CLINICAL STUDY

Effects of short-term administration of low-dose rhGH on IGF-I levels in obesity and Cushing's syndrome: indirect evaluation of sensitivity to GH

M Maccario, F Tassone, C Gauna, S E Oleandri, G Aimaretti, M Procopio, S Grottoli, C D Pflaum¹, C J Strasburger¹ and E Ghigo

Division of Endocrinology, Department of Internal Medicine, University of Turin, Italy and ¹Department of Internal Medicine, University of Munich, Germany

(Correspondence should be addressed to E Ghigo, Division of Endocrinology, Department of Internal Medicine, Ospedale Molinette, C.so A.M. Dogliotti 14, 10126 Torino, Italy; Email: ezio.ghigo@unito.it)

Abstract

Objective: To verify the hypothesis of an increased sensitivity to GH in obesity (OB) and Cushing's syndrome (CS).

Design: We studied the effects of short-term administration of low-dose rhGH on circulating IGF-I levels in patients with simple OB or CS and in normal subjects (NS).

Methods: Nineteen women with abdominal OB aged (mean $\pm$ S.E.M.) 38.2 ± 3.1 years, body mass index 40.7 ± 2.5 kg/m², waist to hip ratio 0.86 ± 0.02 , ten with CS (50.4 ± 4.2 years, 29.7 ± 3.3 kg/m²) and 11 NS (35.0 ± 3.6 years, 20.5 ± 0.5 kg/m²) underwent s.c. administration of 5 μ g/kg per day rhGH at 2200 h for four days. Serum IGF-I, IGF-binding protein-3 (IGFBP-3), GH-binding protein (GHBP), insulin and glucose levels were determined at baseline and 12 h after the first and the last rhGH administration.

Results: Basal IGF-I levels in NS (239.3 ± 22.9 μ g/l) were similar to those in OB (181.5 ± 13.7 μ g/l) and CS (229.0 ± 29.1 μ g/l). Basal IGFBP-3, GHBP and glucose levels in NS, OB and CS were similar while insulin levels in NS were lower ($P < 0.01$) than those in OB and CS. In NS, the low rhGH dose induced a sustained rise of IGF-I levels (279.0 ± 19.5 μ g/l, $P < 0.001$), a non-significant IGFBP-3 increase and no change in GHBP, insulin and glucose levels. In OB and CS, the IGF-I response to rhGH showed progressive increase (246.2 ± 17.2 and 311.0 ± 30.4 μ g/l respectively, $P < 0.01$ vs baseline). Adjusting by ANCOVA for basal values, rhGH-induced IGF-I levels in CS (299.4 μ g/l) were higher than in OB (279.1 μ g/l, $P < 0.01$), which, in turn, were higher ($P < 0.05$) than in NS (257.7 μ g/l). In OB, but not in CS, IGFBP-3 and insulin levels showed slight but significant ($P < 0.05$) increases during rhGH treatment, which did not modify glucose levels in any group; thus, in the OB patient group a significant fall in glucose/insulin ratio was observed.

Conclusions: Short-term treatment with low-dose rhGH has enhanced stimulatory effect on IGF-I levels in OB and, particularly, in hypercortisolemic patients. These findings support the hypothesis that hyperinsulinism and hypercortisolism enhance the sensitivity to GH in humans.

European Journal of Endocrinology 144 251–256

Introduction

Insulin-like growth factor-I (IGF-I) synthesis and release are mostly dependent on growth hormone (GH) but a major role is also played by nutritional status (1, 2). An important influence on IGF-I levels is also played by peripheral hormones such as insulin, cortisol and gonadal hormones (3–7). In fact, insulin directly stimulates IGF-I and enhances its response to GH in animals (3, 4). The influence of cortisol on IGF-I is more controversial. *In vitro* studies reported both stimulatory and inhibitory effects of glucocorticoids on IGF-I synthesis (4, 8), while human *in vivo* studies

showed that even short-term treatment with glucocorticoids clearly stimulates IGF-I levels (5, 6).

