

The effect of chronic treatment with GH on gonadal function in men with isolated GH deficiency

Cesare Carani¹, Antonio R M Granata¹, Michele De Rosa², Cecilia Garau¹, Stefano Zarrilli², Luigi Paesano², Annamaria Colao², Paolo Marrama¹ and Gaetano Lombardi¹

¹Department of Endocrinology, University of Modena and ²Department of Molecular and Clinical Endocrinology and Oncology, University 'Federico II', Naples, Italy

(Correspondence should be addressed to G Lombardi, Department of Molecular and Clinical Endocrinology and Oncology, University 'Federico II', via G. Santacroce 40/a 80129, Naples, Italy)

Abstract

Eleven adult males, previously submitted to neurosurgery because of a pituitary lesion (three with craniopharyngioma, three with clinically non-functioning adenoma and five with macroprolactinoma) were treated with recombinant GH for 12 months after the diagnosis of GH deficiency was made. Circulating FSH, LH, prolactin, testosterone, 17 β -estradiol (E₂), dehydroepiandrosterone (DHEA-S), androstenedione, 17-OH-progesterone (17OHP), IGF-I, and steroid hormone-binding protein (SHBG) levels were assayed before and after CG test at study entry and 6 and 12 months after GH treatment.

A significant increase in plasma IGF-I levels was obtained after 6 and 12 months of GH treatment. In addition, CG-stimulated, but not baseline, testosterone levels showed a significant increase after 6 and 12 months of GH treatment when compared with study entry (9.6 ± 0.5 and 9.9 ± 0.5 vs 7.9 ± 0.5 ng/ml; $P < 0.05$). Baseline, but not CG-stimulated, serum 17OHP levels were significantly increased only after 12 months of GH treatment (1.7 ± 0.1 vs 1.4 ± 0.1 ng/ml; $P < 0.05$). No significant difference was found as far as both basal and CG-stimulated E₂, androstenedione, DHEA-S and SHBG were concerned. With regards to the semen analysis, only seminal plasma volume was significantly increased after 12 months of GH treatment (2.9 ± 0.3 vs 1.7 ± 0.3 ml; $P < 0.05$). No significant change in sperm count, motility and abnormal forms was observed.

These data show that GH treatment displays a clear-cut effect upon Leydig cell function and increases the production of seminal plasma volume in fertile adult males with isolated GH deficiency.

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Introduction

A considerable body of *in vivo* and *in vitro* evidence suggests the existence of a relationship between growth hormone (GH) and the reproductive system both in animals and humans (1–11). In fact, several studies have demonstrated that GH treatment increases ovary sensitivity to gonadotropin stimulation in infertile women with hyposensitive ovaries (12–19). The effect of GH administration on gonadal function in men has been poorly investigated. In a few hypopituitary patients treated with extra-active GH and chorionic gonadotropin (CG), Rivarola *et al.* (3) did not find any significant increase in testosterone levels when compared with CG treatment alone. Conversely, treatment with recombinant GH (rGH) was thought to be an important permissive factor on Leydig cell activity in 11 prepubertal boys with GH deficiency (GHD) (20). On the other hand, a weak synergic effect on steroidogenesis,

without any notable effect on seminal insulin-like growth factor-I (IGF-I) concentration, was demonstrated in eight oligospermic, eugonadal, non-GH-deficient men treated with GH and CG (21). An improvement in spermatogenesis and an increase in serum testosterone levels were reported after combined treatment with pulsatile gonadotropin-releasing hormone (GnRH) and GH (19, 22). In addition, it has been suggested that rGH administration could improve testosterone production induced by CG alone or combined with gonadotropins (23). It should be mentioned that impaired GH secretion and increased GH-binding protein levels were found in infertile males (24). Finally, recent reports concluded that GH treatment either alone or combined with gonadotropins was unable to increase the sperm count in eugonadal men with severe idiopathic oligospermia (25, 26).

In order to evaluate whether and to what extent GH administration plays a role on testicular steroidogenesis

and spermatogenesis, we studied the effect of 12 months of treatment with rGH on the CG-stimulated sex steroid hormone concentration as well as on sperm parameters in adult patients with isolated GHD and integrity of the pituitary–gonadal axis.

