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# Insulin-like growth factor-1 and growth hormone (GH) have distinct and overlapping anabolic effects in GH-deficient rats

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**The anabolic activity of recombinant human growth hormone (rhGH) and insulin-like growth factor 1 (rhIGF-1) given either alone or together were studied in two models of GH deficiency, hypophysectomized and GH-deficient dwarf rats. A range of rhGH doses (0.08 to 50 mg/kg/day, seven daily sc injections) were given either alone or together with one dose of rhIGF-I (2.4 mg/kg/day, sc infusion). When given alone, or co-administered with rhIGF-1, rhGH produced dose dependent increases in weight gain, bone growth and organ weights. Weight gain in response to rhGH given with rhIGF-1 was comparable to that obtained by a 25-fold higher dose of rhGH given alone. In both animal models absolute weights of the kidneys, liver, spleen and thymus were increased by rhIGF-1 while kidney and liver weight were increased by rhGH. In the hypophysectomized rat, spleen and thymus weights were increased by rhGH but the relative potency of the combination was a 1000-fold that of rhGH alone. The effects of rhIGF-1 and rhGH were additive indicating that the effects of GH or IGF-1 can be greatly increased by their co-administration.**

**Keywords:** IGF-1; GH; additive; anabolic; GH-deficiency

## Introduction

The pituitary gland regulates whole body growth, and in particular the growth of the skeleton, by producing growth hormone (GH) in animals and in man. Many of the somatogenic effects of GH are believed to be mediated, at least in part, by the somatomedins (Salmon and Daughaday, 1957), especially by insulin-like growth factor-1 (IGF-1), whose production in many tissues is regulated by GH. Treatment of GH-deficient rats with either recombinant human GH (rhGH) or recombinant human IGF-1 (rhIGF-1) can increase the growth rate toward that of a normal animal, indicating their important somatogenic activity (Moore *et al.*, 1988). However, it is now becoming clear that GH and IGF-1 have not only overlapping but also distinct biological activities.

Other investigators have studied the effects of co-treatment with GH and IGF-1 in the rat and found no additive effects on body weight gain (Skottner *et al.*, 1987) or bone lengthening (Isgaard *et al.*, 1986; Skottner *et al.*, 1987). In mice (Pell & Bates, 1992) an increased activity of the combination of IGF-1 and GH on some measures of metabolism was reported. We have recently shown that the growth responses to low doses of rhGH in hypophysectomized rats are increased if rhIGF-1 is co-administered (Clark *et al.*, 1994).

To test if the additive effects of rhIGF-1 and rhGH on body growth occurred over a broad range of doses of rhGH, or if additivity was restricted to low doses of rhGH, we have now given several doses of rhGH with or without a fixed dose of rhIGF-1 to two animal models of GH deficiency, the hypophysectomized rat and a mutant dwarf rat.

## Results

### *Body weight gain*

In both studies, all doses of rhGH produced a maintained weight gain. Likewise, rhIGF-1 produced a significant body weight gain that was first recognized on Day 1 of dosing. Figure 1 shows the mean body weight gains on Day 7 plotted against the logarithm of rhGH dose for the hypophysectomized rats. Over this range of rhGH doses, whether rhGH was given alone or in combination with rhIGF-1, there was a linear relationship between the logarithm of rhGH dose and weight gain. The combination of rhGH plus rhIGF-1 gave greater weight gains than either hormone alone, and this appeared to be additive for all doses of rhGH. Excipient treated hypophysectomized rats gained 4.5 ± 1.7 g, while rhIGF-1 at 2.4 mg/kg/day resulted in a weight gain of 18.2 ± 2.0 g. For rhGH (at 0.08, 0.4, 2, 10 and 50 mg/kg/day) the mean weight gains were for rhGH alone, 14.5, 20.5, 26.0, 32.6 and 36.1 g, and for rhGH plus rhIGF-1 the gains were

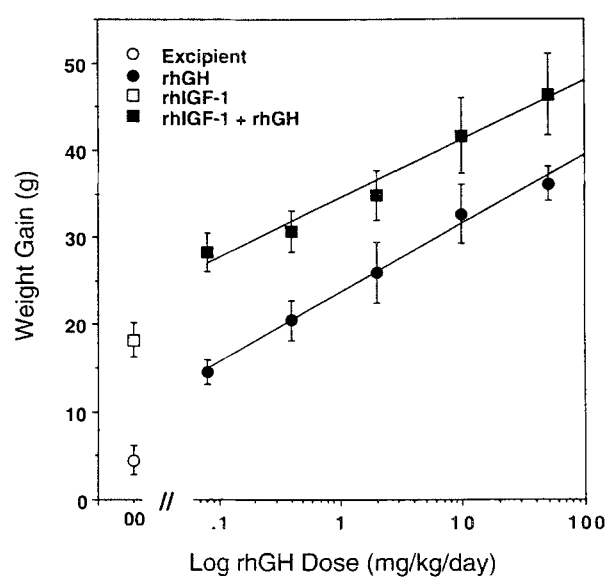


**Figure 1** Weight gain in hypophysectomized rats induced by treatment with excipient, rhIGF-1, rhGH, or rhIGF-1 plus rhGH for 7 days. rhGH (0.08, 0.4, 2, 10 and 50 mg/kg/day) was given alone or with rhIGF-1 (2.4 mg/kg/day). The means and standard deviations are presented ($n = 6$ rats per group)

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28.2, 30.7, 34.8, 41.6 and 46.3 g, respectively, for the 7 days. So at each dose of rhGH there was approximately a 10 gram greater weight gain if rhIGF-1 was also administered.

The weight gain responses to rhGH or rhGH plus rhIGF-1 were analysed as a parallel line bioassay against the logarithm of the dose of rhGH. The two dose response lines fulfilled the criteria for a bioassay, as they proved to be statistically linear and parallel. The potency of the rhGH plus rhIGF-1 was 26.6 times that of the rhGH alone (95% confidence limits 14.8 to 51.7), with the difference between the two dose response lines being highly significant (1.49 degrees of freedom [d.f.], $F = 169.4$, $P < 0.0001$).

