

# Recombinant human insulin-like growth factor I stimulates growth and has distinct effects on organ size in hypophysectomized rats

(growth hormone/body-weight gain/longitudinal bone growth/kidneys/spleen)

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**ABSTRACT** Recombinant human insulin-like growth factor I (rhIGF-I) was infused subcutaneously into hypophysectomized rats for as long as 18 days. Three hundred micrograms (39 nmol) of rhIGF-I per day and 200 milliunits (4.5 nmol) of human growth hormone (hGH) per day increased body weight, tibial epiphyseal width, longitudinal bone growth, and trabecular bone formation similarly. Weight gains of the kidneys and spleen, however, were greater with rhIGF-I than with hGH, whereas the weight of the epididymal fat pads was reduced with rhIGF-I. The weight of the thymus was increased by rhIGF-I treatment. Thus, IGF-I administered over a prolonged period of time mimics GH effects in hypophysectomized rats. Quantitative differences between rhIGF-I and hGH treatment with respect to organ weights may be related to different forms of circulating IGF-I or may be due to independent effects of GH and IGF-I. The results support the somatomedin hypothesis, but they also stress the role of GH as a modulator of IGF-I action.

According to the somatomedin hypothesis, growth hormone (GH) stimulates the endogenous production of insulin-like growth factor I (IGF-I), which in turn is the driving principle of somatic growth (1). On the other hand, direct effects of GH on the growth plate (2) and in cell cultures (3) have been demonstrated under certain conditions. It has been suggested that some effects of GH may be mediated by the local production of IGF-I. It remains to be elucidated whether IGF-I is predominantly an endocrine hormone or a paracrine and autocrine growth factor. Recent reports favor the endocrine hypothesis: subcutaneous infusions of recombinant human IGF-I (rhIGF-I) stimulate the growth of diabetic and hypophysectomized rats and of Snell dwarf mice (4–7). In the present study, distinct effects of rhIGF-I and GH on growth of different organs were obtained by continuous subcutaneous infusions of the two hormones into hypophysectomized rats.

## MATERIALS AND METHODS

Male Tif-RAI rats were hypophysectomized between 40 and 50 days of age (body weight 120–130 g). They were a gift of K. Müller (Ciba-Geigy, Basel). Rats with weight gains of less than 2 g per week during the 3 weeks following the operation were considered to be successfully hypophysectomized. The animals were kept at 25°C on a 12-hr light/dark cycle and had free access to food (Altromin, Lage, F.R.G.) and water. Four animals were housed per box.

rhIGF-I was a gift of W. J. Rutter (Chiron, Emeryville, CA) and J. Nüesch (Ciba-Geigy, Basel). Extracted human GH (hGH, Nanormon) and recombinant human GH (rhGH) were purchased from Nordisk (Gentofte, Denmark).

In a first series of experiments, of 6 days duration, extracted hGH was compared with rhIGF I. The dose range of hGH tested was 12.5–400 milliunits (mu)/day (0.28–9.08 nmol). rhIGF-I was tested in doses of 75–600 µg/day (9.8–78 nmol). In a second series of experiments, maximally effective doses of rhIGF-I (300 µg per day) and of rhGH (200 mu per day) were infused for 18 days.

Alzet minipumps 2001 or 2002 (Alza, Palo Alto, CA) were filled the day before the experiments. rhIGF-I was dissolved in 0.1 M acetic acid. GH was dissolved in 0.9% NaCl. The pumps were allowed to equilibrate overnight in 5% glucose. The implantation of the Alzet pumps under the skin of the abdomen was performed during a short ether anesthesia, and the rats were infused for 6 days. Animals treated for 18 days had an Alzet 2002 pump during the first 12 days, which was then removed and replaced by a new Alzet 2001 pump. Infusion rates were 22 µl/24 hr. On the day of implantation, rats were given a single injection of oxytetracycline (12 mg/kg of body weight; Terravenös, Pfizer, Zürich) intraperitoneally (8).