Patients and methods

Patients

Among 122 patients with pituitary diseases subjected to endocrine testing for GHD, 11 men (age range: 33–54 years) never treated with either testosterone or gonadotropins, agreed to participate in this open prospective study. The patients' profile at study entry is shown in Table 1. All 11 patients had previously undergone surgery because of a pituitary lesion: three patients had a craniopharyngioma, three had a clinically non-functioning pituitary adenoma, and five had a macroprolactinoma (Table 1). All patients underwent surgical treatment at least 2 years before inclusion in the study to allow the exclusion of those with remnant or recurrent tumors at magnetic resonance imaging (MRI). None of the patients with prolactinoma were treated with dopamine agonists at the time of the study. All patients had fathered before surgery. In agreement with the literature, the diagnosis of GHD was made on the basis of a GH response after GH-releasing hormone (GHRH) plus arginine (ARG) (GHRH+ARG), clonidine or insulin tolerance tests (ITT) below $3 \mu\text{g/l}$ (27). A GHRH+ARG test was performed in all patients, while the clonidine test or the ITT were performed in order to confirm the diagnosis of GHD. The individual GH peaks after diagnostic tests are shown in Table 1.

Study protocol

The study design considered four time-points for analysis: at diagnosis (between 6 and 12 months before study entry), at study entry (considered as time 0), and after 6 and 12 months of rGH treatment. Before entering the study, the patients were interviewed for their medical history and underwent physical examination, laboratory evaluation and a complete endocrine screening. As shown in Table 1, all patients had a normal thyroid, adrenal and gonadal profile, evaluated on the basis of normal free thyroxine (fT_4), urinary free cortisol and testosterone levels. To confirm the adequacy of adrenal replacement, the measurement of serum and urinary Na^+ and K^+ levels were performed together with that of free urinary cortisol levels. Circulating testosterone, 17β -estradiol (E_2), 17-OH -progesterone (17OHP), dehydroepiandrosterone-sulphate (DHEA-S), $\Delta 4$ -androstenedione ($\Delta 4$), IGF-I, and steroid hormone-binding protein (SHBG) concentrations were assayed before and after a single i.m. CG test (5000 IU); blood samples were obtained just before CG administration and after 48 h. A standard GnRH ($100 \mu\text{g}$, i.v.) test

was performed at diagnosis, at study entry and after 12 months of GH treatment, in order to exclude the presence of existing or on-going hypogonadism. Increase in serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels one to two and five to six times greater than basal values, respectively, was considered a normal response. A 100% increase in serum testosterone levels after 24 h from the injection of CG was considered a normal response (28).

Gonadal function was evaluated every 6 months during the study both clinically (testicular volume) and by semen analysis which was performed according to the WHO laboratory manual guidelines (29). Seminal fluid samples were obtained by masturbation after 3–5 days of sexual abstinence. After collection, the ejaculates were allowed to liquify at room temperature. Sperm count and motility were evaluated in a Makler counting chamber ($40\times$), while sperm morphology was evaluated after dilution (1:1) in phosphate-buffered saline and Giemsa.

Treatment protocol

The treatment with rGH was carried out as follows: 0.125 IU/kg per week s.c. during the first month and then 0.25 IU/kg per week s.c. for the following months. Six patients were studied in Modena at the Department of Endocrinology, and five patients were studied in Naples at the Department of Molecular and Clinical Endocrinology and Oncology, 'Federico II' University. The protocol was approved by the Local Ethical Committees and informed consent was obtained from each subject.

Assays

Serum samples were kept frozen at -20°C until assayed. Serum thyrotropin (TSH), free tri-iodothyronine (fT_3) and fT_4 levels were measured using the Ciba-Corning Automated Chemiluminescence System using kits provided by Ciba-Corning Diagnostic Corp. (Medfield, USA). The inter- and intra-assay coefficients of variation (C.V.) were respectively 6% and 3.9% for TSH, 8% and 2.1% for fT_3 and 10% and 3.4% for fT_4 . Serum LH, FSH and prolactin levels were measured by a two-site fluorimmunoassay based on the direct sandwich technique (DELPHIA), using kits from Pharmacia and Upjohn (Milan, Italy). The inter- and intra-assay C.V. values were respectively 5.3% and 2.5% for LH, 4.2% and 3.0% for FSH, and 5.0% and 2.6% for prolactin. Serum testosterone levels were measured by RIA in solid phase using kits from DPC (Los Angeles, USA). The inter- and intra-assay C.V. values were 10.7% and 5.9% respectively. Serum E_2 , SHBG and GH were measured by a two-site fluorimmunoassay based on the direct sandwich technique (DELPHIA), using kits from Pharmacia and Upjohn. The inter- and intra-assay C.V. values were respectively 5% and 3.9%

Table 1 Patients' profile at study entry.