Figure 2 and Table 1 show the weight gains of the dwarf rats treated with rhGH and rhGH plus rhIGF-1 for 7 days. The excipient control group of dwarf rats gained the expected small amount of weight (3.9 ± 3.6 g) during the experiment. rhIGF-1 at 2.4 mg/kg/day caused significant weight gain (12.1 ± 3.8 g). The mean body weight gain was increased by rhGH in a dose dependent manner. In dwarf rats, as for hypophysectomized rats, the combination of rhGH plus rhIGF-1 yielded greater body weight gains than either hormone alone, and the effects appeared to be additive over a broad range of doses of rhGH.

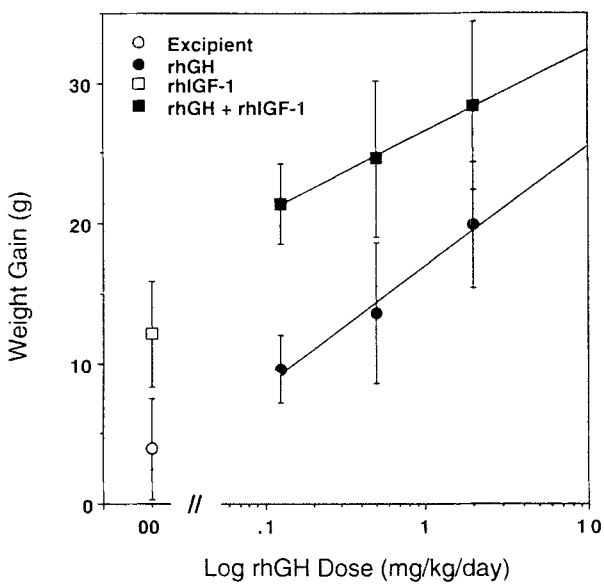


**Figure 2** Weight gain in dwarf rats induced by treatment with excipient, rhIGF-1, rhGH, or rhIGF-1 plus rhGH for 7 days. rhGH (0.125, 0.5, 2 mg/kg/day) was given alone or with rhIGF-1 (2.4 mg/kg/day). The means and standard deviations are presented ($n = 6$ rats per group)

The weight gain responses to rhGH or rhGH plus rhIGF-1 were again analysed as a parallel line bioassay against log dose of rhGH. The two dose response lines fulfilled the criteria for a bioassay, as they proved to be statistically linear and parallel. The relative potency of the rhGH plus rhIGF-1 was 28.9 times that of the rhGH alone (95% confidence limits 7.7 to 514.6), with the difference between the two dose response lines being highly significant (1.30 d.f., $F = 45.75$, $P < 0.001$).

The analysis of the weight gain data in Table 1 confirms that there were clear effects of both rhIGF-1 and rhGH on weight gain, while the lack of a significant interaction between the effects of rhIGF-1 and rhGH again suggests that these effects were additive.

*Bone growth*

Table 1 shows the mean epiphyseal plate width and longitudinal bone growth in the dwarf rats given rhGH and/or rhIGF-1. Both rhIGF-1 and rhGH significantly increased these measures of tibial growth, while the lack of an interaction between the treatments again indicates an additive effect.

*Serum IGF-1 concentration*

Table 1 also shows the serum IGF-1 concentrations in the dwarf rats in blood samples taken at sacrifice (24 h after the last rhGH injection). There were clear effects of both rhIGF-1 and rhGH treatment, and no interaction between the treatments. Treatment with rhIGF-1 increased serum IGF-1 concentrations in the presence and the absence of rhGH treatment. There were decreased IGF-1 concentrations whether the rhGH was given alone or together with rhIGF-1.

*Organ weights*

Table 2 shows the absolute organ weights and the relative organ weights for the dwarf rats. The relative organ weights are expressed as a percentage of body weight multiplied by 10. It is clear that in the dwarf rat the increased body weight caused by rhIGF-1 was associated with increased weights of several internal organs. In contrast, rhGH treatment only increased the absolute weights of the kidney and liver. The relative weights of the kidneys, liver, spleen and thymus were increased by rhIGF-1. The relative weight of the liver was

**Table I** Body growth parameters in dwarf rats

| *Group (dose mg/kg/day)* | *Body weight gain (g)* | *Plate width (μm)* | *Bone growth (μm/day)* | *Serum IGF-1 (ng/ml)* |
|---|---|---|---|---|
| Excipient | 3.9 ± 3.6 | 193 ± 26 | 31 ± 16 | 151 ± 64 |
| rhGH (0.125) | 9.6 ± 2.4 | 201 ± 29 | 33 ± 17 | 88 ± 16 |
| rhGH (0.5) | 13.6 ± 5.0 | 237 ± 21 | 58 ± 19 | 90 ± 49 |
| rhGH (2.0) | 19.9 ± 4.5 | 244 ± 20 | 51 ± 14 | 80 ± 17 |
| rhIGF-1 (2.4) | 12.1 ± 3.8 | 223 ± 21 | 46 ± 11 | 291 ± 63 |
| rhGH (0.125) + rhIGF-1 | 21.4 ± 2.9 | 257 ± 15 | 63 ± 17 | 242 ± 67 |
| rhGH (0.5) + rhIGF-1 | 24.6 ± 5.6 | 261 ± 31 | 61 ± 23 | 213 ± 37 |
| rhGH (2.0) + rhIGF-1 | 28.4 ± 6.0 | 256 ± 12 | 66 ± 15 | 226 ± 21 |
| | | **P** *values* | | |
| Effect of rhIGF-1 | 0.001 | 0.001 | 0.003 | 0.001 |
| Effect of rhGH | 0.001 | 0.001 | 0.014 | 0.002 |
| Interaction | 0.684 | 0.157 | 0.298 | 0.869 |

Weight gain, tibial epiphyseal plate width, longitudinal growth of the tibia and total serum IGF-1 at sacrifice in dwarf rats treated for 7 days with excipient, rhGH, rhIGF-1 or rhGH plus rhIGF-1 (2.4 mg/kg/day). Table shows the means ± SD's ($n = 6$/group), the lower portion shows the $P$ values from the 2 way ANOVA of these data

increased by rhGH treatment. There were no significant interactions between the treatments suggesting that these effects of rhIGF-1 and rhGH were independent of one another. So in dwarf rats, rhIGF-1 had a greater effect than did rhGH on the absolute and relative growth of the spleen, thymus and kidney, while the liver was affected by both agents.