Body weight and food and water consumption were recorded daily between 0700 and 0800. At the end of the experiments, the rats were killed by aortic puncture while under general anesthesia (intramuscular injection of 0.2–0.3 ml of Innovar Vet, Pitman Moore, Washington Crossing, NJ). The implantation sites of the pumps showed no signs of inflammation. Blood glucose was determined (YSI glucose analyzer). A tibial epiphysis was silver-stained, and the width was measured as described by Greenspan *et al.* (9). Methacrylate sections of the contralateral tibia were examined microscopically under a mercury lamp with fluorescence excitation wavelength of 400–450 nm and a tetracycline filter (Leitz, Wetzlar, F.R.G.) (8). The sections were photographed and measurements were obtained from photographic prints. Kidneys, spleen, heart, liver, thymus, epididymal fat pads, and the soleus and gastrocnemius muscle were removed, dissected free of connective tissue, blotted on a filter paper, and weighed. Endogenous IGF-I and exogenous IGF-I in serum were separated from binding proteins by chromatography on Sephadex G-50 (Pharmacia) columns (2 × 50 cm) with 0.1 M acetic acid as eluent and were analyzed by radioimmunoassay (10, 11).

## RESULTS

**Both rhIGF-I and hGH Stimulate Bone Growth and Weight Gain in Hypophysectomized Rats Treated for 6 Days.** Serum levels of IGF-I were  $6 \pm 3$  ng/ml in hypophysectomized control rats and  $316 \pm 44$  ng/ml after 6 days of infusion of 300 µg of rhIGF-I per day (Fig. 1). The highest dose of hGH (400 mu per day, 9.0 nmol) raised endogenous IGF-I to 88 ng/ml.

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Abbreviations: IGF-I, insulin-like growth factor I; GH, growth hormone; hGH, human GH; rhGH, recombinant hGH; rhIGF-I, recombinant human IGF-I; mu, milliunit(s).

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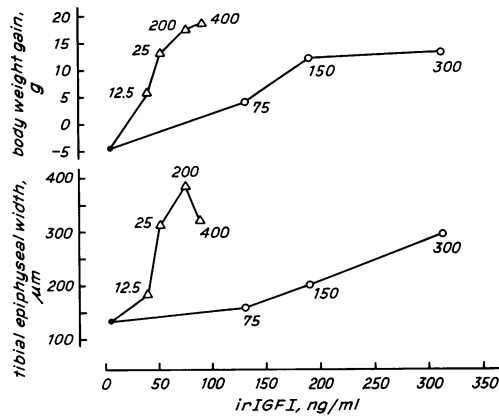


FIG. 1. Serum levels of immunoreactive (ir) IGF-I and body-weight gain and tibial epiphyseal width in hypophysectomized rats infused with rhIGF-I at 75, 150, or 300  $\mu\text{g}$  per day ( $\circ$ ) or with hGH at 12.5, 25, 200, or 400  $\mu\text{u}$  per day ( $\Delta$ ) for 6 days. Hypophysectomized control rats were infused with saline. Mean values are shown ( $n = 4$ ); means  $\pm$  SD are given in the text.

The administration of rhIGF-I led to a dose-dependent weight gain of  $13.6 \pm 3.4$  g with 300  $\mu\text{g}$  (39 nmol) of rhIGF-I per day. With 600  $\mu\text{g}$  of rhIGF-I per day, body weights did not increase further ( $15.0 \pm 1.4$  g). Out of eight hypophysectomized rats treated with 600  $\mu\text{g}$  of rhIGF-I, five died within hours after the implantation of the pumps, probably of hypoglycemia. Infusions of hGH also led to dose-dependent weight gains of up to  $18.2 \pm 1.2$  g per day obtained with 200  $\mu\text{u}$ . Four hundred milliunits of hGH did not raise the weights further ( $18.5 \pm 2.4$  g). Normal Tif-RAI rats with a body weight of about 135 g have a tibial epiphyseal width of  $497 \pm 52$   $\mu\text{m}$ ; untreated hypophysectomized rats have one of only  $135 \pm 14$   $\mu\text{m}$ . Infusions of rhIGF-I led to a dose-dependent widening of the tibial epiphyseal width up to  $297 \pm 60$   $\mu\text{m}$  obtained with 300  $\mu\text{g}$  of rhIGF-I per day. Tibial epiphyseal widths were also increased dose-dependently up to  $383 \pm 32$   $\mu\text{m}$  with hGH.

