

Research paper

The Influence of Serum Cortisol Levels on Growth Hormone Responsiveness to GH-Releasing Hormone Plus GH-Releasing Peptide-6 in Patients with Hypocortisolism

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ABSTRACT

The aim of this study was to evaluate the influence of circulating cortisol levels on the somatotroph responsiveness to the most potent stimuli of growth hormone (GH) secretion, the GHRH+GHRP-6 test. We studied 12 patients with hypocortisolism (10 with Addison's disease and 2 with isolated ACTH deficiency) before and after glucocorticoid (GC) replacement therapy and compared them with 14 healthy subjects. In the 10 patients with Addison's disease, GH responses (GH peak, $\mu\text{g/L}$) to GHRH+GHRP-6 were similar both during GC, (68.2 ± 12.8) and off GC (60.3 ± 14.1) therapy and did not differ from those in controls (61.5 ± 6.0). In a subgroup of 4 patients with newly diagnosed Addison's disease, GH responsiveness to GHRH+GHRP-6 prior to GC replacement (26.4 ± 4.1) was significantly lower than in the 6 patients with long-standing Addison's disease after short-term GC withdrawal (82.9 ± 18.2). In the newly diagnosed Addison's patients, after one month of GC replacement, mean GH peak value increased to 40.7 ± 11.8 . In the 2 patients with isolated ACTH deficiency, GH responses to GHRH+GHRP-6 did not differ off and on GC therapy (60.3 ± 14.1 and 41.5 ± 2.0 , respectively). Our data suggest that short-term GC deprivation does not have a major impact on GH responsiveness to GHRH+GHRP-6. However, in patients with long-standing hypocortisolism, GH response is blunted but still within normal range ($> 15 \mu\text{g/L}$).

Key words: growth hormone, hypocortisolism, GHRH+GHRP-6

INTRODUCTION

Endogenous glucocorticoids play an important

physiological role in the regulation of somatotroph function¹. These steroids regulate growth hormone (GH) synthesis and secretion by modulating both hypothalamic and pituitary function². Chronic glucocorticoid excess causes growth retardation and inhibits GH secretion and GH responsiveness to several stimuli, including GHRH and GHRP-6³⁻⁸. The precise mechanisms by which this occurs are unknown. Glucocorticoids inhibit GHRH and/or stimulate soma-

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tostatin synthesis and release^{9,10,11}. At the pituitary level, glucocorticoids increase the GH and GH-releasing hormone receptor (GHRH-R) and pituitary GH secretagogue receptor (GHS-R) synthesis and gene expression and enhance the stability of GH mRNA¹²⁻¹⁶. Therefore, physiological amounts of glucocorticoids are important for the hypothalamic-pituitary somatotroph axis.

The effect of glucocorticoid deprivation on GH release has been less extensively studied in both animals and man. In rats, adrenalectomy decreases GH responsiveness to GHRH, whereas glucocorticoid treatment has the opposite effect, probably as a result of a decrease in GHRH receptor number and changes in pituitary sensitivity to GHRH^{17,18}. Patients with ACTH deficiency and prolonged hypocortisolism also have reduced GH responsiveness to several stimuli, including GHRH^{19,20}. Studies on the effects of short-term hypocortisolism on GH secretion in man are conflicting. In normal subjects pretreated with metyrapone and in patients with Addison's disease, an acute decrease in circulating cortisol level does not alter GH responsiveness to GHRH or GHRP-6^{21,22}. An increase in GHRH-induced GH release in normal subjects was found by lowering cortisol levels^{23,24}.

GH-releasing peptide-6 (GHRP-6) is a synthetic hexapeptide that releases GH through GHS-R by a mechanism which differs from that of GHRH²⁵⁻²⁷. This hexapeptide activates hypothalamic and pituitary GHS-R whose natural ligand, ghrelin, was recently discovered²⁸⁻³⁰. We demonstrated that the combined administration of GHRH + GHRP-6 is a useful test

of GH reserve in adult patients and may become the new gold standard test in a variety of clinical settings, particularly in the evaluation of adults suspected of growth hormone deficiency³¹⁻³⁴.

