Effect of Recombinant Human Growth Hormone (GH) Replacement on the Hypothalamic-Pituitary-Adrenal Axis in Adult GH-Deficient Patients

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The aim of the study was to evaluate the hypothalamic-pituitary-adrenal (HPA) axis in patients (nine males, three females; mean age ± SEM 51 ± 2 yr) with adult-onset GH deficiency (GHD) due to surgically treated pituitary tumors with preserved HPA function and without evidence of tumor recurrence before and during recombinant human (rh) GH replacement therapy (duration 31 ± 6 months). HPA function was assessed by urinary free cortisol and morning serum cortisol levels as well as cortisol responses to 1 μg ACTH test (n = 7 patients) or insulin tolerance test (n = 5 patients) before and during rhGH therapy, the cut-off for the diagnosis of hypothalamic-adrenalism being a cortisol peak less than 18 μg/dl (<500 nmol/liter) after stimulatory tests. Serum cortisol and urinary free cortisol levels were significantly lower on therapy than before [7.6 ± 0.8 vs. 11.5 ± 0.9 μg/dl (208 ± 22 vs. 317 ± 24 nmol/liter), P < 0.01, and 19.8 ± 2.5 vs. 32.2 ± 3.2 μg per 24 h (54 ± 7 vs. 89 ± 9 nmol per 24 h), P < 0.05, respectively], whereas no change in cortisol-binding globulin levels was observed. Cortisol peak after either ACTH test or insulin tolerance test was lower on rhGH therapy than before [15.9 ± 1.5 vs. 20.2 ± 1.1 μg/dl (437 ± 43 vs. 557 ± 31), P = 0.01, and 13.1 ± 2.6 vs. 20.4 ± 1.4 μg/dl (362 ± 71 vs. 564 ± 37 nmol/liter), P = 0.03, respectively]. Accordingly, central hypoadrenalism was detected in nine of 11 patients. In conclusion, low GH and IGF-I levels, likely enhancing the conversion of cortisone to cortisol, may mask a condition of central hypoadrenalism. Therefore, the reassessment of HPA function in GHD patients during rhGH therapy is mandatory. (J Clin Endocrinol Metab 89: 5397–5401, 2004)

ADULT-ONSET GH DEFICIENCY (GHD) is mostly due to organic lesions of the pituitary-hypothalamic region and is frequently associated with multiple pituitary hormone deficiencies. Recent evidence indicate that a state of untreated GHD may cause other pituitary defects to be undisagnosed. In particular, in our experience replacement therapy with recombinant human (rh) GH unmasked a state of central hypothyroid in a consistent number of adults with GHD due to central organic lesions (1, 2). Conversely, little information on the impact of GHD on the assessment of central hypoadrenalism in these patients is available, although complex and often contradictory relationships between the GH-IGF-I system and the hypothalamus-pituitary-adrenal axis (HPA) at both central and peripheral levels have been reported (3–9). In particular, although ACTH is the primary regulator of adrenal cortex growth and function, the GH-IGF-I system seems to exert a positive effect on cortisol biosynthesis, probably by inducing the expression of steroidogenenic acute regulatory protein (3). In contrast to this positive effect, it has been suggested that the acute increase of IGF-I may cause, via a negative feedback mechanism, a reduction of both GH and cortisol in animals, whereas these data have not been confirmed by a recent study reporting the effect of recombinant human IGF-I on HPA axis in healthy subjects (4, 5).

At the peripheral level, previous studies reported contradictory data on the reduction of cortisol-binding globulin (CBG) induced by rhGH therapy, these discrepancies probably reflecting different rhGH regimens (6–9). Moreover, GH may influence the interconversion of hormonally active cortisol and inactive cortisone by modulating the activity of 11β-hydroxysteroid dehydrogenase, particularly the type 1 isoenzyme (11βHSD1) (10). In fact, the activity of 11βHSD1, which acts predominantly as a reductase that converts cortisone to cortisol (11–13), is inhibited in conditions of GH excess, such as acromegaly, and restored after successful treatment of the disease (14, 15). Conversely, 11βHSD1 activity is enhanced, leading to increased tissue exposure to glucocorticoids, in patients with GHD and reduced by rhGH replacement therapy (6, 16, 17).

The aim of the present study was to evaluate the impact of appropriate rhGH replacement therapy on HPA axis in patients with adult-onset GHD due to surgically treated pituitary tumors and preserved HPA function.