**Bone Growth in Hypophysectomized Rats Treated with rhIGF-I or rhGH for 18 Days.** Hypophysectomized control rats consumed 5.9 g of food per day, and rhGH- and rhIGF-I-treated animals consumed 7.8 g per day and 8.7 g per day, respectively. Water consumption was 20.3 g per day for the control rats and 23.7 g per day and 21.7 g per day for the rhGH- and rhIGF-I-treated animals, respectively. Serum levels of IGF-I were  $12 \pm 3$  ng/ml in the control animals and were increased to as much as  $402 \pm 158$  ng/ml in rhIGF-I-treated rats and to as much as  $48 \pm 25$  ng/ml in rhGH-treated rats. Blood glucose levels at the time of sacrifice did not differ in the three groups of rats ( $118 \pm 20$  mg/dl with rhIGF-I,  $97 \pm 9$  mg/dl with rhGH, and  $121 \pm 14$  mg/dl in the controls). In the rhIGF-I- and the rhGH-treated rats, body weights were increased to the same extent (Fig. 2). The weights of the control rats remained stable. Fig. 3 and the microscopic pictures of the tetracycline stainings in Fig. 4 show the accumulated longitudinal bone growth. After 18 days, longitudinal growth of the tibia was  $73 \pm 12$   $\mu\text{m}$  in saline-treated control rats,  $666 \pm 36$   $\mu\text{m}$  with 300  $\mu\text{g}$  of rhIGF-I per day, and  $945 \pm 194$   $\mu\text{m}$  with 200  $\mu\text{u}$  of rhGH per day. The difference between rhIGF-I- and rhGH-treated rats was not statistically significant. In both rhIGF-I- and rhGH-treated rats the epiphysis appeared normal (Fig. 5). Stacks of chondrocytes were again visible, and trabecular bone was normal. Cartilage and bone structure of rats treated with rhGH could not be differentiated from cartilage and bone structure of rats treated with rhIGF-I.

**Distinct Effects of rhIGF-I and rhGH on Organ Weights in Hypophysectomized Rats Treated for 18 Days.** Hypophysec-

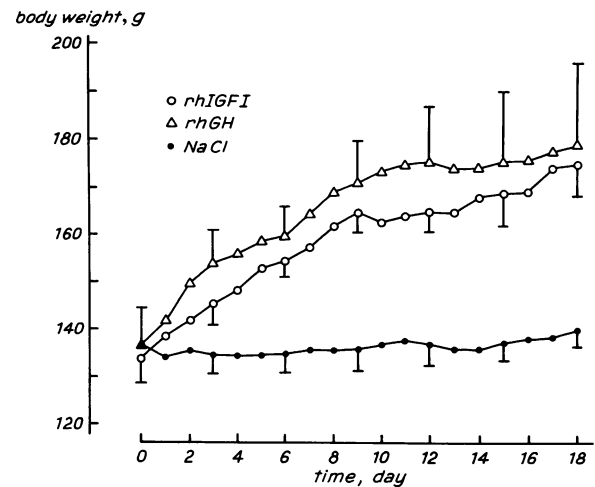


FIG. 2. Body-weight gain in hypophysectomized rats infused for 18 days with rhIGF-I (300  $\mu\text{g}/\text{day}$ ), rhGH (200  $\mu\text{u}/\text{day}$ ), or 0.9% NaCl. Mean values ( $\pm$  SD for representative points) are shown ( $n = 4$ ).

tomized rats treated with rhGH or rhIGF-I attained different weights of individual organs. Kidney weights were only increased from  $0.679 \pm 0.057$  g to  $0.825 \pm 0.092$  g with rhGH ( $P < 0.05$ ) (Fig. 3). With rhIGF-I, however, kidney weights were increased to  $1.176 \pm 0.087$  g ( $P < 0.01$  vs. rhGH treatment). rhGH-treated rats had larger spleens than saline-infused control rats ( $0.410 \pm 0.155$  g vs.  $0.248 \pm 0.015$  g; not significant). The effect of rhIGF-I on this parameter was significantly greater than that of rhGH ( $0.666 \pm 0.037$  g,  $P < 0.05$  vs. rhGH). On microscopic examination, the spleens and kidneys of rhGH-treated rats could not be distinguished from those of rhIGF-I-treated rats. rhIGF-I increased the weight of the thymus from  $0.274 \pm 0.034$  g to  $0.738 \pm 0.150$  g ( $P < 0.01$ ). The weights of the kidneys, spleen, and thymus, expressed as percentage of total body weight, were reduced in hypophysectomized rats (Table 1). rhIGF-I administered for 18 days normalized the fractional weights of the kidneys and the spleen, whereas the thymus tended to be heavier than that of nonhypophysectomized rats (not significant). rhGH, on the other hand, did not completely normalize the fractional weights.