Obesity (OB) and Cushing's syndrome (CS) are characterized by marked decrease of both spontaneous and stimulated GH secretion (9, 10), but nevertheless total IGF-I levels have been reported normal or low normal (11–15) and free IGF-I levels even increased, at least in OB (16). It has also been reported that total IGF-I levels in patients with subcutaneous OB are higher than those in patients with visceral OB (17). In the latter patient group a hyperactivity of the hypothalamo–pituitary–adrenal axis has been reported (18). It has been hypothesized that hyperinsulinism

and/or hypercortisolism could lead to peripheral 'hepatic' GH hypersensitivity allowing for maintained IGF-I synthesis and release in spite of clear-cut reduction of GH secretion in both OB and CS. Circulating GH-binding protein (GHBP) levels, which likely reflect the GH receptor status (19), have been reported increased in OB by some authors (19) and normal in hypercortisolism (14).

Based on the foregoing, in order to verify the hypothesis that hyperinsulinism and hypercortisolism enhance the sensitivity to GH in humans, we studied the IGF-I response to low-dose recombinant human GH (rhGH) in patients with abdominal OB or with CS; these responses were compared with those in normal lean subjects. As a low rhGH dose we chose the lowest one able to increase IGF-I levels in normal adults (20). The effects of this rhGH dose on IGF-binding protein-3 (IGFBP-3), GHBP, insulin and glucose levels were also evaluated.

Subjects and methods

Nineteen women with abdominal OB aged (mean $\pm$ S.E.M.) 38.2 ± 3.1 years, body mass index (BMI) 40.7 ± 7.0 kg/m², waist to hip ratio 0.86 ± 0.02 , ten with CS (50.4 ± 4.2 years, 29.7 ± 3.3 kg/m²) and 11 normal women (NS) (35.0 ± 3.6 years, 20.5 ± 0.5 kg/m²) were studied. All subjects gave informed consent to participate in the study, which had been approved by the local Ethical Committee.

Regarding clinical and hormonal of the patients with CS: (i) two patients had classical cortisol-secreting adrenal adenoma as shown by hormonal and imaging findings; (ii) eight had classical pituitary adrenocorticotropin-dependent CS demonstrated by hormonal and magnetic resonance imaging findings; (iii) mean urinary cortisol levels in the two subgroups were similar (levels in the whole group were 530.4 ± 173.6 μ g/24 h).

All subjects underwent short-term treatment by low-dose rhGH administration (5 μ g/kg per day s.c. at 2200 h for 4 days). Blood samples for IGF-I, IGFBP-3, GHBP, insulin and glucose measurements were drawn in basal conditions and then 12 h after the first and fourth rhGH injections.

Serum IGF-I levels were measured in duplicate by RIA (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). To avoid interference by binding proteins, all plasma samples were treated with acid ethanol. The sensitivity of the assay was 0.3 μ g/l. The inter- and intra-assay coefficient of variation were 10.1–15.7% and 7.6–15.5% respectively. IGF-I concentrations were expressed as absolute values (μ g/l) with reference to a recombinant IGF-I preparation.

IGFBP-3 levels were measured in duplicate by RIA (Nichols Institute Diagnostics). The sensitivity of the assay was 0.25 ng/ml. The inter- and intra-assay coefficients of variation were 5.3–6.3% and 3.4–8.0% respectively.

Serum GHBP levels (pmol/l) were measured by a ligand immunofunctional assay (21) with a monoclonal anti-GHBP antibody (22, 23). Within-assay coefficient of variation was 3.4% at 115 pmol/l and 1550 pmol/l. Between-assay coefficients of variation were 8.5 and 10.9% respectively.

Serum insulin levels were measured in duplicate by IRMA (INSIK-5; Sorin Biomedica, Saluggia, Italy). The sensitivity was 2.5 ± 0.3 mU/l. Inter- and intra-assay coefficients of variation were between 6.2 and 10.8% and between 5.5 and 10.6% respectively.

Plasma glucose levels were measured by a glucose oxidase colorimetric method (GLUCOFIX; Menarini Diagnostics, Firenze, Italia).