Patient age (years)	Weight (kg)	Height (cm)	Diagnosis	Diagnosis of GHD: Serum GH levels (ng/ml)			Plasma IGF-I levels (ng/ml)	Serum FSH levels (IU/l)	Serum LH levels (IU/l)	Serum testosterone levels (ng/ml)	Serum prolactin levels (ng/ml)	Serum FT ₄ levels (pg/ml)	Urinary free cortisol levels (µg/24 h)
				GHRH+ARG	Clonidine	ITT							
1	32	81	NFA	0.1	1.2	n.d.	190	5.3	3.4	5.9	5.6	11.8	104
2	35	75	Craniopharyngioma	1.1	n.d.	0.1	170	3.3	3.2	4.5	4.5	10.5	197
3	37	71	Prolactinoma	2.2	2.5	n.d.	116	1.7	1.5	4.3	2.8	8.9	225
4	39	85	Prolactinoma	3	n.d.	1.1	133	3.5	3.8	4.4	7.2	9.4	175
5	40	70	Prolactinoma	2.8	n.d.	0.1	145	3.8	4.0	4.2	6.3	11.7	183
6	43	65	Craniopharyngioma	1.1	n.d.	0.5	180	2.9	3.0	4.8	4.3	12.7	155
7	45	70	Craniopharyngioma	0.5	1.1	n.d.	106	3.9	1.5	4.5	6.0	10.8	149
8	47	58	Prolactinoma	0.8	n.d.	0.1	195	4.2	4.3	4.0	5.5	10.5	163
9	49	88	NFA	0.1	0.5	n.d.	118	4.9	3.2	3.8	2.3	11.3	154
10	50	85	NFA	0.1	0.1	n.d.	86	5.6	3.1	3.6	4.5	11.7	144
11	53	86	Prolactinoma	2.2	2.5	n.d.	95	2.4	2.8	3.8	15.1	10.3	120

NFA, clinically non-functioning pituitary adenoma.

Normal ranges: IGF-I, 110–385 ng/ml in 20–40 year old adults and 65–320 ng/ml in 40–60 year old adults; FSH and LH, 5–18 IU/l; testosterone 3.5–9 ng/ml; prolactin, 5–15 ng/ml; FT₄, 7.1–18.5 pg/ml; free urinary cortisol, 35–135 µg/24 h. n.d., not defined.

for E₂, 10% and 7.7% for SHBG, and 5% and 3.2% for GH. Serum 17OHP was measured by RIA in solid phase using kits from DPC. The inter- and intra-assay C.V. values were 25% and 5.6%. Serum DHEA-S levels were measured by a solid-phase competitive binding enzyme immunoassay, using kits from BIO-RAD Nova-Path. The inter- and intra-assay C.V. values were respectively 15% and 7.6%. Serum Δ4 levels were measured by coated-tube RIA, using kits from Diagnostic System Laboratories Inc., (Webster, USA). The inter- and intra-assay C.V. values were respectively 15% and 5.6%. Plasma IGF-I levels were measured by coated-tube IRMA, using kits from Diagnostic Systems Laboratories Inc. The inter- and intra-assay C.V. values were respectively 10% and 3.4%.

Statistical analysis

Data are shown as mean ± s.e.m. ANOVA followed by the Newman–Keuls test and Student's *t*-test for paired data were used where appropriate. Significance was set at 5%.

Results

No significant difference was found as far as hormone parameters and sperm parameters were concerned, between the evaluation performed at diagnosis and at study entry. In particular, all 11 patients, though having serum FSH, LH and testosterone levels in the low normal range (Table 1), had a normal gonadotropin response to GnRH. The maximal percent increment was 245 ± 21% and 542 ± 51% for FSH and LH respectively. Before starting rGH treatment, the response to CG was normal (Table 2).

In all 11 patients serum IGF-I concentrations were normal but close to the low normal range (Table 1). After 6 and 12 months of treatment, serum IGF-I concentrations significantly increased in all patients, reaching values higher than 2 s.d. in eight out of eleven patients. A significant increase in the CG-stimulated response of testosterone was shown after 6 and 12 month of GH treatment when compared with baseline response ($P < 0.05$, Fig. 1). Moreover, a significant increase in baseline serum 17OHP levels was found after 12 months of GH treatment in respect to pretreatment period ($P < 0.05$, Fig. 1). No significant difference in the response of E₂, Δ4, DHEA-S and SHBG levels to the CG test was found before and after GH treatment (Table 2). No significant change of gonadotropin response to GnRH was found after 6 and 12 months of GH treatment (data not shown).