The rhIGF-I-infused rats had lighter fat pads than the saline-treated hypophysectomized control rats ( $0.515 \pm 0.113$  g vs.  $0.714 \pm 0.083$  g;  $P < 0.05$ ) (Table 2). The results were similar when the weights were expressed as percentage of total body weight ( $P < 0.01$ ) (Table 1). rhGH increased the

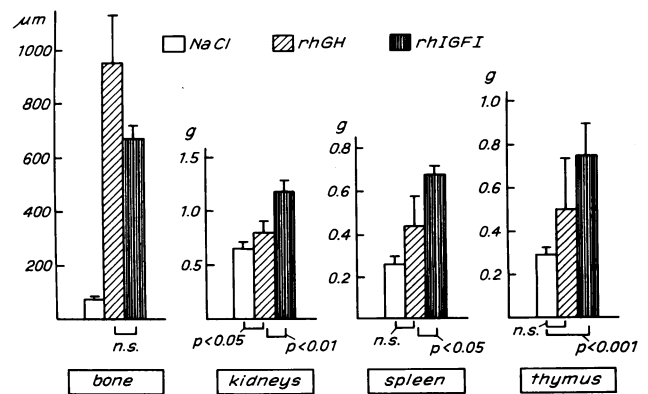


FIG. 3. Accumulated longitudinal bone growth and weights of kidneys, spleen, and thymus in hypophysectomized rats treated for 18 days with rhIGF-I (300  $\mu\text{g}/\text{day}$ ), rhGH (200  $\mu\text{u}$  per day), or 0.9% NaCl. Mean values  $\pm$  SD are shown ( $n = 4$ ). n.s., Not significant.

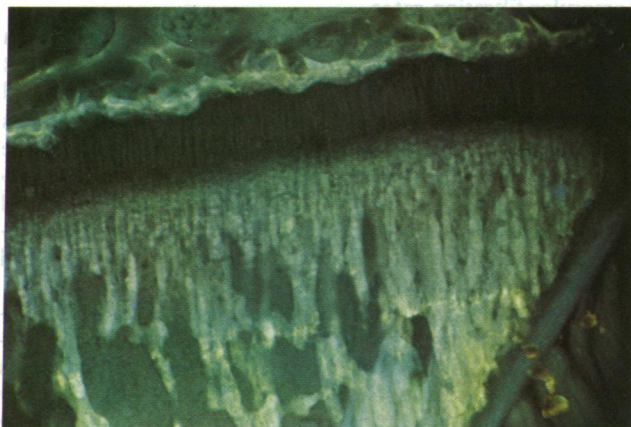


FIG. 4. Microscopic examination of tetracycline-stained tibia of hypophysectomized rats treated for 18 days with saline (Top), rhGH (Middle), or rhIGF-I (Bottom). Distances between the lower end of the epiphysis and the border of fluorescent tetracycline were  $73 \pm 12 \mu\text{m}$  (Top),  $945 \pm 194 \mu\text{m}$  (Middle), and  $666 \pm 36 \mu\text{m}$  (Bottom). ( $\times 10$ .)

weight of gastrocnemius ( $P < 0.01$  vs. saline-treated controls) and soleus muscles ( $P < 0.05$  vs. saline-treated controls) (Table 2). The increase in muscle weight brought about by rhIGF-I did not reach significance. Administration of either rhIGF-I or rhGH increased the weight of the heart of hypophysectomized rats ( $P < 0.05$ ). The weight of the liver remained unchanged.

#### DISCUSSION

Here we have addressed the question of whether IGF-I substitutes for GH in hypophysectomized rats treated for a prolonged time period. This appeared relevant because