Table 3 shows the absolute organ weights, and Table 4 shows the relative organ weights, for the hypophysectomized rats. Both rhIGF-1 and rhGH significantly increased the absolute size of every organ weighed. rhIGF-1 increased the relative weights of the kidney, spleen and thymus, but did not significantly affect relative heart and liver size. In contrast, rhGH showed a different spectrum of activity. The relative size of the spleen, thymus and liver were all increased by rhGH, an effect on the heart is also suggested ($P = 0.052$). The relative size of the kidney was not affected by rhGH treatment. These results are consistent with the results in the dwarf rat, as there were no statistically significant interactions between the effects of rhGH and rhIGF-1 for either absolute or relative measures of organ weight.

Figure 3 illustrates the effects of rhGH and rhIGF-1 on the growth of the spleen in hypophysectomized rats. The combination of rhIGF-1 and the highest dose of rhGH gave a

**Table 2** Absolute and relative organ weights in dwarf rats

| *Group (dose mg/kg/day)* | *Spleen* (mg) | *Thymus* (mg) | *Absolute weights* *Kidneys* (g) | *Heart* (mg) | *Liver* (g) |
|---|---|---|---|---|---|
| Excipient | 305 ± 24 | 181 ± 55 | 1.03 ± 0.10 | 532 ± 92 | 4.84 ± 0.53 |
| rhGH (0.125) | 345 ± 43 | 206 ± 65 | 1.10 ± 0.05 | 619 ± 156 | 5.26 ± 0.40 |
| rhGH (0.5) | 369 ± 64 | 246 ± 40 | 1.14 ± 0.12 | 615 ± 132 | 5.46 ± 0.82 |
| rhGH (2.0) | 395 ± 64 | 208 ± 46 | 1.12 ± 0.04 | 586 ± 64 | 6.04 ± 0.77 |
| rhIGF-1 (2.4) | 665 ± 197 | 255 ± 51 | 1.40 ± 0.12 | 664 ± 89 | 5.34 ± 0.53 |
| rhGH (0.125) + rhIGF-1 | 612 ± 80 | 280 ± 46 | 1.42 ± 0.05 | 588 ± 58 | 6.18 ± 0.30 |
| rhGH (0.5) + rhIGF-1 | 684 ± 39 | 292 ± 79 | 1.54 ± 0.05 | 750 ± 255 | 6.13 ± 0.22 |
| rhGH (2.0) + rhIGF-1 | 662 ± 80 | 278 ± 67 | 1.48 ± 0.07 | 633 ± 37 | 6.66 ± 0.65 |
| | | | P *values* | | |
| Effect of rhIGF-1 | 0.001 | 0.001 | 0.001 | 0.065 | 0.001 |
| Effect of rhGH | 0.379 | 0.202 | 0.010 | 0.343 | 0.001 |
| Interaction | 0.526 | 0.918 | 0.715 | 0.348 | 0.827 |
| | | | *Relative weights (% Bwt × 10)* | | |
| | *Spleen* | *Thymus* | *Kidneys* | *Heart* | *Liver* |
| Excipient | 2.3 ± 0.1 | 1.4 ± 0.4 | 7.9 ± 0.5 | 4.0 ± 0.4 | 37 ± 2 |
| rhGH (0.125) | 2.5 ± 0.3 | 1.5 ± 0.5 | 8.1 ± 0.6 | 4.5 ± 1.1 | 39 ± 2 |
| rhGH (0.5) | 2.6 ± 0.2 | 1.8 ± 0.3 | 8.1 ± 0.4 | 4.3 ± 0.6 | 39 ± 3 |
| rhGH (2.0) | 2.7 ± 0.2 | 1.4 ± 0.3 | 7.7 ± 0.9 | 4.0 ± 0.1 | 41 ± 3 |
| rhIGF-1 (2.4) | 4.7 ± 1.2 | 1.8 ± 0.4 | 10.0 ± 0.9 | 4.8 ± 0.9 | 38 ± 1 |
| rhGH (0.125) + rhIGF-1 | 4.2 ± 0.3 | 1.9 ± 0.4 | 9.7 ± 0.7 | 4.0 ± 0.5 | 42 ± 2 |
| rhGH (0.5) + rhIGF-1 | 4.6 ± 0.4 | 2.0 ± 0.6 | 10.3 ± 0.8 | 5.0 ± 1.6 | 41 ± 3 |
| rhGH (2.0) + rhIGF-1 | 4.3 ± 0.4 | 1.8 ± 0.5 | 9.6 ± 0.9 | 4.1 ± 0.4 | 43 ± 3 |
| | | | P *values* | | |
| Effect of rhIGF-1 | 0.001 | 0.005 | 0.001 | 0.321 | 0.003 |
| Effect of rhGH | 0.699 | 0.423 | 0.375 | 0.351 | 0.001 |
| Interaction | 0.204 | 0.893 | 0.730 | 0.251 | 0.713 |

The absolute and relative wet weights of the spleen, thymus, kidneys, heart and liver obtained at sacrifice are shown in dwarf rats treated for 7 days with excipient, rhGH, rhIGF-1 or rhGH plus rhIGF-1 (2.4 mg/kg/day). The upper portion of the Table shows the means ± SD's ($n = 6$/group), the lower portion shows the P values from the two way ANOVA of these data