As far as semen analysis is concerned, a significant increase in seminal plasma volume was found after 6 and 12 months of GH treatment (2.4 ± 0.1 and 2.9 ± 0.3 ml respectively) when compared with basal volume (1.7 ± 0.3 ml; $P < 0.05$). No significant change in sperm count (93.6 ± 53.3, 89.4 ± 111.4 and 77.2 ±

Table 2 Hormone response after CG test before and after 6 and 12 months of treatment in 11 adult males with isolated GHD.

	At diagnosis		At study entry		6 months GH treatment		12 months GH treatment		P
	Basal	After 48 h	Basal	After 48 h	Basal	After 48 h	Basal	After 48 h	
	Testosterone (ng/ml)	4.2 ± 0.4	7.3 ± 0.8	4.1 ± 0.2	7.9 ± 0.5	4.4 ± 0.2	9.6 ± 0.5	4.8 ± 0.3	
Androstenedione (ng/ml)	211 ± 11	288 ± 31	209 ± 11	295 ± 28	205 ± 11	276 ± 20	219 ± 17	291 ± 23	n.s.
17OHP (ng/ml)	1.4 ± 0.1	4.3 ± 0.6	1.4 ± 0.1	4.2 ± 0.5	1.6 ± 0.1	4.4 ± 0.4	1.7 ± 0.1	3.9 ± 0.2	< 0.05
DHEA-S (µg/dl)	0.8 ± 0.3	1.8 ± 0.4	0.9 ± 0.1	1.7 ± 0.2	1.1 ± 0.1	1.7 ± 0.2	1.3 ± 0.1	1.8 ± 0.2	n.s.
E ₂ (pg/ml)	12.9 ± 1.5	38.5 ± 4.8	12.7 ± 1.3	40.7 ± 4.3	13.7 ± 1.4	40.1 ± 5	15.5 ± 1.5	35.1 ± 2.3	n.s.
SHBG (ng/ml)	34.8 ± 6.5	33.3 ± 4.1	35.4 ± 6.2	30.1 ± 3.4	36.1 ± 5.2	38.9 ± 4.8	34.4 ± 4.6	31.6 ± 4.5	n.s.

n.s., not significant.

51.4×10⁶), motility (13.2±5.6, 12.6±8.3 and 10.9±12.6%), and abnormal forms (52.7±14.3, 53.2±9.9 and 53.9±10.9%) was recorded at baseline and 6 and 12 months after GH treatment.

Discussion

The results of the present study demonstrated that rGH treatment at a dose of 0.125–0.25 IU/kg stimulated Leydig cell activity in adult males with isolated GHD. In fact, a significant increase in CG-stimulated testosterone levels was observed in our patients during treatment. However, it is worthwhile underlining that the high GH doses used in this study induced very high IGF-I levels (+2 s.d. in eight out of eleven patients) which suggests that the Leydig cell activity was stimulated because of supraphysiological GH levels. In a preliminary study in GHD prepubertal boys, Kulin *et al.* (20) found an increase in CG-stimulated testosterone and dihydrotestosterone levels after GH treatment. This finding supports the hypothesis that GH plays a permissive role in Leydig cell activity. Recently, the combined treatment with gonadotropins and GH was shown to improve testosterone production induced by gonadotropin treatment, even if only in four hypopituitary adult men, still confirming a role of GH on testosterone production (23).

The present study was designed to evaluate the effect of standard replacement GH treatment on gonadal function in a particular group of fertile eugonadal males with isolated GHD acquired in adulthood. It should be noted that all our patients had serum testosterone levels in the low normal range as well as a normal response of FSH and LH to the GnRH test, although some of them had subnormal gonadotropin levels in basal conditions. In experimental animals, GH has been shown to affect gonadal function. Using the immunohistochemically localized GH receptor-binding protein in Leydig cells, Lobie *et al.* (30) suggested that GH may stimulate local production of IGF-I. This is in agreement with the results of Chatelain *et al.* (11) who reported that GH and IGF-I treatment increased testicular LH receptors and steroidogenic responsiveness in GHD dwarf mice. This finding suggested that IGF-I can induce Leydig cell maturation and that the effect of GH is probably mediated by IGF-I. Nevertheless, up to now the mechanism of the GH action on gonadal function has not been fully clarified. The finding of an increased testosterone response after CG administration in our patients confirms the results of Chatelain *et al.* (11), who found that during GH treatment in deficient dwarf mice the acute administration of CG increased the testicular steroidogenic response without affecting basal hormone levels. On the other hand, serum Δ4 and DHEA-S levels were not modified both in basal condition and 48 h after CG injection. These data are partially in disagreement with those reported by Balducci *et al.* (23), who found that serum Δ4 levels increased during GH treatment while serum 17OHP levels were higher during GH

