Dose-response study of GH effects on circulating IGF-I and IGFBP-3 levels in healthy young men and women

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Ghigo, E., G. Aimaretti, M. Maccario, G. Fanciulli, E. Arvat, F. Minuto, G. Giordano, G. Delitala, and F. Camanni. Dose-response study of GH effects on circulating IGF-I and IGFBP-3 levels in healthy young men and women. *Am. J. Physiol.* 276 (*Endocrinol. Metab.* 39): E1009–E1013, 1999.—The aim of our study was to define the dose-response effect of a short-term treatment with different recombinant human growth hormone (rhGH) doses (1.25, 2.5, 5.0, 10.0, and 20.0 $\mu g \cdot kg^{-1} \cdot day^{-1}$ for 4 days) on insulin-like growth factor I (IGF-I) and insulin-like growth factor-binding protein (IGFBP)-3 levels in 21 normal young adults of both sexes. The dose of 1.25 μg/kg rhGH did not modify IGF-I levels. The dose of 2.5 μg/kg rhGH significantly increased IGF-I levels in men ($P < 0.05$) but not in women, whereas the higher doses increased IGF-I levels in both sexes ($P < 0.002$). IGFBP-3 levels were not modified by 1.25 or 2.5 μg/kg rhGH in either sex. On the other hand, 5.0 μg/kg increased IGFBP-3 levels in men ($P < 0.05$) but not in women, whereas the higher doses increased IGFBP-3 levels similarly in both sexes ($P < 0.02$). In conclusion, our results demonstrate that IGF-I and IGFBP-3 responses to rhGH are dose and sex dependent. However, IGFBP-3 is less sensitive than IGF-I to rhGH stimulation.

recombinant human growth hormone; insulin-like growth factor I; insulin-like growth factor-binding protein-3; insulin-like growth factor I generation test

GROWTH HORMONE (GH) is the major hormonal regulator of insulin-like growth factor I (IGF-I; see Ref. 10). In fact, serum IGF-I levels reflect the GH secretory status, being low in GH deficiency and elevated in acromegalic patients (4, 28). However, nutritional impairment leads to peripheral GH resistance, with low IGF-I levels in spite of elevated GH levels (10).

The evaluation of the IGF-I response to exogenous GH administration was proposed in the investigation of short stature (5, 8, 13, 25). However, the IGF-I generation test has never been defined in terms of GH dose, length of treatment, and timing in IGF-I assay as well as of normative values. Particularly, it is still unknown what is the lowest GH dose able to increase IGF-I levels in normal young subjects. As in GH-deficient adults, the increase in IGF-I levels during GH replacement was reported to be higher in males than in females (21), and whether the stimulatory effect of recombinant human GH (rhGH) on IGF-I levels is dependent on gender has to be verified in normal subjects. Interestingly, although basal IGF-I levels are similar in both sexes, spontaneous GH secretion over 24 h is clearly higher in young women than in men (20).

The assessment of the normative IGF-I responses to rhGH is fundamental to verify the possible changes in GH sensitivity during life span and in various pathophysiological conditions such as GH deficiency, obesity, malnutrition, catabolic states, Cushing's syndrome, and dilated cardiomyopathy. To this purpose, aging in male and oral estrogens therapy in postmenopausal women has been reported, accompanied by a reduced sensitivity to GH (24).

Hence, the aim of our study was to define the dose-response effect of a short-term treatment with different rhGH doses on IGF-I and insulin-like growth factor-binding protein (IGFBP)-3 levels in normal young adults of both sexes. Specifically, we aimed to define the lowest rhGH dose able to increase IGF-I and IGFBP-3 levels. The effects of rhGH administration on GH, insulin, glucose, free-triiodothyronine (fT_3), and free-thyoxine (fT_4) levels were also studied.

SUBJECTS AND METHODS

Twenty-one Caucasian normal healthy young adult volunteers [12 males [age ± SE: 30.5 ± 2.7 yr; body mass index (BMI): 22.7 ± 0.5 kg/m^2] and 9 female (age: 30.5 ± 1.3 yr; BMI: 21.0 ± 0.5 kg/m^2)] were studied. In females, the study was performed only in the early follicular phase. All subjects gave informed consent to enter the study, which had been approved by the Ethical Committee of the University of Turin.