The statistical analysis was performed using ANOVA and Newman-Keuls test. Particularly, the responses to rhGH were analyzed by ANCOVA adjusting for basal values. The results are expressed as means $\pm$ S.E. and 'adjusted means'. These are the means obtained after removing all differences that can be accounted for by the covariate in the ANOVA design (24). The general formula is: $Y - \bar{y}_{j(\text{adj})} = Y - \bar{y}_j - b(X - \bar{x}_j - X - \bar{x})$, where: $Y - \bar{y}_{j(\text{adj})}$ is the adjusted mean of group j ; $Y - \bar{y}_j$ the mean of group j before adjustment; b the common regression coefficient; $X - \bar{x}_j$ the mean of the covariate for group j ; $X - \bar{x}$ the grand mean of the covariate.

Results

Basal IGF-I levels in NS (239.3 ± 22.9 μ g/l) were similar to those in OB (181.5 ± 13.7 μ g/l) and CS (229.0 ± 29.1 μ g/l) (Table 1).

Basal IGFBP-3, GHBP and glucose levels in NS, OB and CS were similar while insulin levels in NS were lower ($P < 0.01$) than those in OB and CS (Table 1).

In NS, the low rhGH dose induced prompt IGF-I increase (12 h after the first rhGH administration: 270.4 ± 22.4 μ g/l, $P < 0.01$ vs baseline); this increase persisted similarly at the end of rhGH treatment (12 h after the fourth administration: 279.0 ± 19.5 μ g/l, $P < 0.01$ vs baseline) (Fig. 1).

In NS, treatment with rhGH induced non-significant IGFBP-3 increase and no change in GHBP, insulin and glucose levels (Table 1).

In OB and CS, during rhGH treatment, IGF-I levels showed a pattern similar to that in NS; in fact there was prompt increase in IGF-I levels (12 h after the first administration: OB 209.8 ± 14.0 and CS 261.7 ± 24.8 μ g/l, $P < 0.01$ vs baseline), which, however, were further increased at the end of rhGH treatment (12 h after the fourth administration: OB 246.1 ± 17.1 and CS 311.0 ± 30.4 μ g/l, $P < 0.001$ vs baseline) (Fig. 1).

Adjusting by ANCOVA for basal values, rhGH-induced IGF-I levels in CS (299.4 μ g/l) were higher than in OB (279.1 μ g/l, $P < 0.01$), which, in turn, were higher ($P < 0.05$) than in NS (257.7 μ g/l) (Fig. 1).

Table 1 Mean ($\pm$ S.E.M.) hormonal and biochemical variables before and 12 h after the first and last administration of rhGH (5 μ g/kg per day for 4 days) in DB and CS patients and in NS.

Group	Measured values			Adjusted for baseline values	
	Baseline	After 1st dose	After 4th dose	After 1st dose	After 4th dose
IGFBP-3 (mg/l)					
NS	3.6 $\pm$ 0.2	4.0 $\pm$ 0.2	3.8 $\pm$ 0.2	3.5	3.5
OB	3.0 $\pm$ 0.2	3.1 $\pm$ 0.3	3.6 $\pm$ 0.2 ^{oo}	3.1	3.6
CS	2.7 $\pm$ 0.3	2.9 $\pm$ 0.3	3.1 $\pm$ 0.5	3.3	3.4
GHBP (pmol/l)					
NS	2335 $\pm$ 253	2668 $\pm$ 504	2734 $\pm$ 265	3135	3208
OB	3151 $\pm$ 471	3820 $\pm$ 477	3775 $\pm$ 448	3467	3407
CS	3134 $\pm$ 952	2419 $\pm$ 410	2550 $\pm$ 494	2293	2579
Glucose (mg/l)					
NS	81.3 $\pm$ 2.4	80.4 $\pm$ 1.3	83.2 $\pm$ 3.5	86.9	87.0
OB	89.1 $\pm$ 2.0	88.1 $\pm$ 3.2	88.3 $\pm$ 2.9	89.8	89.3
CS	105.6 $\pm$ 10.3	109.2 $\pm$ 8.1	106.1 $\pm$ 7.7	101.0	101.2
Insulin (mU/l)					
NS	10.4 $\pm$ 1.0	12.2 $\pm$ 1.0	11.3 $\pm$ 1.1	23.1	18.7
OB	24.7 $\pm$ 3.1*	30.7 $\pm$ 4.6	36.3 $\pm$ 5.0 ^{oo}	25.4	32.7
CS	24.9 $\pm$ 3.5*	28.2 $\pm$ 3.7	28.0 $\pm$ 3.7	22.6	24.2
Glucose/insulin ratio					
NS	8.3 $\pm$ 0.7	7 $\pm$ 0.6	7.8 $\pm$ 0.6	5.4	6.9
OB	5.1 $\pm$ 0.8	4.1 $\pm$ 0.7	3.3 $\pm$ 0.4 ^{oo}	4.8	3.6*
CS	4.6 $\pm$ 0.3	4.2 $\pm$ 0.3	4.1 $\pm$ 0.4	5.2	4.7*