The aim of the present study was to investigate the possible influence of serum cortisol levels on GH response to the combined GHRH+GHRP-6 test in adult patients with primary and secondary adrenal insufficiency, in which we do not expect GH deficiency. Specifically, there were patients without clinical symptoms or signs of GH deficiency, and without organic pathology in the hypothalamic/pituitary region.

SUBJECTS AND METHODS

Subjects

1. Addison's disease

Ten patients with autoimmune Addison's disease (1 man and 9 women) were studied (Tables 1 and 2). Their mean age was 43.1 ± 4.2 yrs (range 22 - 68) and the mean body mass index (BMI) was 22.5 ± 1.2 kg/m² (range 16.7 - 30.0). Six patients had long-standing hypocortisolism and had been receiving glucocorticoid replacement therapy with hydrocortisone acetate for more than two years (Table 1), while 4 patients had newly diagnosed Addison's disease (Table 2). Hydrocortisone was administered at a dose of 30 µg/day, divided into 2 doses (at 0800 and 1600 h). Five patients also had mineralocorticoid deficiency and received replacement therapy with fludrocortisone at a dose of 0.1 µg/day. One patient with Addison's disease (patient 4, Table 2) also had associated prema-

Table 1. Clinical and laboratory data of six patients with long-standing Addison's disease, compared with healthy control subjects

Patient no.	Sex	Age (yr)	BMI (kg/m ²)	Hydrocortisone (µg/day)	Fludrocortisone (µg/day)	Peak GH (µg/L)	
						On Hydrocortisone	Off Hydrocortisone (72h)
1.	F	44	20.2	25	0.1	111.7	99.5
2.	F	68	30.1	30	-	31.4	46.2
3.	F	58	24.6	30	0.1	97.7	103.6
4.	F	26	21.5	25	0.1	59.3	42.9
5.	F	45	23.3	30	0.1	74.2	50.2
6.	F	46	25.0	30	-	144.6	155.0
PATIENTS							
Mean values ± SE		47.8±5.8	24.1±1.4	28.3±1.1	0.1±0.0	86.5±16.4	82.9±18.2
CONTROLS							
Mean values ± SE		46.0±5.0	21.6±1.1	-	-	61.5±6.0	

Table 2. Clinical and laboratory data of four patients with newly diagnosed Addison's disease with and without hydrocortisone, compared with healthy control subjects

Patient no.	Sex	Age (yr)	BMI (kg/m ²)	Hydrocortisone (µg/day)	Fludrocortisone (µg/day)	Peak GH (µg/L)	
						Before Hydrocortisone	After Hydrocortisone (1 month)
1.	M	22	18.5	25	-	26.1	75.7
2.	F	41	16.7	20	-	35.3	32.8
3.	F	39	20.2	30	-	15.6	29.9
4.	F	42	25.0	30	0.1	28.5	24.7
PATIENTS							
Mean values ± SE		36.0±4.7	20.1±1.8	26.3±2.4	0.1±0.0	26.4±4.1**	40.8±11.8
CONTROLS							
Mean values ± SE		46.0±5.0	21.6±1.1	-	-	61.5±6.0	

**p <0.01 (Patients before Hydrocortisone vs. Controls)

ture ovarian insufficiency and primary hypothyroidism and was receiving adequate replacement therapy with L-thyroxine at doses of 100 µg/day.

The results obtained in the patients were compared with those of a control group consisting of 14 healthy female subjects (age 40.4 ± 3.2 yrs, range 22 – 64 yrs; BMI 23.2 ± 0.8 kg/m², range 18.1 – 30.3). They were free of any medication at the time of the study. The women were tested in the early follicular phase of their menstrual cycles.

2. Isolated ACTH deficiency

Two patients with newly diagnosed isolated ACTH deficiency (both women) were studied. Their mean age was 46.0 ± 5.0 yrs and mean BMI was 21.6 ± 1.1 kg/m².