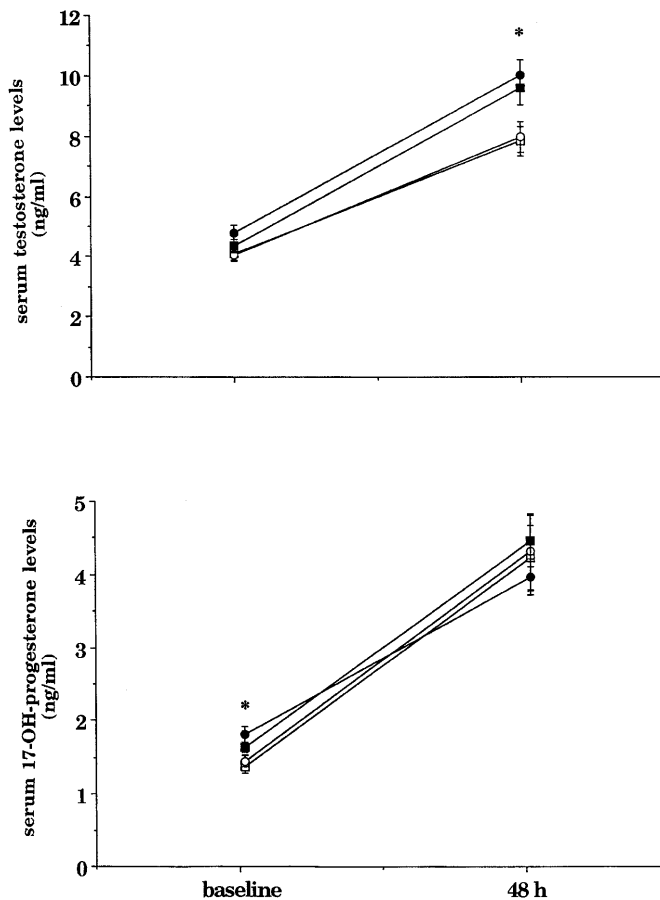


Figure 1 Effect of CG administration (5000 IU i.m.) on serum testosterone (top), and 17OHP levels (bottom) before and after 6 and 12 months of treatment with rGH in 11 adult patients with GHD. * $P < 0.01$ vs basal values. □, at diagnosis; ○, at study entry; ■, after 6 and ● after 12 months of GH replacement.

withdrawal. They suggested that this different response to CG during GH treatment was due to increased enzyme activity (31). Similarly, plasma E_2 levels were in the normal range before treatment and did not show any significant increase during treatment. On the other hand, Anapliotou *et al.* (21) demonstrated the absence of variation in 17OHP, $\Delta 4$, DHEA, DHEA-S and E_2 during treatment with CG and/or GH. In our study, serum 17OHP levels were significantly increased before CG administration only after 12 months of GH treatment. The discrepancy between our results and those of Anapliotou *et al.* (21) is likely to be due to the different dose and duration of treatment with GH. However, the possibility that the observed greater serum testosterone response to CG after GH replacement was only apparent and determined by an alteration in the metabolic clearance rate cannot be ruled out.

Sperm count and motility did not improve while seminal plasma volume significantly increased after GH treatment in our patients. However, it should be noted that the increase in seminal plasma volume was detected in the absence of a simultaneous increase in sperm count, indicating that the total number of

spermatozoa in the ejaculate increased. None of the patients included in this study was sterile or oligospermic before rGH treatment. These data are in agreement with those reported by Lee *et al.* (25), who did not find any change in sperm count in men with severe idiopathic oligospermia after GH therapy and by Pedersen *et al.* (33) who showed that GH treatment for 4 months did not change semen quality in isolated GHD patients. On the other hand, Ovesen *et al.* (34) showed an increase of sperm motility without any modification of sperm count in nine oligozoospermic patients treated with GH for 12 weeks. In addition, only two out of seven patients with hypogonadotropic hypogonadism showed improved sperm count after GH treatment (19, 22, 32). The increase of plasma seminal volume after GH treatment in our patients can be attributed to a direct action of IGF-I on prostate epithelial cells (35, 36).

In conclusion, chronic GH administration in fertile male patients with isolated GHD acquired in adulthood increased the testosterone response to CG and the seminal plasma volume, without any change in sperm count, motility and morphology.

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