All subjects underwent six tests with different rhGH doses (Genotropin vials, 4 IU = 1.33 mg in 1 ml, 1.25, 2.5, 5.0, 10.0, and 20.0 μg/kg; Pharmacia, Stockholm, Sweden) or placebo (0.9% saline solution) given subcutaneously every evening at 2100 for 4 days. Each test was performed in random order at least 1 mo apart. Blood samples for hormonal and biochemical assays were drawn after an overnight fast, basally, and 12 h after each rhGH administration. A blood sample was also taken 24 h after the last rhGH administration. IGF-I, IGFBP-3, GH, insulin, and glucose were assayed at each time point. fT_3 and fT_4 were assayed basally and 12 h after the last administration.

Serum IGF-I was measured by RIA (Nicholls Institute) after acid-ethanol extraction to avoid interference by binding proteins. The sensitivity of the assay was 0.013 nmol/l. The inter- and intra-assay coefficients of variation were 5.2–8.4% and 2.4–3.0%, respectively. Serum IGFBP-3 was measured by RIA (Nicholls Institute). The sensitivity of the assay was 0.008 nmol/l. The inter- and intra-assay coefficients of variation were 5.3–6.3% and 3.4–8.0%, respectively. Serum GH

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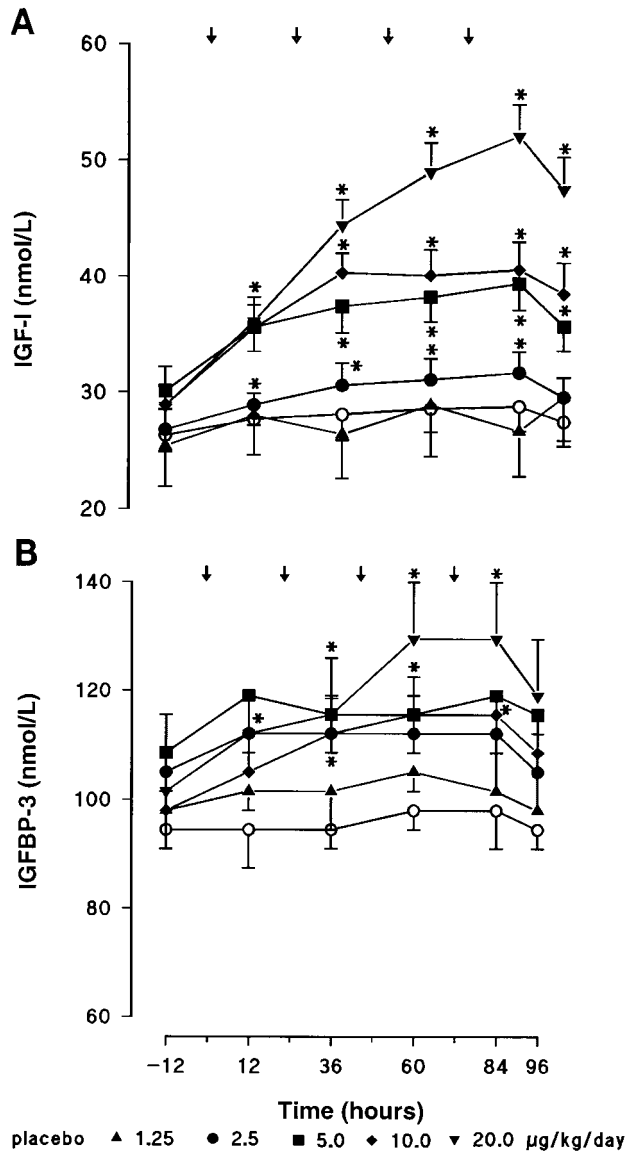

Fig. 1. Dose-related effect of 4-day treatment with recombinant human growth hormone (rhGH) on insulin-like growth factor I (IGF-I) and insulin-like growth factor-binding protein (IGFBP)-3 levels in 21 normal young subjects (*$P < 0.001$ vs. baseline). ↓rhGH administration.

