Systems). This GH assay was designed to specifically measure 22-kDa GH while excluding PegV or GHBP interference, and was calibrated with International Standard 98/574 in line with recent recommendations on GH assays with good analytical sensitivity, linearity and recovery. Other limitations with the PegV-GST include the high cost of PegV and that subjects are required to attend an additional clinic visit to receive their PegV injection 72 h before the GST. Furthermore, our present study does not exclude the postulate that the presence of estrogen in premenopausal females and oral estrogen use may augment endogenous GH secretion by inducing systemic IGF-I deprivation.

In this study, the accompanying analyses of GH/IGF parameters indicated that a single high PegV dose priming can rapidly lower total IGF-I levels and increased basal (but not peak) GH levels. Before PegV priming, only subject 10 had an IGF-I SDS $< -2.0$, but after PegV priming, the IGF-I SDS of another five subjects (3 non-obese and 2 obese subjects) decreased to $< -2.0$ (Table 4). Our data are consistent with those in normal subjects by Veldhuis et al. [22] and Berg et al. [38], who reported PegV-induced IGF-I lowering by 30–40 % and GH increase by 70–260 %, but contrasts to adults with GHD and low IGF-I levels in the Berg study [38] where IGF-I and GH levels were unchanged. This may be explained by the lower and different PegV dosing regimens (PegV loading dose of 80 mg followed by 20 mg daily for 14 days) of the Berg study [38] compared to our study (a single weight-based PegV dose). Furthermore, as the subjects with low IGF-I levels in the Berg study [38] had higher BMIs and much lower IGF-I levels than our subjects, it is plausible that obesity and very low IGF-I levels dampens the effects of PegV in blocking GH actions, thereby affecting the negative feedback mechanism between GH and IGF-I at the pituitary and hypothalamus.

We also found that single high dose PegV rapidly decreased bioactive IGF, but did not alter IGF-II, IGFBP-1, IGFBP-2 and IGFBP-3 levels. Nonetheless, a strong positive correlation between serum PegV levels and IGFBP-1 and IGFBP-2 levels was observed, suggesting that the modulatory effects of PegV on bioactive IGF may be mediated through concomitant changes in IGFBP-1 and IGFBP-2 levels with increases in serum PegV levels. High levels of IGFBP-1 and IGFBP-2 may also inhibit IGF-I signaling and IGF-I-stimulated actions by blocking IGF-I binding to its receptor [39]. Furthermore, because PegV can improve insulin sensitivity by reducing GH-induced lipolysis [40] and fasting insulin levels [41], the rise in IGFBP-1 levels is likely to be the result of low prevailing insulin levels in inducing an up-regulation of hepatic secretion of IGFBP-1.

Consistent with the data by Berg et al. [38], our study also demonstrated that PegV markedly increased GHBP levels. Circulating GH is partly bound to GHBP [42], which in humans is derived from proteolysis of the extracellular domain of the GH receptor. It has been proposed that GHBP levels are reflective of available cell surface GH receptors, and thereby possibly regulation of GH bioactivity. As GH binding to the GH receptor is the initial step that leads to the physiological functions of the hormone, the rise in GHBP levels suggests that GHBP was saturated with PegV resulting in an increase in the half-life of GH, but also may explain the reduction of GH action by the increased GH binding to GHBP that impedes GH access to its receptor [42]. Nonetheless, the true clinical significance of the rise of GHBP levels following PegV priming remains unclear and requires verification with further studies.

In conclusion, we have demonstrated that single dose PegV priming the GST in adults suspected of GHD rapidly increased basal GH and GHBP levels, in response to concomitant Peg-V-induced rapid reductions in IGF-I levels and bioactive IGF, but did not alter total and free cortisol levels. PegV priming did not appear to improve the diagnostic accuracy of the GST, but a strong positive correlation between serum PegV levels and basal and peak GH levels was observed. Further studies involving larger subject numbers are needed to verify the clinical utility of PegV-GST in evaluating for adult GHD before this test can be recommended in routine clinical practice.

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