**Table 3** Absolute organ weights in hypophysectomized rats

| *Group (dose mg/kg/day)* | *Spleen* (mg) | *Thymus* (mg) | *Absolute weights* *Kidneys* (g) | *Heart* (mg) | *Liver* (g) |
|---|---|---|---|---|---|
| Excipient | 206 ± 24 | 296 ± 61 | 704 ± 58 | 384 ± 44 | 4.42 ± 0.41 |
| rhGH (0.08) | 238 ± 25 | 313 ± 105 | 777 ± 69 | 416 ± 34 | 4.46 ± 0.34 |
| rhGH (0.40) | 278 ± 13 | 409 ± 75 | 847 ± 57 | 428 ± 70 | 4.91 ± 0.24 |
| rhGH (2.0) | 320 ± 22 | 404 ± 64 | 847 ± 47 | 414 ± 28 | 5.28 ± 0.25 |
| rhGH (10.0) | 346 ± 66 | 468 ± 79 | 913 ± 69 | 499 ± 70 | 5.47 ± 0.37 |
| rhGH (50.0) | 385 ± 49 | 541 ± 133 | 984 ± 40 | 440 ± 19 | 5.90 ± 0.52 |
| rhIGF-1 (2.4) | 441 ± 68 | 494 ± 67 | 1029 ± 71 | 454 ± 49 | 5.00 ± 0.59 |
| rhGH (0.08) + rhIGF-1 | 479 ± 55 | 617 ± 66 | 1107 ± 52 | 474 ± 42 | 5.30 ± 0.29 |
| rhGH (0.40) + rhIGF-1 | 442 ± 35 | 522 ± 80 | 1083 ± 40 | 449 ± 34 | 5.14 ± 0.67 |
| rhGH (2.0) + rhIGF-1 | 480 ± 20 | 573 ± 73 | 1201 ± 97 | 501 ± 104 | 5.57 ± 0.70 |
| rhGH (10.0) + rhIGF-1 | 612 ± 95 | 677 ± 110 | 1276 ± 111 | 582 ± 94 | 5.91 ± 0.57 |
| rhGH (50.0) + rhIGF-1 | 619 ± 128 | 746 ± 184 | 1311 ± 114 | 523 ± 60 | 6.65 ± 0.85 |
| | | | P *values* | | |
| Effect of rhIGF-1 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| Effect of rhGH | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| Interaction | 0.185 | 0.325 | 0.352 | 0.756 | 0.649 |

The absolute wet weights of the spleen, thymus, kidneys, heart and liver obtained at sacrifice are shown in hypophysectomized rats treated for 7 days with excipient, rhGH, rhIGF-1 or rhGH plus rhIGF-1 (2.4 mg/kg/day). The upper portion of the Table shows the means ± SD's ($n = 6$/group), the lower portion shows the $P$ values from the two way ANOVA of these data

**Table 4** Relative organ weights in hypophysectomized rats

| *Group (dose mg/kg/day)* | *Absolute weights (% Bwt × 10)* | | | | |
|---|---|---|---|---|---|
| | *Spleen* | *Thymus* | *Kidneys* | *Heart* | *Liver* |
| Excipient | 2.1 ± 0.2 | 3.1 ± 0.6 | 7.3 ± 0.4 | 4.0 ± 0.4 | 46 ± 3 |
| rhGH (0.08) | 2.2 ± 0.2 | 2.9 ± 0.9 | 7.3 ± 0.5 | 3.9 ± 0.3 | 42 ± 2 |
| rhGH (0.40) | 2.5 ± 0.1 | 3.6 ± 0.7 | 7.5 ± 0.4 | 3.8 ± 0.7 | 44 ± 2 |
| rhGH (2.0) | 2.7 ± 0.2 | 3.4 ± 0.5 | 7.2 ± 0.3 | 3.5 ± 0.2 | 45 ± 1 |
| rhGH (10.0) | 2.8 ± 0.5 | 3.8 ± 0.6 | 7.3 ± 0.5 | 4.0 ± 0.6 | 44 ± 2 |
| rhGH (50.0) | 3.0 ± 0.3 | 4.2 ± 1.0 | 7.7 ± 0.3 | 3.4 ± 0.2 | 46 ± 4 |
| rhIGF-1 (2.4) | 4.0 ± 0.5 | 4.5 ± 0.7 | 9.3 ± 0.4 | 4.1 ± 0.4 | 45 ± 4 |
| rhGH (0.08) + rhIGF-1 | 4.0 ± 0.3 | 5.1 ± 0.6 | 9.2 ± 0.5 | 4.0 ± 0.4 | 44 ± 3 |
| rhGH (0.40) + rhIGF-1 | 3.6 ± 0.3 | 4.3 ± 0.7 | 8.8 ± 0.5 | 3.7 ± 0.3 | 42 ± 5 |
| rhGH (2.0) + rhIGF-1 | 3.8 ± 0.3 | 4.5 ± 0.5 | 9.4 ± 0.6 | 3.9 ± 0.8 | 44 ± 3 |
| rhGH (10.0) + rhIGF-1 | 4.6 ± 0.6 | 5.1 ± 0.6 | 9.6 ± 0.6 | 4.4 ± 0.6 | 44 ± 4 |
| rhGH (50.0) + rhIGF-1 | 4.4 ± 0.7 | 5.3 ± 1.0 | 9.5 ± 0.7 | 3.8 ± 0.5 | 48 ± 5 |
| | | *P values* | | | |
| Effect of rhIGF-1 | 0.001 | 0.001 | 0.001 | 0.098 | 0.765 |
| Effect of rhGH | 0.001 | 0.015 | 0.460 | 0.052 | 0.031 |
| Interaction | 0.064 | 0.193 | 0.204 | 0.677 | 0.588 |

The relative wet weights of the spleen, thymus, kidneys, heart and liver obtained at sacrifice are shown for hypophysectomized rats treated for 7 days with excipient, rhGH, rhIGF-1 or rhGH plus rhIGF (2.4/mg/kg/day). The Table shows the relative organ weights, calculated as a percentage of body weight (Bwt) multiplied by 10. The upper portion of the Table shows the means ± SD's ($n = 6$/group), the lower portion shows the $P$ values from the two way ANOVA of these data

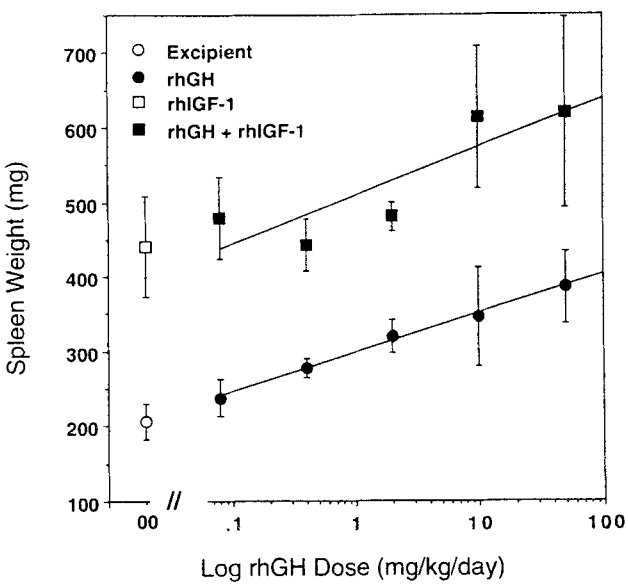


**Figure 3** Spleen weight in hypophysectomized rats treated with excipient, rhIGF-1, rhGH, or rhIGF-1 plus rhGH for 7 days. rhGH (0.08, 0.4, 2, 10 and 50 mg/kg/day) was given alone or with rhIGF-1 (2.4 mg/kg/day). The means and standard deviations are presented ($n = 6$ rats per group)