Patients and Methods

Patients

Twelve consecutive patients [nine males and three females; mean age 51 ± 2 yr, range 38–57; mean body mass index (BMI) 25.6 ± 1.6 kg/m²] with severe adult-onset GHD and preserved HPA function before the start of rhGH therapy were studied. Severe GHD was defined as a peak response of serum GH less than 3 μg/liter to a GH provocative test.
[insulin tolerance test (ITT) or arginine+GHRH] as previously reported (18). All patients showed IGF-I levels below the normal range. In all patients, GHD was due to surgically treated pituitary tumors; three patients had a nonfunctioning pituitary adenoma, seven prolactinoma, one craniopharyngioma, and one GH-secreting adenoma. None of the patients underwent conventional or stereotactic radiotherapy. GHD was isolated in three patients, whereas in the remaining nine, it was associated with other multiple deficiencies. The HPA function was assessed before and during rhGH therapy, as described in Study protocol. When necessary, conventional hormone replacement therapy for other pituitary hormone deficiencies was given at stable doses for at least 3 months before the start of the study. None of the women was receiving estrogen therapy before and during the study. The main basal characteristics of GHD subjects, as well as the primary pituitary disorder and the hormone replacement therapies other than rhGH, are shown in Table 1. Informed consent was obtained from all participants and the study was approved by the local ethics committee.

Study protocol

All the patients were evaluated at baseline and on rhGH therapy (mean duration 31 ± 6 months, range 6–72 months; mean dose 0.3 ± 0.01 mg/d). HPA function was assessed by serum cortisol levels before and after appropriate provocative stimuli, i.e. ITT (insulin dose 0.15 U/kg, n = 5 patients, blood samples for cortisol measurement were collected at ~30, 0, 30, 45, 60, 90, and 120 min; adequate hypoglycemia < 40 mg/dl, 22 nmol/liter) or, when ITT was contraindicated, short ACTH test 1 µg (n = 7 patients, blood samples for cortisol measurement were collected at ~30, 0, 30, 40, and 60 min). All subjects were evaluated after an overnight fast and after 1 h of bed rest, with an iv catheter inserted in a forearm vein between 0800 and 0900 h and kept patent by slow saline infusion. All patients were evaluated with the same stimulation test either before and during rhGH therapy. The accepted cut-off for the diagnosis of hypoadrenalism was a cortisol peak less than 18 µg/dl (<500 nmol/liter) after both tests (19, 20). Urinary free cortisol (UFC), serum CBG, plasma ACTH, serum TSH, free T₄ (FT₄), and IGF-I levels were determined. Blood glucose, electrolytes, systolic and diastolic blood pressure, and physical examination were also performed. Anthropometric measurements, such as BMI and percent of body fat (BF%), were performed in all patients. Weight and height were measured following the Anthropometric Standardization Reference Manual. BMI was calculated as weight (kilograms)/height (square meters).

Methods

Serum IGF-I was measured by a RIA method supplied by Mediagnost (Tübingen, Germany). Plasma ACTH and cortisol levels were measured by chemoluminescence immunometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA) and immunofluorometric assay (AutoDELFIA, Wallac Oy), respectively. UFC was assayed after dichloromethane extraction by the same immunofluorimetric assay (AutoDELFIA, Wallac Oy). Plasma ACTH and cortisol levels were measured by chemoluminescence immunometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA) and immunofluorometric assay (AutoDELFIA, Wallac Oy). Serum CBG levels were evaluated by RIA method (Biosource, Nivelles, Belgium). Serum TSH, FT₄, and free T₃ levels were measured by an immunofluorimetric assay (AutoDELFIA, Wallac Oy). Body composition was evaluated by whole-body bioelectrical impedance analysis, using a portable impedance analyser (RIL Systems, Detroit, MI), following the instruction given by the manufacturer. BF% was calculated using Segal’s regression equation (21), and the results were compared with those reported by Pichard et al. (22) in normal subjects matched for age and sex.

Statistics

The data are expressed as mean ± SEM. Differences between parameters evaluated before and on therapy were assessed by the two-tailed Student’s t test for paired observations. Area under the curve (AUC) was calculated by trapezoidal integration, and comparison between AUCs was made by paired Student’s t test. For each variable, after testing normality of distribution by Kolmogorov-Smirnov test, two-way ANOVA was used to compare cortisol response to each test (1 µg ACTH and ITT) at baseline and after human H replacement therapy. P < 0.05 was accepted as significant.