Study protocol

The GHRH+GHRP-6 test was performed after an overnight fast, and the subjects remained recumbent throughout. One hour before starting the test (0800h), an indwelling catheter was inserted into an antecubital vein and was kept patent by a slow saline infusion. After 3 basal blood samples (-30, -15 and 0 minutes), all subjects received GHRH (1 µg/kg, GRF 1-29 NH₂, Geref Serono, Madrid, Spain) + GHRP-6 (1 µg/kg, His-D-Trp-Ala-Trp-D-Phe-Lys-NH₂; Clinalfa Laufelfinger, Switzerland). Blood samples were subsequently obtained at 15, 30, 45, 60, 90 and 120 minutes for GH measurements. Normal growth hormone response to GHRH+GHRP-6 was previously defined as a peak GH concentration greater than 15 µg/L³². Serum cortisol level was measured at baseline on and

off glucocorticoid therapy.

Adrenal insufficiency. Six patients with long-standing Addison's disease were studied on two occasions, with an interval of at least 30 days between the tests. On the first occasion, they received GHRH+GHRP-6 during their regular hydrocortisone replacement therapy on an out-patient basis. To avoid a possible stimulating effect of acute glucocorticoid administration on GHRH+GHRP-6 induced GH release, on the morning of the test the patients received their dose of hydrocortisone after the test. The second GHRH+GHRP-6 test was performed after 72h withdrawal of hydrocortisone therapy, and the patients were hospitalized. All patients developed clinical signs of adrenal insufficiency during hydrocortisone withdrawal.

Four patients who had newly diagnosed Addison's disease were studied on two occasions: before hydrocortisone replacement and after one month of hydrocortisone therapy.

Isolated ACTH deficiency. Two patients who had newly diagnosed isolated ACTH deficiency were studied twice with GHRH+GHRP-6 test: before hydrocortisone replacement and after one month of hydrocortisone therapy (30 µg/day in two doses).

Each control subject underwent the GHRH+GHRP-6 test as described above. The local ethical committee approved the protocol and all the subjects gave informed consent.

Methods

Serum GH was measured with a time-resolved flu-

oroimmunoassay (Wallac, Turcu, Finland, $\mu\text{g/L}$) with sensitivity of assay $0.011 \mu\text{g/L}$ and with CV 6.3% ($0.4 \mu\text{g/L}$), 5.3% ($10.2 \mu\text{g/L}$), 4.2% ($43.4 \mu\text{g/L}$). Serum cortisol was measured with a radioimmunoassay (RIA, INEP, Zemun, nmol/L) with CV 16.6% (115 nmol/L), 2.5% (584 nmol/L), 5.9% (1001 nmol/L).

Statistical analysis

Results are reported as the mean \pm SE. Growth hormone responses to the combined test were quantified by determining the area under the curve (AUC), calculated using trapezoidal integration. Data were analysed using non-parametric methods including the Mann-Whitney rank sum test for independent samples and the Wilcoxon rank test for data.

RESULTS

1. Addison's disease

In all patients with Addison's disease, GH responses to GHRH+GHRP-6 were similar both during standard replacement therapy (GH peak, $68.2 \pm 12.8 \mu\text{g/L}$; AUC, $3601 \pm 694 \mu\text{g/L/120min}$) and without hydrocortisone (GH peak, $60.3 \pm 14.1 \mu\text{g/L}$; AUC, $3201 \pm 692 \mu\text{g/L/120min}$, $p > 0.05$) and did not differ from those in controls (GH peak, $61.5 \pm 6.0 \mu\text{g/L}$; AUC, $3500 \pm 413 \mu\text{g/L/120min}$, $p > 0.05$). In the six patients with long-standing Addison's disease, a similar GH response after the GHRH+GHRP-6 test was seen both during replacement therapy (GH peak, $86.5 \pm 16.4 \mu\text{g/L}$; AUC, $4721 \pm 768 \mu\text{g/L/120min}$) and after short-term glucocorticoid withdrawal (GH peak, $82.9 \pm 18.2 \mu\text{g/L}$; AUC, $4336 \pm 863 \mu\text{g/L/120min}$, $p > 0.05$; Figures 1a and 1b). Moreover, the patients with long-standing Addison's disease both on and off replacement therapy did not show significant changes in GH release compared to the normal subjects (GH peak, $61.5 \pm 6.0 \mu\text{g/L}$; AUC, $3500 \pm 413 \mu\text{g/L/120min}$, $p > 0.05$). All the patients had peak GH values above $15 \mu\text{g/L}$ on and off replacement therapy. As expected, hydrocortisone withdrawal was associated with a decrease in circulating cortisol ($962.0 \pm 117.0 \text{ nmol/L}$ vs. $23.9 \pm 12.3 \text{ nmol/L}$; $p < 0.05$). In our group of patients, glucocorticoid withdrawal for 72h caused clinical signs of adrenal insufficiency, but all manifestations were well tolerated. None of the patients had to interrupt the study protocol.