and insulin were measured in duplicate by immunoradiometric assay (HGH-CTK IRMA and INSIK-5; Sorin, Saluggia, Italy). The sensitivity of the assay was 0.15 μg/l for GH and 2.5 μU/l for insulin. The inter- and intra-assay coefficients of variation were 4.9–6.5% and 1.5–2.9% for GH and 6.5–15.0% and 4.5–13.4% for insulin, respectively. Serum fT_3 and fT_4 were measured by RIA (Amerlex-MAB; Johnson & Johnson Clinical Diagnostic). The sensitivity of the assay was 0.5 pmol/l for fT_3 and 0.6 pmol/l for fT_4. The inter- and intra-assay coefficients of variation were 6.5–9.8% and 3.5–5.8% for fT_3 and 5.0–15.0% and 3.7–6.5% for fT_4, respectively. Plasma glucose was determined by the glucose oxidase colorimetric method (GLUCOFIX; Menarini Diagnostics, Firenze, Italy).

Data are expressed, either in absolute values or in incremental area under the curve, as means ± SE. The statistical analysis of the data was carried out by ANOVA and paired and unpaired Student's *t*-test when appropriate.

RESULTS

Mean basal GH, IGF-I, and IGFBP-3 levels were 4.0 ± 0.8 μg/l, 27.7 ± 0.7 nmol/l, and 101.5 ± 3.5 nmol/l, respectively, and did not significantly differ among various testing sessions. GH levels were higher in women than in men (6.6 ± 2.1 vs. 0.5 ± 0.3 μg/l, $P <$ 0.001), whereas no sex difference was shown in IGF-I and IGFBP-3 levels.

Placebo and the dose of 1.25 μg/kg rhGH failed to modify IGF-I levels at any time. On the other hand, the values significantly increased 12 h after the first administration of 2.5, 5.0, 10.0, and 20.0 μg/kg rhGH (IGF-I level at 12 h vs. basal: 28.9 ± 1.7 vs. 26.8 ± 1.9, 35.6 ± 2.1 vs. 30.1 ± 2.1, 35.5 ± 2.0 vs. 28.9 ± 1.6, and 36.1 ± 2.1 vs. 28.8 ± 1.8 nmol/l, respectively, $P < 0.001$). However, the increases in IGF-I levels observed after 5.0, 10.0, and 20.0 μg/kg rhGH were higher ($P < 0.001$) than that recorded after the 2.5 μg/kg rhGH dose (Fig. 1).

After the second, third, and fourth administration of 2.5, 5.0, 10.0, and 20.0 μg/kg rhGH, IGF-I levels further increased, showing a clear dose-response relationship ($P < 0.001$). Twenty-four hours after the last administration of each rhGH dose, IGF-I levels were decreased but still higher than basal levels ($P < 0.007$).

The dose of 1.25, 2.5, and 5.0 μg/kg rhGH failed to change IGFBP-3 levels at any time. IGFBP-3 levels were increased after the first administration of 20.0 μg/kg (112.0 ± 7.0 vs. 101.5 ± 7.0 nmol/l, $P < 0.02$) but not after 10.0 μg/kg rhGH. On the other hand, IGFBP-3 levels increased after the second, third, and fourth administration of both 10.0 ($P < 0.01$) and 20.0 μg/kg rhGH ($P < 0.02$-$P < 0.001$; Fig. 1).