Results

Hormonal and auxological changes on rhGH therapy

Serum IGF-I levels normalized during rhGH therapy [from 71.7 ± 5.3 to 177.8 ± 13.4 ng/ml (from 9.6 ± 0.7 to 23.8 ± 1.8 nmol/liter, P < 0.001], and BF% significantly decreased from 32.9 ± 2.4 to 30.1 ± 2.5% (P < 0.001), whereas BMI did not change (25.6 ± 1.5 vs. 25.2 ± 1.5%, P = NS). Although no modification of both TSH and free T₃ levels was recorded, a significant reduction in serum FT₄ levels was observed [1.1 ± 0.03 to 0.9 ± 0.03 ng/dl (13.1 ± 0.4 and 11.4 ± 0.4 pmol/liter) at baseline and during rhGH, respectively, P < 0.001], and when necessary, L-T₄ substitutive doses were promptly adjusted. The mean FT₄, after LT-4 adjustment, was not significantly different with regard to the mean FT₄ before rhGH therapy (12.9 ± 0.5 vs. 13.1 ± 0.4 pmol/liter (1.0 ± 0.03 vs. 1.1 ± 0.03 ng/dl, P = NS). Thus, evaluation of HPA axis was performed during optimal L-T₄ replacement, at both the beginning and end of the study. No alteration of blood glucose, electrolytes, or systolic/diastolic blood pressure and no clinical signs of adrenal insufficiency were observed in any subject at baseline and during the treatment period. None of the patients showed recurrence of the pituitary lesion at magnetic resonance imaging throughout the study.

<table>
<thead>
<tr>
<th>TABLE 1. Clinical characteristics of GH-deficient patients</th>
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<td>Patient</td>
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M, Male; F, female; NFP, nonfunctioning pituitary adenoma; PRL, prolactin-secreting adenoma; GH, GH-secreting adenoma; CRANIO, craniopharyngioma; HRT, hormone replacement therapy.
**HPA changes on rhGH therapy**

Serum cortisol levels, as well as UFC concentrations, were significantly lower on rhGH than before replacement therapy (7.6 ± 0.8 vs. 11.5 ± 0.8 μg/dl [208 ± 22 vs. 317 ± 24 nmol/liter], P < 0.003, and 19.6 ± 2.5 vs. 32.2 ± 3.2 μg per 24 h [54 ± 7 vs. 89 ± 9 nmol per 24 h], P = 0.006, respectively), whereas no change in mean serum CBG levels was observed (45.2 ± 3.1 vs. 44 ± 5 μg/ml, P = NS) (Table 2 and Fig. 1). Plasma morning ACTH levels did not vary significantly [25 ± 7 and 21 ± 5 ng/liter (5.5 ± 1.6 and 4.6 ± 1.1 pmol/liter) at baseline and during rhGH, respectively, P = NS]. As far as cortisol responses to provocative tests were concerned, serum cortisol peak either after 1 μg ACTH or ITT on rhGH therapy resulted in significantly lower levels than pretreatment (Table 2 and Fig. 2). Moreover, during rhGH therapy cortisol levels at each test time evaluated by two-way ANOVA as well as cortisol responses measured as AUC value after either 1 μg ACTH or ITT were significant lower (P < 0.005) in comparison with pretreatment values (Table 2 and Fig. 1).

According to the diagnostic criteria, central hypoadrenalism was detected in nine of 11 patients. Among these nine subjects, two had UFC levels below the lower limit of the normal range (Fig. 1).

**Discussion**

The present report demonstrated that rhGH replacement therapy was associated with significant changes in the HPA axis in patients with GHD due to pituitary lesion and preserved HPA function. Indeed, a significant decrease in basal serum cortisol and UFC levels together with a blunted cortisol response to provocative tests was observed in almost all patients during rhGH replacement therapy. Moreover, new diagnosis of central hypoadrenalism was assessed during rhGH therapy in a consistent number of patients of the present series that included only patients with preserved HPA function before the therapy start. However, it is worth noting that no patient had signs or symptoms of adrenal insufficiency, the hormonal parameters being consistent with a condition of subclinical central hypoadrenalism. The impairment of HPA function was not due to recurrence of hypothalamic-pituitary lesion, which was excluded by nuclear magnetic resonance imaging in all patients.