In the 4 patients with newly diagnosed Addison's

disease, GH responsiveness to GHRH+GHRP-6 before hydrocortisone replacement was significantly lower in comparison with patients in whom hydrocortisone was acutely withdrawn (GH peak, $26.4 \pm 4.1 \mu\text{g/L}$ vs. $82.9 \pm 18.2 \mu\text{g/L}$, $p < 0.01$; AUC, $1498 \pm 335 \mu\text{g/L/120min}$ vs. $4336 \pm 863 \mu\text{g/L/120min}$, $p < 0.01$; Figures 1a and 1b). No differences in age and BMI were seen between the two groups. GH response to the GHRH+GHRP-6 test increased after one month of replacement therapy with hydrocortisone (GH peak, $40.7 \pm 11.8 \mu\text{g/L}$; AUC, $1920 \pm 756 \mu\text{g/L/120min}$), statistically not different from the patients with long-standing Addison's disease on standard hydrocortisone

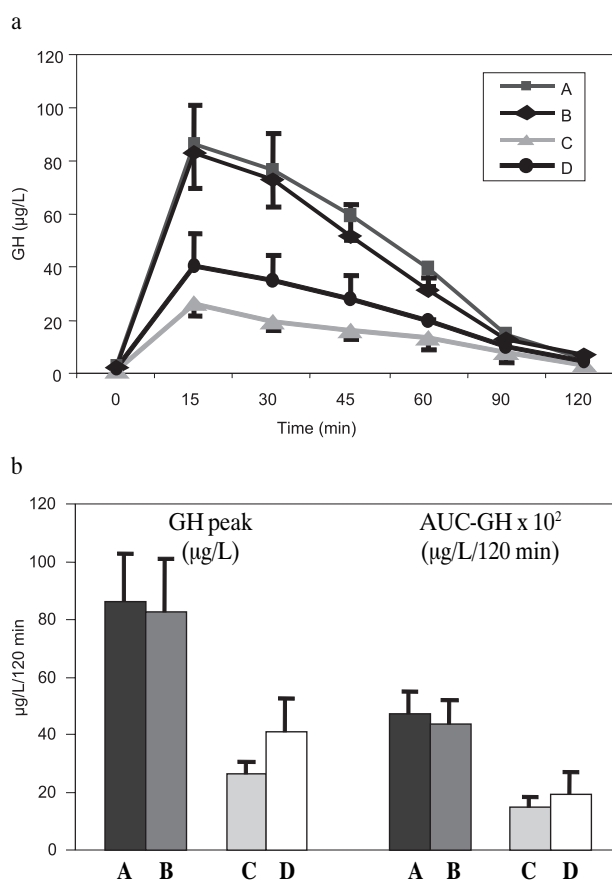


Figure 1. Mean plasma GH levels (a: mean peak \pm SE, b: mean peak and mean area under the curve \pm SE) after GHRH+GHRP-6 administration in: **A** – Patients with long-standing Addison's disease on hydrocortisone therapy; **B** – Patients with long-standing Addison's disease 72h off hydrocortisone therapy; **C** – Patients with newly diagnosed Addison's disease before hydrocortisone therapy; **D** – Patients with newly diagnosed Addison's disease after one month of hydrocortisone therapy.

** C vs. B $p < 0.01$

therapy (GH peak, $86.5 \pm 16.4 \mu\text{g/L}$; AUC, $4721 \pm 768 \mu\text{g/L/120min}$, $p = 0.088$; *Figures 1a and 1b*). Serum cortisol levels at the time of diagnosis were $20.8 \pm 3.8 \text{ nmol/l}$, increasing after one month of stable hydrocortisone replacement therapy ($613.0 \pm 60.0 \text{ nmol/L}$; $p < 0.05$).