* $P < 0.01$ vs normal subjects.° $P < 0.05$ vs baseline.°° $P < 0.05$ vs baseline.

The IGF-I/IGFBP-3 ratio showed a non-significant ($P > 0.05$) trend towards increase in all groups (84 h vs baseline: NS 73.2 ± 3.3 vs 65.7 ± 5.1 ; OB 67.0 ± 6.3 vs 60.5 ± 5.6 ; CS 123.6 ± 28.2 vs 101.4 ± 12.7).

In OB, but not in CS, IGFBP-3 and insulin levels showed a significant ($P < 0.05$) increase during rhGH treatment, which did not modify glucose levels in any group; thus, in the OB patient group a significant fall in glucose/insulin ratio was observed (Table 1).

There was no correlation between morning insulin or cortisol levels and rhGH-induced IGF-I increase. However, when the area under the curve over 84 h for insulin was taken into account a positive association with the IGF-I response to rhGH was found ($P < 0.05$). IGFBP-3 levels did not show any correlation with either insulin or cortisol levels.

Neither IGF-I nor IGFBP-3 changes after rhGH treatment were associated with GHBP or glucose levels in any group.

Figure 1 IGF-I levels before and 12 h after the first and the last administration of rhGH (5 μ g/kg per day for 4 days) in patients with simple OB or CS and in NS. The left panel reports the absolute curves of IGF-I response while the right panel reports the IGF-I response after adjusting for basal values. Arrows represent rhGH administrations. * $P < 0.05$ vs NS.

No side-effects were recorded during rhGH administration.

Discussion

The results of the present study demonstrate that very low-dose rhGH is able to increase IGF-I levels in both patients with simple OB or CS to an extent higher than in NS. In OB patients worsening of the glucose/insulin ratio was observed during treatment with low-dose rhGH.

Clear reduction of both spontaneous (9, 10) and stimulated (25, 26) GH secretion has been demonstrated in both OB and CS, though in the latter condition normal GH secretion has been recently reported by some authors (27). In spite of reduced GH secretion, IGF-I levels have been shown normal (11), high normal (28) and low normal (12, 13, and present data) in OB patients and normal or high normal in hypercortisolemic patients (14, 15, and present data).

To explain the preserved IGF-I synthesis and release despite reduced GH secretion in these pathophysiological conditions, an increase in peripheral, namely hepatic, sensitivity to GH activity has been hypothesized. However, high-affinity GHB levels, which likely reflect the peripheral GH receptor status (19), have been found unchanged in CS (14), but increased in OB by some (19), but not by others (10) including ourselves in the present study. As GHB levels are reported to be positively associated with BMI and fat mass (29), may be it mainly reflects to a major extent the adipose tissue sensitivity to GH.