300% increase in average spleen weight (Table 3: from 206 ± 24 mg in excipient treated rats to 619 ± 128 mg in the high dose combination group), while body weight increased by less than 50%. The thymus (Table 3) showed a change in size of a similar magnitude to that of the spleen in response to the combination treatment of the highest dose of rhGH and rhIGF-1. The thymus increased from 296 ± 61 mg in excipient treated rats to 494 ± 67 mg with rhIGF-1 and 746 ± 184 mg with combination treatment. Table 3 also shows that the relative weight of the thymus was significantly increased by both rhIGF-1 and rhGH, and the lack of an interaction between their effects indicates that their effects were additive.

The increase in size of the spleen and thymus in response to rhGH or rhGH plus rhIGF-1 was analysed as a parallel line bioassay against the logarithm of rhGH dose. For both the spleen and thymus the dose response lines fulfilled the criteria for a bioassay, as they were statistically linear and parallel. For the spleen the relative potency of the combination of rhGH plus rhIGF-1 was 4931 times that of the rhGH alone (95% confidence limits 516 to 227039), with the difference between the two dose response lines being highly significant (1.49 d.f., $F = 171.9$, $P < 0.0001$). For the thymus the relative potency of the combination of rhGH plus rhIGF-1 was 1096 times that of the rhGH alone (95% confidence limits 78.6 to 286042), with the difference between the two dose response lines being highly significant (1.49 d.f., $F = 54.4$, $P < 0.0001$).

*Blood chemistry*

Table 5 shows blood glucose, blood urea nitrogen (BUN), serum phosphate, serum calcium, serum creatinine, and total serum protein in the hypophysectomized rats. The BUN (Figure 4) was high (35.0 ± 8.6 mg/dl) in excipient treated rats and was decreased by rhIGF-1, rhGH, and by rhIGF-1 plus rhGH. Treatment with rhGH caused a dose related fall in BUN, but rhGH given at 50 mg/kg only reduced BUN to 22.8 ± 1.7 mg/dl whereas much lower BUN's were found after rhIGF-1 (15.2 ± 2.2 mg/dl). There was a statistically significant interaction between the effects of rhGH and rhIGF-1, which was due (Figure 4 and Table 5) to the reduction in BUN caused by rhIGF-1 not being further increased by co-treatment with rhGH.

Blood creatinine (Table 5) and serum total protein concentrations were significantly ($P < 0.001$) decreased by rhIGF-1 but not by rhGH treatment. Serum phosphate concentrations were increased by both rhIGF-1 and rhGH, while the combination did not have an additive effect. Glucose and calcium concentrations in blood were not significantly altered by any treatment.

Similar treatment related changes in serum chemistries were seen in the dwarf rats. For example the BUN was reduced from 22.8 ± 2.2 mg/dl in excipient treated dwarf rats to 16.8 ± 2.2 mg/dl in rhIGF-1 treated rats, whereas in the rhGH treated rats (2.0 mg/kg/day) the BUN remained high (25.2 ± 1.8 mg/dl), while in the rats given the combination of rhGH (2.0 mg/kg/day) plus rhIGF-1 the mean BUN was 17.2 ± 2.0 mg/dl.

## Discussion

Body weight gain and bone growth in hypophysectomized rats are used widely as bioassays for GH activity (Groesbeck *et al.*, 1987). The hypophysectomized rat also grows when

**Table 5** Blood chemistries in hypophysectomized rats

| *Group (dose mg/kg/day)* | *Glucose (mg/dl)* | *Calcium (mg/dl)* | *Creatinine (mg/dl)* | *Phosphate (mg/dl)* | *BUN (mg/dl)* | *Total protein (g/dl)* |
|---|---|---|---|---|---|---|
| Excipient | 124 ± 14 | 10.9 ± 1.5 | 0.70 ± 0.07 | 7.5 ± 1.0 | 35.0 ± 8.6 | 5.5 ± 0.1 |
| rhGH (0.08) | 129 ± 18 | 10.4 ± 1.2 | 0.66 ± 0.11 | 7.7 ± 0.9 | 32.6 ± 3.4 | 5.7 ± 0.7 |
| rhGH (0.40) | 132 ± 19 | 10.5 ± 0.6 | 0.67 ± 0.05 | 7.6 ± 0.7 | 27.7 ± 3.1 | 5.6 ± 0.4 |
| rhGH (2.0) | 124 ± 7 | 11.0 ± 1.0 | 0.67 ± 0.05 | 8.5 ± 0.8 | 29.4 ± 3.4 | 5.0 ± 0.2 |
| rhGH (10.0) | 135 ± 14 | 11.1 ± 1.0 | 0.65 ± 0.05 | 8.9 ± 1.0 | 25.8 ± 3.6 | 5.2 ± 0.3 |
| rhGH (50.0) | 134 ± 10 | 11.7 ± 0.8 | 0.63 ± 0.05 | 9.2 ± 0.6 | 22.8 ± 1.7 | 5.3 ± 0.5 |
| rhIGF-1 (2.4) | 131 ± 23 | 10.8 ± 0.7 | 0.55 ± 0.05 | 8.9 ± 0.7 | 15.2 ± 2.2 | 5.3 ± 0.3 |
| rhGH (0.08) + rhIGF-1 | 130 ± 15 | 12.1 ± 1.9 | 0.53 ± 0.05 | 10.7 ± 1.7 | 16.8 ± 2.9 | 4.5 ± 0.1 |
| rhGH (0.40) + rhIGF-1 | 132 ± 25 | 10.8 ± 1.0 | 0.53 ± 0.08 | 9.9 ± 1.0 | 15.5 ± 2.9 | 4.6 ± 0.5 |
| rhGH (2.0) + rhIGF-1 | 130 ± 16 | 10.5 ± 1.0 | 0.57 ± 0.08 | 9.0 ± 1.0 | 12.8 ± 2.6 | 4.7 ± 1.1 |
| rhGH (10.0) + rhIGF-1 | 128 ± 11 | 10.7 ± 0.6 | 0.52 ± 0.04 | 9.6 ± 0.9 | 13.8 ± 2.3 | 4.6 ± 0.3 |
| rhGH (50.0) + rhIGF-1 | 128 ± 13 | 10.9 ± 1.2 | 0.58 ± 0.08 | 10.1 ± 0.9 | 14.0 ± 2.3 | 4.9 ± 0.9 |
| | | | *P values* | | | |
| Effect of rhIGF-1 | 0.928 | 0.992 | 0.001 | 0.001 | 0.001 | 0.001 |
| Effect of rhGH | 0.946 | 0.669 | 0.719 | 0.009 | 0.001 | 0.212 |
| Interaction | 0.870 | 0.084 | 0.524 | 0.018 | 0.016 | 0.184 |