**TABLE 2. Evaluation of the HPA at baseline and on rhGH therapy (mean ± SEM)**

<table>
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<tr>
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<th>Baseline</th>
<th>rhGH</th>
<th>P</th>
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<tbody>
<tr>
<td>ACTH (ng/liter)</td>
<td>25 ± 7 (6–85)</td>
<td>21 ± 5 (5–64)</td>
<td>NS</td>
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<tr>
<td>Serum cortisol (μg/dl)</td>
<td>11.5 ± 0.8 (6.5–19.1)</td>
<td>7.6 ± 0.8 (4.1–10.1)</td>
<td>&lt;0.005</td>
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<tr>
<td>Serum cortisol peak (μg/dl)</td>
<td>20.2 ± 1.1</td>
<td>15.9 ± 1.5</td>
<td>&lt;0.01</td>
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<tr>
<td>1 μg ACTH testa</td>
<td>20.4 ± 1.4</td>
<td>15.9 ± 1.5</td>
<td>&lt;0.01</td>
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<tr>
<td>ITT</td>
<td>1300 ± 74</td>
<td>1037 ± 83.7</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>AUC serum cortisol</td>
<td>2147 ± 194</td>
<td>1157 ± 303</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>1 μg ACTH test (μg/dl-60 min)b</td>
<td>32.2 ± 3.2 (20–56)</td>
<td>19.6 ± 2.5 (6.9–31)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>ITT (μg/dl-120 min)b</td>
<td>45.2 ± 3.1 (28.7–65.5)</td>
<td>44.0 ± 5.0 (28.2–67.5)</td>
<td>NS</td>
</tr>
<tr>
<td>UFC (μg/dl)</td>
<td>3.1 (28.7–65.5)</td>
<td>44.0 ± 5.0 (28.2–67.5)</td>
<td>NS</td>
</tr>
<tr>
<td>CBG (μg/ml)</td>
<td>23–149</td>
<td>23–149</td>
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Normal ranges: ACTH, 3–60 ng/liter; serum cortisol, 5–25 μg/dl; UFC, 12.7–100 μg/24 h; CBG, 23–149 μg/ml.

Note: Conventional × CF (conversion factor) = SI. ACTH, CF = 0.22; Serum cortisol, CF = 27.59; UFC, CF = 2.759; FT4, CF = 12.87.

a Test performed in seven of 12 patients.

b Test performed in five of 12 patients.
8), this discrepancy being probably due to the higher doses of rhGH used in the past. Moreover, the significant reduction in both UFC levels and cortisol response to provocative stimuli is consistent with a poor, if any, impact of CBG on GH activity of 11β-HSD1, which is consistent with a poor, if any, impact of CBG on GH activity. In fact, it has been observed that in human disorders characterized by GH excess or deficiency, 11β-HSD1 activity is inhibited or enhanced, and the resulting tissue exposure to glucocorticoids reduced or increased, respectively (14–17). As far as the clinical impact of GHD and rhGH therapy on cortisol metabolism in patients with hypopituitarism is concerned, previous studies mostly investigated the effects of rhGH administration on glucocorticoid replacement therapy. In particular, studies carried out in patients receiving hydrocortisone replacement demonstrated that rhGH therapy caused a decrease in the ratio of cortisol/cortisone metabolites in urine, likely reflecting the inhibition of 11β-HSD1 activity by GH (6, 16). In a more recent study carried out in patients with severe GHD taking different forms of glucocorticoids (hydrocortisone or cortisone acetate), supraphysiological tissue exposure to glucocorticoids was documented in patients receiving hydrocortisone, this situation being ameliorated by rhGH therapy (23). On the other hand, patients receiving cortisone acetate were more susceptible to the inhibitory effect of GH on 11β-HSD1, thus making necessary a reassessment of glucocorticoid doses during rhGH therapy to ensure an adequate replacement (25).

The above-mentioned studies reported the effect of rhGH replacement on the availability of hydrocortisone or cortisone acetate in patients with GHD and central adrenal insufficiency and focused on the need of careful monitoring of cortisol levels in these patients to adjust substitutive therapy (6, 16, 25). The present study first demonstrated that rhGH replacement therapy, likely normalizing the overactivity of 11β-HSD1 induced by GHD, unmasked a condition of previously undiagnosed central hypoadrenalism in patients with adult-onset GHD due to organic lesions of the pituitary-hypothalamic region. Therefore, it is likely that, in these patients, HPA insufficiency prevented the achievement of a new steady state of the axis, once GH replacement normalized 11β-HSD1 activity and reduced cortisone to cortisol conversion. Although the underlying mechanisms are different, this situation is reminiscent of our previous experience reporting that about half of euthyroid patients with GHD due to central organic lesions showed FT4 levels under the normal range after 6 months of rhGH therapy (1). Therefore, it is of paramount importance to take into account that GHD could be a pitfall in the diagnosis of central hypoadrenalism as well as central hypothyroidism.

In conclusion, this study first demonstrated in a homogeneous cohort of GHD patients with preserved HPA function that GHD may mask a central hypoadrenal state in a consistent number of patients and that rhGH replacement therapy, likely by normalizing 11β-HSD1 activity and reducing cortisone to cortisol conversion, may cause central hypoadrenalism to be manifest and diagnosed. Therefore, in these patients a careful reassessment of adrenal function through appropriate testing is mandatory to start or adjust glucocorticoid replacement therapy.

Acknowledgments

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![Figure 2](https://example.com/figure2.png)
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