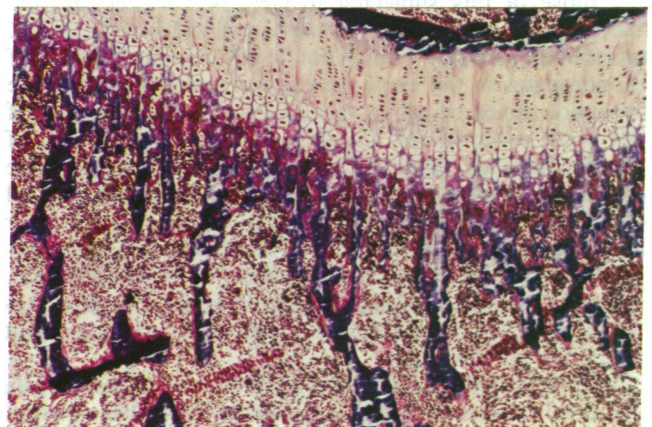
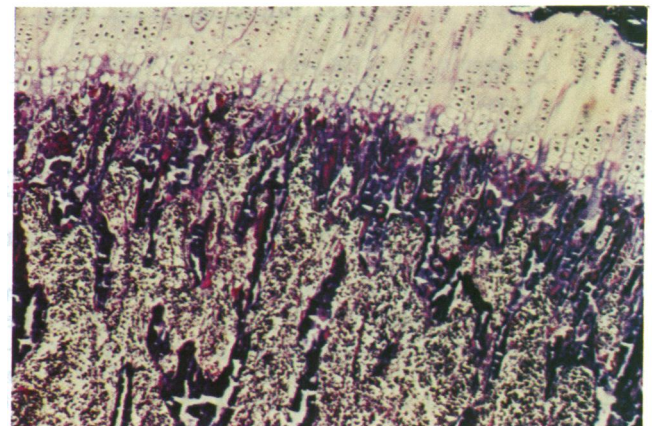
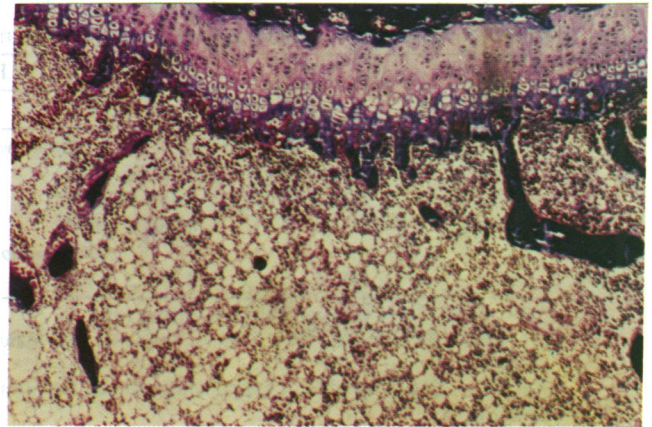


FIG. 5. Ladewig staining of the tibia of hypophysectomized rats treated for 18 days with saline (Top), rhGH (Middle), or rhIGF-I (Bottom).

Isaksson and coworkers (12) demonstrated that unilateral injections of GH into epiphyseal plates were only effective locally and not in a systemic way (e.g., on the contralateral epiphyseal plate). GH was thought to affect prechondrocytes at an early stage of differentiation through stimulation of IGF-I production, which in turn enhances the replication of chondrocytes and matrix formation. This idea is supported by results obtained in cell cultures, where IGF-I or IGF-I-like molecules appear to be released, thereby conditioning the medium (3, 13). Moreover, dual actions of GH and of IGF-I on growth and on differentiation have been proposed by Green and coworkers (14–16), who performed experiments with cultured preadipocytes and muscle cells. GH directly promoted differentiation of those cultured cells, and the

Table 1. Organ weights expressed as percentage of total body weight (fractional weight) in hypophysectomized rats infused for 18 days with saline, rhGH (200 mu/day), or rhIGF-I (300 µg/day)

Treatment	Body weight, g	Fractional weight, % (mean ± SD, n = 4)			
		Kidneys	Spleen	Thymus	Fat pads
<i>Hypophysectomized rats</i>					
Saline	139 ± 4	0.487 ± 0.033	0.178 ± 0.010	0.196 ± 0.022	0.512 ± 0.046
rhGH	177 ± 17	0.465 ± 0.017*	0.231 ± 0.067†	0.264 ± 0.113	0.384 ± 0.068‡
rhIGF-I	173 ± 6	0.680 ± 0.054§	0.386 ± 0.034§	0.424 ± 0.070§	0.297 ± 0.064¶
<i>Nonhypophysectomized rats</i>					
Saline	148 ± 5	0.799 ± 0.021	0.341 ± 0.021	0.361 ± 0.038	0.428 ± 0.024

\**P* < 0.001, †*P* < 0.01 vs. rhIGF-I-treated rats.‡*P* < 0.05, §*P* < 0.001, ¶*P* < 0.01 vs. saline-treated hypophysectomized rats.