Serum chemistries (glucose, calcium, creatinine, phosphate, blood urea nitrogen, and total protein) were measured in serum taken at sacrifice from hypophysectomized rats treated for 7 days with excipient, rhGH, rhIGF-1 or rhGH plus rhIGF-1 (2.4 mg/kg/day). The upper portion of the Table shows the means ± SD's ($n = 6$/group), the lower portion shows the $P$ values from the two way ANOVA of these data

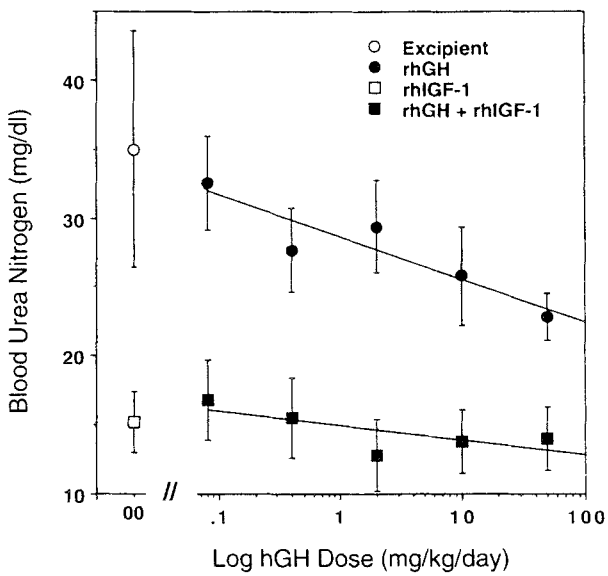


**Figure 4** Blood urea nitrogen (BUN) in serum obtained at sacrifice from hypophysectomized rats after 7 days treatment with excipient, rhIGF-1, rhGH, or rhIGF-1 plus rhGH. rhGH (0.08, 0.4, 2, 10 and 50 mg/kg/day) was given alone or with rhIGF-1 (2.4 mg/kg/day). The means and standard deviations are presented ($n = 6$ rats per group)

given hIGF-1 derived from natural sources (Schoenle *et al.*, 1982) or rhIGF-1 (Skottner *et al.*, 1987; Guler *et al.*, 1988). The present study confirms and extends our previous report (Clark *et al.*, 1994) in which we showed that the effects of a small dose of rhGH on whole body growth, organ growth and bone growth were enhanced by the co-administration of rhIGF-1. We have now tested the anabolic effects of rhIGF-1 given together with rhGH over a very wide dose range. A surprising finding was that there was a clear additive effect of rhGH and rhIGF-1 on weight gain even when rhGH was given at very large doses.

Other investigators have co-administered rhGH and rhIGF-1 to rodents, but they obtained different results. In one study, GH and methionyl-IGF-1 given in combination intravenously to rats induced no greater response than treatment with either hormone alone (Skottner *et al.*, 1987). Methionyl-IGF-1 alone increased the epiphyseal cartilage width and lengthened the tibia, while having no effect on body weight (Skottner *et al.*, 1987). The local, direct administration of either GH or IGF-1 to the proximal tibia stimulated longitudinal bone growth, but there was no additive effect of the IGF-1 plus GH combination compared to GH alone (Isgaard *et al.*, 1986). Several differences in experimental design between the present experiments and previous studies could account for these differences. For example, the met-rhIGF-1 used in some earlier experiments (Skottner *et al.*, 1987) was not as pure as that used in the present studies. In addition the effects of the combination treatment were tested in rats primed with GH (Skottner *et al.*, 1987) and the GH was infused rather than injected.

Earlier studies suggest that in the hypophysectomized rat (Skottner *et al.*, 1987; Guler *et al.*, 1988) and in the dwarf rat (Skottner *et al.*, 1989) IGF-1 and GH have different relative activities on different organs and tissues. In the dwarf rat, rhGH inconsistently increased some organ weights whereas rhIGF-1 given with or without rhGH, clearly increased several organ weights relative to the excipient group. In the dwarf rat, after correcting for body weight, rhIGF-1 increased the organ-to-body weight ratios for liver, kidneys, spleen and thymus while rhGH only affected the relative liver weight. This confirmed earlier observations (Skottner *et al.*, 1987, 1989; Guler *et al.*, 1988) that there is a preferential effect of rhIGF-1, of kidney, spleen and thymus size. This effect of rhIGF-1 on relative organ size occurred in both the presence or the absence of rhGH. These findings indicate that at least part of the body weight gain induced by the hormone combination can be attributed to each agent causing the growth of different tissues.

The mechanism behind the different relative activities of rhIGF-1 and rhGH on growth is unknown. There are many possible explanations, including that there may be different numbers of receptors for IGF-1 and GH in different tissues, or that GH has differing abilities to generate IGF-1 in different tissues. Another possibility is that other proteins direct GH and IGF-1 to different tissues. Specific binding proteins have been described for both GH and IGF-1 and the concentration of these binding proteins in different tissues may influence the local concentration of GH and IGF-1. For whole body growth a larger response was noted with rhGH treatment, whereas for the growth of the spleen and the thymus, the largest effect was due to rhIGF-1 treatment. Potency estimates showed that rhIGF-1 plus rhGH was orders of magnitude (more than 1000-fold) more potent than rhGH alone at inducing thymic and splenic growth in the hypophysectomized rat. Similar dramatic effects of rhIGF-1

on spleen and thymus were seen in the dwarf rat. The data for both absolute and the relative growth of the thymus indicate that both rhIGF-1 and rhGH have independent and additive effects. The preferred therapy to stimulate thymic growth in rodents would appear to be the combination of rhIGF-1 and rhGH.