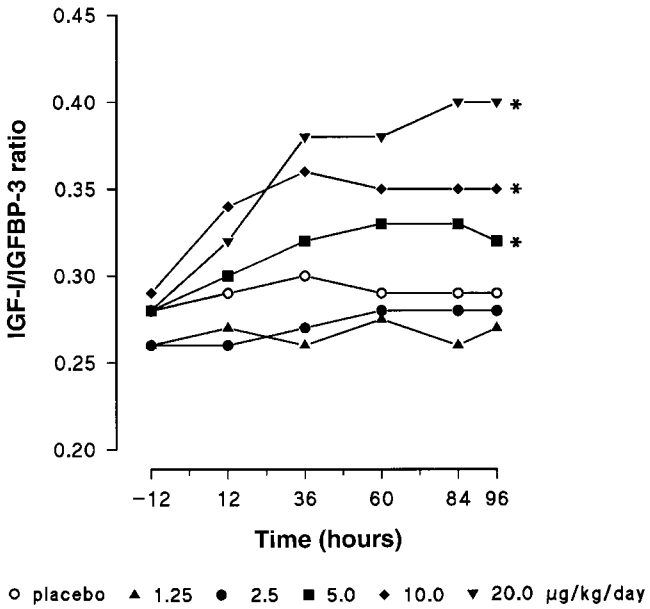

Fig. 2. Changes in IGF-I-to-IGFBP-3 ratio after 4-day treatment with various rhGH doses in 21 normal young subjects (*$P < 0.01$ vs. placebo).

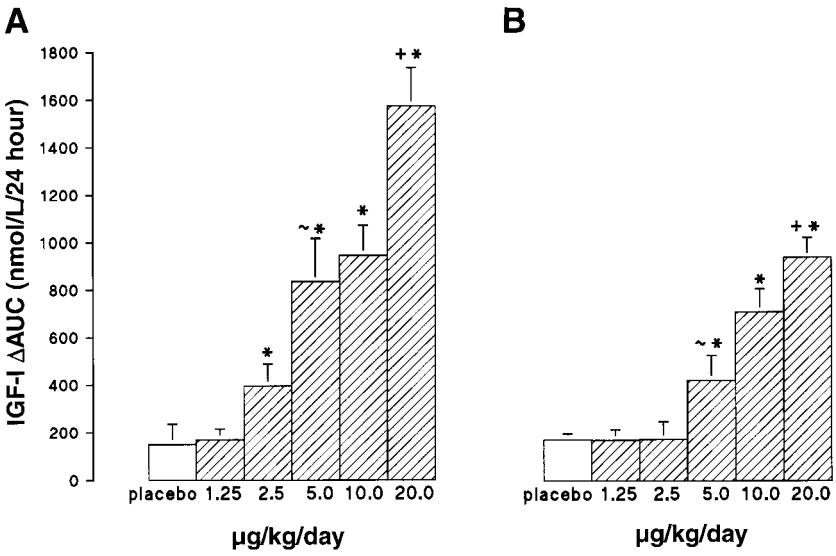

Fig. 3. Dose-related effect of 4-day treatment with rhGH on IGF-I levels in 12 normal men (*A*) and 9 normal women (*B*; $*P < 0.001$ vs. placebo; $\sim P < 0.05$ and $+P < 0.004$, males vs. females). ΔAUC, change in area under the curve.

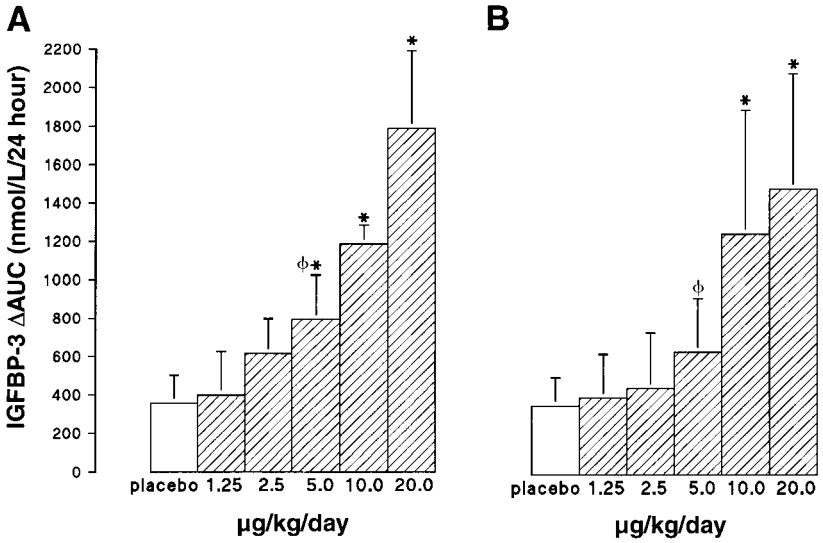

Fig. 4. Dose-related effect of 4-day treatment with rhGH on IGFBP-3 levels in 12 normal men (*A*) and 9 normal women (*B*; $*P < 0.05$ vs. placebo; $\phi P < 0.05$, males vs. females).