It is widely accepted that IGF-I synthesis and release in man are mostly dependent on GH (2) and even an rhGH dose as low as 5 µg/kg is able to stimulate IGF-I levels in young NS (20). However, IGF-I is also dependent on the nutritional status and peripheral hormones such as insulin, cortisol, thyroid and gonadal hormones (3–7). Among them, insulin and cortisol play a major role. In fact, insulin directly stimulates IGF-I and enhances its response to GH in animals (3, 4). On the other hand, though an inhibitory effect of cortisol on IGF-I synthesis has been found *in vitro* (8), in humans *in vivo* even short-term treatment with glucocorticoids clearly stimulates IGF-I levels (5, 6). Thus, it could be anticipated that either hyperinsulinemic or hypercortisolemic states show an increase of rhGH-induced IGF-I production. Our present findings agree with the working hypothesis; in fact, treatment with a very low rhGH dose increased IGF-I levels in OB and hypercortisolemic patients to an extent higher than in controls. Thus, these results support the existence of increased hepatic sensitivity to GH in OB and in CS.

Interestingly, the highest IGF-I response to rhGH was recorded in patients with CS in whom a progressive increase was more pronounced than in OB subjects in spite of a similar degree of basal hyperinsulinism but

lower BMI. Evidence that the highest IGF-I response to rhGH was recorded in the group having intermediate BMI makes unlikely the hypothesis that administering rhGH on a weight basis biases the present results. In this context, however, also differences in body composition could have been influenced the results.

Evidence that hypercortisolemic patients show the highest IGF-I response to rhGH suggests that cortisol hypersecretion could have an additive effect with hyperinsulinism in enhancing the IGF-I response to low-dose rhGH. This hypothesis agrees with data showing the stimulatory effect of glucocorticoids on IGF-I levels in animals and in man (4, 8), but does not fit with recent findings showing that IGF-I levels are more clearly reduced in patients with visceral OB (17), in whom hyperactivity of the hypothalamo–pituitary–adrenal axis has been reported (18). In the present study, the IGF-I response to rhGH was positively associated with insulin levels during treatment while it did not correlate with either morning levels of insulin or cortisol at baseline; it is widely accepted, however, that single-point measurements do not reflect the 24 h production rate, which could have an impact on peripheral GH sensitivity.

The possibility that factors other than insulin and cortisol play a role in the enhanced GH sensitivity in OB and hypercortisolemic patients has, however, to be taken into account. In fact, alterations in androgen (30) and leptin levels could have a role. For instance, leptin levels are elevated in OB (31), and are stimulated by glucocorticoids (32), while leptin stimulates IGF-I synthesis and release in animals *in vitro* (33).

In agreement with the assumption that IGF-I is more sensitive to GH than IGFBP-3 (20, 34), in hypercortisolemic patients as well as in NS we found no significant increase in IGFBP-3 levels, which, in turn, showed a slight but significant rise in OB patients.

Another point raised by our results is that rhGH treatment worsened the glucose/insulin ratio in OB while it had only a slightly non-significant negative effect in NS and in CS patients. The well-known anti-insulin effect of GH (35) accounts for this finding, but it is noteworthy that in OB patients this effect occurs even during short-term treatment with a very low rhGH dose. The GH-induced worsening of insulin resistance could, however, be transient. In fact, in OB patients as well as in adults with severe GH deficiency long-term treatment with an rhGH dose even higher than that in our present study has been reported able to improve peripheral insulin sensitivity (36, 37).

In conclusion, short-term treatment with low-dose rhGH has an enhanced stimulatory effect on IGF-I levels in OB and, particularly, in hypercortisolemic patients. These findings suggest that hyperinsulinism and hypercortisolism enhance the hepatic sensitivity to GH in humans, though no alterations in GHB levels as a possible reflection of GH receptor status were observed.

Acknowledgements

This study was supported by Ministero dell'Università e della Ricerca Scientifica e Tecnologica and Fondazione per lo Studio delle Malattie Endocrino-Metaboliche. The authors wish to thank Prof. F. Camanni for his support and for revising the manuscript, Drs G Corneli, C Ganzaroli, R Rossetto, M Bidlingmaier and K Morrison for their participation to the study and Mrs M Taliano, Mrs R Schwaiger and Mrs S Freihöfer for their skillful technical assistance.

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Received 21 March 2000
Accepted 13 November 2000