Both rhGH and rhIGF-1 stimulated the absolute growth of the kidney in dwarf and hypophysectomized rats, but only rhIGF-1 caused the kidney to increase in size relative to the rest of the body. A recent report comparing the effects of rhGH and rhIGF-1 in normal female SD rats (Mehls *et al.*, 1993) also found that rhIGF-1, but not rhGH, caused a selective stimulation of renal growth. Both rhIGF-1 and rhGH caused a significant fall in serum BUN, perhaps indicating their potent anabolic activity. However rhIGF-1 had a greater effect on BUN than did rhGH, and the serum creatinine was decreased by rhIGF-1 but not by rhGH. rhIGF-1 had clear effects on both the size and the function of the kidney, probably increasing renal creatinine and BUN excretion, and because it also increased blood phosphate levels it may have caused renal phosphate retention.

The additive effects of rhIGF-1 and rhGH on body growth might also be explained by the two agents causing an additive increase in total serum IGF-1 concentrations. However, the present results in dwarf rats confirm the results of an earlier study (Clark *et al.*, 1994) in hypophysectomized rats, in that the effects of rhGH and rhIGF-1 on the statural measurements of growth were not reflected in a similar pattern of changes in serum IGF-1 concentrations. In the present study (Table 1) clear growth responses were induced by rhGH but serum IGF-1 concentrations at sacrifice in the rhGH treated rats were lower than in excipient controls. There was a significant increase in total serum IGF-1 concentrations in the rhIGF-1 treated rats and in the rats receiving rhIGF-1 plus rhGH. However, when rhGH was given with rhIGF-1 serum IGF-1 concentrations fell compared to the rhIGF-1 alone group. An explanation could be that there was a transient rise in serum IGF-1 concentrations following each GH injection, which we did not detect by sampling 24 h after the injection. The fall in total serum IGF-1 concentrations 24 h following an injection of rhGH agrees with our previous studies (Clark *et al.*, 1991, 1994). No change in serum IGF-1 following an injection of GH has been reported by others in the rat (Orlowski & Chernausek, 1988) and in the mouse (Woodall *et al.*, 1991).

All the animals in our studies appeared to be in good health and survived through the 7 day treatment period. Groesbeck *et al.* (1987) have reported that very large doses of rat GH (5 mg/rat/day), purified from pituitaries, had 'toxic effects' in prepubertal and growth plateaued adult female rats. We treated hypophysectomized rats with similar doses of rhGH and did not observe any deaths, so our results do not support the idea that large doses of rhGH have toxic effects in GH deficient rats. Even when a high dose of rhIGF-1 was given in combination with high doses of rhGH the animals appeared to be in good health. For example, in our study the maximum growth rate achieved by rhGH plus rhIGF-1 in hypophysectomized rats was 46 grams in seven days. Normal intact female SD rats of 100 g grow at about 35 g/week, and male rats at about 50 g/week. Therefore the weight gains we obtained fall within the normal range for rats of this strain and age. There are several differences between the experimental designs and reagents used in the present study and the study by Groesbeck *et al.* (1987). Treatment in the present study was for 7 days; the earlier study treated for 24 days and observed toxicity toward the end of the study. The present study used recombinant hGH whereas the earlier study used pituitary-derived rat GH.

Characterization of the distinct effects of GH and IGF-1 is important not only for the understanding of the control of normal growth and anabolism but also to improve the diagnosis and treatment of growth disorders and catabolic states. Currently GH is used for the treatment of short stature in children with GH deficiency. However, other uses for GH are being investigated. For example, clinical trials in GH-deficient adults suggest that GH has positive anabolic effects in adult humans (Bengtsson *et al.*, 1993). Clinical experience with IGF-1 is less extensive, but the number of studies is increasing. The growth promoting effects of IGF-1 are currently being evaluated in children with GH insensitivity (Rosenfeld *et al.*, 1992) and metabolic effects studied in adults (Sherwin, 1992). Our studies in the rat and a recent study in humans (Kupfer *et al.*, 1993) show that the combination of GH and IGF-1 is more efficacious than either hormone alone. The side effects of both GH and IGF-1 include adverse effects on carbohydrate metabolism. It is possible that the combination treatment reduces the risk for this particular side effect, since GH and IGF-1 have opposite effects on blood glucose levels. Thus, theoretically, treatment with the combination of GH and IGF-1 may be both more efficacious and safer than treatment with either hormone alone.

There are other reasons why rhGH and rhIGF-1 given in combination may be a logical anabolic and somatogenic therapy. There is evidence in the rat (Tannenbaum *et al.*, 1983) and in man (Hartman *et al.*, 1991) that IGF-1 can inhibit GH secretion from the pituitary. It is therefore possible that long-term treatment with rhIGF-1 may lead to long-term suppression of endogenous GH secretion, resulting in relative GH deficiency. A logical extension of this reasoning is that rhIGF-1 treatment should be accompanied by rhGH treatment to correct the relative GH deficiency induced by the rhIGF-1, especially if there are beneficial GH efficacies that are independent of IGF-1.

Another rationale for using rhGH in combination with rhIGF-1 is that a potential benefit of rhIGF-1 as a treatment for reversing catabolic states is to decrease protein breakdown (Ballard *et al.*, 1991; Kupfer *et al.*, 1993). rhGH and rhIGF-1 may also differ in their relative activities on protein synthesis and breakdown. In the present study the relative effects of rhGH and rhIGF-1 on BUN (an indicator of protein breakdown) were quite different. There was a fall in BUN with rhGH, but in the presence of rhIGF-1, which had much greater activity, little effect of additional rhGH could be demonstrated. Therefore, in catabolic states it would be logical to deliver an agent to inhibit protein breakdown (rhIGF-1) and an agent to stimulate protein synthesis (rhGH).

We conclude that co-treatment of hypophysectomized or dwarf rats with continuous infusions of rhIGF-1 and injections of rhGH amplifies anabolic responses compared to responses obtained with either hormone alone. This finding indicates for the first time that exogenous rhIGF-1 can increase specific growth responses initiated by rhGH over a wide dose range in a manner that is at least additive.

## Materials and methods

### *Animals*

The experimental procedures and design were approved by the institutional Animal Care and Use Committee.