Table 1. *Insulin, glucose, fT_3, and fT_4 levels before and 84 h after the first administration of different rhGH doses*

	Insulin, μU/l		Glucose, mg/dl		fT_3, ng/dl		fT_4, ng/dl	
	Basal	84 h	Basal	84 h	Basal	84 h	Basal	84 h
Saline	10.6 ± 1.3	11.3 ± 1.5	80.6 ± 2.0	81.4 ± 1.8	2.8 ± 0.3	3.1 ± 0.2	12.8 ± 1.5	14.1 ± 1.3
rhGH doses, $\mu g \cdot kg^{-1} \cdot day^{-1}$								
1.25	9.5 ± 1.1	11.5 ± 1.5	82.3 ± 1.5	81.7 ± 2.5	2.6 ± 0.3	3.3 ± 0.2	13.9 ± 1.2	13.6 ± 1.2
2.5	10.5 ± 1.2	12.6 ± 1.8	83.5 ± 1.7	83.8 ± 2.1	2.9 ± 0.3	3.2 ± 0.2	14.0 ± 3.4	14.1 ± 3.2
5.0	13.0 ± 1.3	11.6 ± 0.8	82.6 ± 2.5	83.6 ± 2.1	3.4 ± 0.3	3.0 ± 0.5	14.4 ± 2.4	14.1 ± 1.5
10.0	11.5 ± 1.4	13.6 ± 2.5	79.5 ± 1.5	82.1 ± 2.1	2.7 ± 0.3	2.9 ± 0.5	12.3 ± 1.1	13.6 ± 1.2
20.0	13.1 ± 2.0	16.5 ± 2.4	82.5 ± 2.9	86.2 ± 2.5	2.6 ± 0.4	3.1 ± 0.6	13.1 ± 1.2	12.9 ± 1.3

Data are means ± SE. rhGH, recombinant human growth hormone; fT_3 and fT_4, free-3,5,3′-triiodothyronine and -thyroxine, respectively.

The ratio of IGF-I over IGFBP-3 (Fig. 2) was significantly increased during GH treatment with the dose of 5.0, 10.0, and 20.0 $\mu g \cdot kg^{-1} \cdot day^{-1}$.

When the data, evaluated as changes in area under the curve ($nmol \cdot l^{-1} \cdot 24\ h^{-1}$) from baseline to 84 h, are examined dividing subjects by sex, the dose of 2.5 μg/kg rhGH significantly stimulated IGF-I levels in men ($P < 0.05$) but not in women. The higher doses increased IGF-I levels in both sexes ($P < 0.001$), but the IGF-I responses to the administration of 5.0, 10.0, and 20.0 μg/kg rhGH were lower in women than in men. However, this difference attained statistical significance only after the 5.0 and 20.0 μg/kg rhGH doses ($P < 0.05$ and 0.0004, respectively; Fig. 3). IGFBP-3 levels were not modified by 1.25 and 2.5 μg/kg rhGH in either sex. On the other hand, 5.0 μg/kg rhGH increased IGFBP-3 levels in men ($P < 0.05$) but not in women, whereas the higher rhGH doses similarly increased IGFBP-3 levels in both sexes ($P < 0.01$; Fig. 4).

All rhGH doses did not significantly modify fasting GH (data not reported), glucose, insulin, fT_3, and fT_4 levels (Table 1).

DISCUSSION

The results of our study in normal humans demonstrate first that the lowest rhGH doses effective to induce an increase in IGF-I and IGFBP-3 levels are 2.5 and 5.0 μg/kg, respectively. Moreover, the IGF-I-releasing effect of various rhGH doses shows a clear dose-response relationship.