2. Isolated ACTH deficiency

In the two patients with newly diagnosed isolated ACTH deficiency, GH responsiveness to GHRH+GHRP-6 were similar both before hydrocortisone therapy (GH peak, $64.9 \pm 4.6 \mu\text{g/L}$) and while on hydrocortisone (GH peak, $41.5 \pm 2.0 \mu\text{g/L}$; $p > 0.05$) and did not differ from those in controls (GH peak, $61.5 \pm 6.0 \mu\text{g/L}$; $p > 0.05$).

DISCUSSION

The aim of the present study was to investigate whether the GH response to the most potent GH stimuli, the combined GHRH+GHRP-6 test is affected by low serum cortisol levels in two groups of patients in whom we did not suspect GH deficiency. Patients with primary adrenal insufficiency and those with isolated ACTH deficiency served as a model for the investigation of the influence of serum cortisol levels on GH responsiveness to the provocative stimuli.

In the patients with Addison's disease on stable replacement therapy, GH responsiveness to the GHRH+GHRP-6 test was normal. In these patients, an acute decrease in circulating cortisol levels, caused by 72h-withdrawal of hydrocortisone, did not significantly modify the GH response to GHRH+GHRP-6. GH responses to GHRH+GHRP-6 both with and without hydrocortisone therapy were similar to and not different from the control subjects.

The patients with newly diagnosed Addison's disease (i.e. prolonged glucocorticoid deprivation), who were chronically severely depleted of glucocorticoids, had a blunted GH response to GHRH+GHRP-6, but still within the normal range (a GH response over $15 \mu\text{g/L}$), indicating subtle changes in somatotroph function. After one month of glucocorticoid replacement therapy, GH response to the GHRH+GHRP-6 test increased, suggesting the possibility of transitory reduction of GH secretion, possibly caused by low cortisol levels.

Our data are in agreement with the results of Pin-

to et al, demonstrating that somatotroph function is preserved in states of acute glucocorticoid deprivation²¹. In their study, an acute decrease in circulating cortisol levels, achieved with metyrapone pretreatment, did not change the GH response to GHRP-6 in normal subjects. Patients with chronic hypoadrenalism on hydrocortisone therapy had normal increase in GH levels after GHRP-6, and after glucocorticoid interruption for 72h, no significant change in GH responsiveness was observed. They concluded that short-term glucocorticoid deprivation does not have a major impact on GHRP-6-dependent GH release.

In patients with ACTH deficiency, prolonged glucocorticoid deprivation reduced GH responsiveness to several stimuli, including insulin-induced hypoglycemia, arginine and GHRH^{19,20} and glucocorticoid replacement therapy restored GH release after GHRH administration^{19,20}. The authors concluded that in patients with idiopathic ACTH deficiency, an impaired GH response to stimuli, reversible during glucocorticoid replacement therapy, may be the consequence of the low cortisol levels. These patients were studied with an insulin tolerance test and an arginine infusion test, which are less potent GH releasers than the combined GHRH+GHRP-6 test. In our two patients with isolated ACTH deficiency, GH response to the GHRH+GHRP-6 test was normal before and after one month of hydrocortisone therapy. These data are in agreement with the results of Sakai et al, who demonstrated complete ACTH deficiency after radiation therapy for brain tumor with a normal GH reserve³⁵.

It has been shown that glucocorticoids have a stimulatory effect on pituitary GHRH and GHS-R synthesis, at least in the rat³⁶⁻³⁸. Adrenalectomy markedly decreases GHS-R gene expression, whereas glucocorticoid treatment has the opposite effect^{36,37}. It is possible that in Addison's patients with prolonged hypocortisolism before glucocorticoid replacement, GHRH-R and GHS-R are down-regulated with consequent attenuation of GHRH+GHRP-6-induced GH release. It is likely that in these hypoadrenal patients, even after adequate glucocorticoid replacement therapy, circulating glucocorticoid levels are inadequate to maintain a completely normal pituitary GHRH-R and GHS-R synthesis and somatotroph function.

In conclusion, our data suggest that short-term glucocorticoid deprivation does not modify the GH responsiveness to GHRH+GHRP-6. However, in long-

standing hypocortisolism, GH response to the combined test is blunted, yet still within the normal range, suggesting the presence of subtle changes in somatotroph function.

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