Female dwarf rats (60–70 days of age, 100–140 g) were bred (Simonsen, Gilroy, CA) by homozygous mating (Charlton *et al.*, 1988). Young female hypophysectomized rats (85–105 g, Taconic Farms, Germantown, NY) were received 10 days after surgery, and then weighed every 2–3 days for 10 days to meet entry criteria of a weight gain of less than 7 grams and no overall body weight loss. The hypophysectomized rats were not replaced with corticosterone or thyroxine. The rats for both studies were group housed (five per cage) on polystyrene chips and fed a standard laboratory animal chow and distilled water *ad libitum*, and kept in a room of constant humidity and temperature with controlled lighting (12 h light: 12 h dark). The animals were randomized

by standard procedures into their treatment groups and cages to give groups of equal initial body weights.

*Surgery*

All the rats were anesthetized with a mixture of ketamine (75 mg/kg, ip, Aveco, Ft. Dodge, Iowa) and xylazine (15 mg/kg, Rugby Labs, Rockville Center, N.Y.), shaved on the neck, and two osmotic minipumps (Alzet 2001, delivery rate 1 μL/h, Alza, Palo Alto, CA) were placed subcutaneously. The wounds were closed with metal clips and the animals were placed on heated pads until recovery (within 2 h) and then returned to their cages. Body weights were recorded daily.

*Drug delivery*

Two osmotic minipumps were used per animal to deliver the rhIGF-1 (Genentech, Inc., 5 mg/ml) or its excipient (10 mM citrate buffer, 126 mM NaCl, pH 6.0). Together the two pumps delivered 2.4 mg/kg/day. The rhGH (Genentech, Inc., 5 mg/vial) or its excipient 5 mM phosphate buffer (pH 7.8) were given daily as a single 0.1 ml subcutaneous injection.

To attain the high concentrations of rhGH needed in these studies the solubility of rhGH was increased by including 0.1% Tween 20 in the buffer. In the study in dwarf rats, an ip injection of 1 ml of sterile saline containing 1 mg of oxytetracycline (Liquamycin, Pfizer, 50 mg/ml) was given as an intravital marker for subsequent measurement of the longitudinal growth of the tibia.

*Experimental designs*

The study in dwarf rats consisted of eight groups ($n = 6$/group):

1. Excipient pumps, excipient injections
2. Excipient pumps, rhGH injections (0.125 mg/kg/day)
3. Excipient pumps, rhGH injections (0.5 mg/kg/day)
4. Excipient pumps, rhGH injections (2.0 mg/kg/day)
5. rhIGF-1 pumps (2.4 mg/kg/day), excipient injections
6. rhIGF-1 pumps (2.4 mg/kg/day), rhGH injections (0.125 mg/kg/day)
7. rhIGF-1 pumps (2.4 mg/kg/day), rhGH injections (0.5 mg/kg/day)
8. rhIGF-1 pumps (2.4 mg/kg/day), rhGH injections (2.0 mg/kg/day)

The study in hypophysectomized rats consisted of 12 groups ($n = 6$/group):

1. Excipient pumps, excipient injections
2. Excipient pumps, rhGH injections (0.08 mg/kg/day)
3. Excipient pumps, rhGH injections (0.4 mg/kg/day)
4. Excipient pumps, rhGH injections (2.0 mg/kg/day)
5. Excipient pumps, rhGH injections (10.0 mg/kg/day)
6. Excipient pumps, rhGH injections (50.0 mg/kg/day)
7. rhIGF-1 pumps (2.4 mg/kg/day), excipient injections
8. rhIGF-1 pumps (2.4 mg/kg/day), rhGH injections (0.08 mg/kg/day)
9. rhIGF-1 pumps (2.4 mg/kg/day), rhGH injections (0.4 mg/kg/day)
10. rhIGF-1 pumps (2.4 mg/kg/day), rhGH injections (2.0 mg/kg/day)
11. rhIGF-1 pumps (2.4 mg/kg/day), rhGH injections (10.0 mg/kg/day)
12. rhIGF-1 pumps (2.4 mg/kg/day), rhGH injections (50.0 mg/kg/day)

*Tissue harvest*

Twenty-four hours after the last injection, the rats were asphyxiated with carbon dioxide, and plasma and serum samples taken by cardiac puncture. The liver, kidneys, heart, spleen and thymus were then removed, blotted dry and immediately weighed. In the study in dwarf rats, both tibia were dissected and one placed in 10% neutral buffered formalin and one placed in 70% alcohol. The bone placed in formalin was later decalcified, bisected longitudinally and embedded in paraffin for sectioning and staining with toluidine blue. The epiphyseal plate width was measured as the distance between the germinal cell layer and the transition from active chondrocytes to new bone deposits. This distance was measured microscopically with the aid of a calibrated ocular micrometer. The tibia placed in alcohol was sectioned longitudinally without being decalcified and the distance between the growth plate and the oxytetracycline band was measured under fluorescence using a calibrated ocular micrometer. The microscopist was blinded to the identity of the animal or its treatment.

*Blood analyses*

Blood glucose (coupled hexokinase procedure), total protein, blood urea nitrogen, creatinine, calcium and phosphorus, were measured using a Monarch 2000 Chemical Systems instrument (Allied Instrument Laboratories, Lexington, MA). Serum was extracted using acid-ethanol (12.5% 2N HCl, 87.5% EtOH at 4°C for 30 min, with a 1:15 ratio of serum to acid-ethanol) and total IGF-1 measured by radioimmunoassay in the neutralized supernatant, using rhIGF-1 as standard. Absence of BP contamination in the supernatants was confirmed by ligand blot. The acceptable range was 1.25–40 ng/ml, the intra- and interassay variability were 5–9% and 6–15%, respectively (Lieberman *et al.*, 1992).

*Statistical analyses*

The balanced experimental designs allowed the data to be statistically analysed by two way analysis of variance (ANOVA), using rhIGF-1 and rhGH as the main effect classifications. The $P$ values for the two main effects and their interaction are shown in the Tables. The relationships between the responses and the logarithm of the dose of rhGH, given with or without rhIGF-1, were also analysed as a parallel line bioassay, to establish the potency of the combination treatment with respect to rhGH given alone. A $P$ value of less than 0.05 was considered significant. All data are represented as the mean ± standard deviation of six animals per group.

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