The stimulatory effects of rhGH on IGF-I and IGFBP-3 levels in normal subjects have been assessed before by Skjaerbaek and co-workers (26), who, however, used two rhGH doses markedly higher than those employed in our study.

Indeed, the minimal rhGH dose that we found able to increase IGF-I levels in normal subjects is much lower than that usually proposed for IGF-I generation tests (5, 8, 13). Moreover, the lowest effective dose of rhGH administered in our study is very close to the daily GH production rate estimated in normal young adults (29).

Thus testing with this dose is fundamental to verify the possible changes in hepatic GH sensitivity during life span and in various pathophysiological conditions, such as GH deficiency, obesity, malnutrition, catabolic states, Cushing's syndrome, and dilated cardiomyopathy.

On the other hand, the GH dose needed for replacement in severe GH-deficient adults still seems really low (3, 14, 15, 21).

Interestingly, our data demonstrate that the IGF-I and IGFBP-3 response to rhGH is dependent on gender; in fact, the lowest effective rhGH dose is higher in women than in men.

This evidence indicates that the peripheral GH sensitivity in men is higher than that in women. In agreement with our findings, it has been recently reported that, in GH-deficient adults, the IGF-I increase during GH chronic treatment was higher in men than in women (9, 14, 15, 21). The existence of a sex-dependent effect of rhGH on IGF-I synthesis and release is not surprising. In fact, although basal IGF-I levels are generally reported to be similar in both sexes (18, 23), spontaneous GH secretion over 24 h is higher in young women than in men (17, 20).

The GH-receptor number is probably gender independent, as indicated by evidence that GH-binding protein levels, a marker of GH-receptor status, in men and women are similar (2). On the other hand, there is evidence indicating that estradiol is able to reduce IGF-I levels, likely impairing the postreceptor mechanisms underlying the stimulatory effect of GH on IGF-I synthesis and release (22, 30).

Our findings also demonstrate that IGFBP-3 is less sensitive than IGF-I to rhGH stimulation, in agreement with previous results in GH-deficient adults (14, 15). Actually, IGFBP-3 synthesis and release depend on GH but probably also on IGF-I (4, 11, 12, 27). This could also explain why the timing of the IGFBP-3 response is delayed with respect to that of IGF-I.

The evidence that IGFBP-3 is less sensitive than IGF-I to the stimulation by rhGH implies that the assay of IGF-I is more reliable than that of IGFBP-3 for investigating the GH secretory status and monitoring the adequacy of GH therapy in GH-deficient patients (19); notice also that IGF-I more than IGFBP-3 reflects the GH status in acromegalic patients (10, 28).

Indeed IGFBP-3 is also less sensitive than IGF-I to the physiological increase and decrease in GH secretion that occurs during puberty and aging, respectively (6, 7, 10).

Interestingly, an rhGH dose able to increase IGF-I but not IGFBP-3 levels also results in an increase in the IGF-I-to-IGFBP-3 ratio, which reflects an increase in the free, biologically active IGF-I (14, 16).

Finally, in spite of evidence that chronic rhGH treatment influences insulin and glucose levels as well as the thyroxine-to-3,5,3′-triiodothyronine conversion ratio both in normal and GH-deficient subjects (1, 15), we did not find any effect on these parameters. This could be due to the short-term treatment performed in our study. Alternatively, this evidence suggests that the previously reported effects were due to high rhGH doses.

In conclusion, the results of the present study in normal young adults demonstrate that the IGF-I and IGFBP-3 responses to rhGH are dose and gender dependent. Moreover, the minimum rhGH dose able to increase IGF-I and IGFBP-3 levels is unexpectedly low, and IGFBP-3 is less sensitive than IGF-I to rhGH stimulation.

We thank Dr. J. Bellone, G. Corneli, and J. Ramunni for cooperation.

This study was performed under the auspices of the Italian Society of Endocrinology, Study Group for Growth Hormone Secretion During Life Span, and was supported (40%) by Grant 9706151106 from Ministero Universitá e Ricerca Scientifica Tecnologica.

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Received 20 October 1998; accepted in final form 23 February 1999.

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