Table 2. Organ weights in hypophysectomized rats infused for 18 days with saline, rhGH (200 mu/day), or rhIGF-I (300 µg/day)

Treatment	Weight, g (mean ± SD, n = 4)				
	Fat pads	Heart	Gastrocnemius muscle	Soleus muscle	Liver
Saline	0.714 ± 0.083	0.381 ± 0.023	1.665 ± 0.135	0.126 ± 0.014	4.565 ± 0.551
rhGH	0.690 ± 0.180	0.427 ± 0.067*	2.259 ± 0.206†	0.163 ± 0.016*	5.779 ± 1.030
rhIGF-I	0.515 ± 0.113*	0.515 ± 0.054†	1.954 ± 0.213	0.140 ± 0.014	5.705 ± 0.574

\**P* < 0.05; †*P* < 0.01 vs. saline-treated hypophysectomized rats.

preadipocytes were shown to be more sensitive to treatment with IGF-I than precursor cells.

In the present study, IGF-I was shown to act directly on peripheral organs through the circulation and not to require GH to be effective. Thus, body-weight gains of hypophysectomized rats treated with maximal doses of GH or IGF-I over periods of up to 18 days were similar. Moreover, the tibia of the rats treated with IGF-I had grown  $660 \pm 36 \mu\text{m}$  in length, and those of rats subjected to GH,  $945 \pm 194 \mu\text{m}$ . On microscopic examination, the epiphyseal width of the tibia of rats treated with IGF-I could not be differentiated. Stacks of chondrocytes in the epiphyseal plates and trabecular bone formation appeared similar in IGF-I- and GH-treated rats. If GH were essential for an early step of differentiation of prechondrocytes, treatment with IGF-I alone in the absence of GH would have resulted in a block of differentiation and of cell replication. Thus, our data imply that IGF-I is a major hormone leading to differentiation and replication of epiphyseal cartilage and, as a consequence, to bone formation. rhIGF-I administered systemically reached chondrocytes and bone cells via the circulation and, therefore, is a potent endocrine growth factor.

In GH-treated rats, dose-response curves of weight gain and tibial epiphyseal width were steeper than those obtained with IGF-I. With IGF-I, stimulation of growth occurred over a wide range of IGF-I serum concentrations. This may be circumstantial evidence for a local action of GH, be it direct or, alternatively, through local production of IGF-I. That rat IGF-I is 2–3 times more potent than IGF-I in rat osteoblasts (17) does not explain the difference in the slope of the dose-response curves. This discrepancy may be due to different modes of presentation of IGF-I to cells and tissues. In hypophysectomized rats treated with IGF-I, the hormone is mostly bound to a 50-kDa binding protein, and small amounts circulate in the free form (10). In contrast, GH treatment restored a normal binding pattern where endogenous IGF-I is mostly bound to a 150-kDa carrier protein (18). GH appears to modulate the effects of IGF-I by changing the binding pattern and may also affect IGF receptors. Not only does GH lead to skeletal growth, but it also increases the weight of several organs and enhances the reduced immune response of hypophysectomized animals (19–21). Here we present evidence that the weights of the spleen and the thymus are restored to normal under the influence of rhIGF-I. GH was

less potent than rhIGF-I. rhIGF-I also increased the weight of the kidneys, which doubled in size in the rats treated for 18 days with rhIGF-I. Again, GH was less potent. Similar findings have been obtained with the Snell dwarf mouse (7). Results obtained during continuous subcutaneous infusion of rhIGF-I in healthy human volunteers have revealed that creatinine clearance is increased (unpublished data). Therefore, the increased kidney weights may reflect increased glomerular filtration rates.

Striking effects of rhIGF-I and of rhGH were observed on the cell content of the bone marrow. There, ubiquitous fat droplets observed in hypophysectomized rats were replaced by bone marrow cells, and the histological appearance was normalized. The effects of rhIGF-I and rhGH on the bone marrow *in vivo* are consistent with the stimulation of erythropoiesis by rhIGF-I *in vitro* (22) and with the increased serum levels of erythropoietin, iron incorporation into erythrocytes, and number of reticulocytes in hypophysectomized rats under treatment with rhIGF-I (23).

In conclusion, rhIGF-I is a potent anabolic and growth-promoting hormone in hypophysectomized rats in the absence of GH. That IGF-I acts as a circulating hormone does not exclude direct effects of GH on chondrocytes, possibly mediated by the local production of growth factors including IGF-I. Differences between the effectiveness of rhIGF-I and of rhGH on organ weights of the kidneys, spleen, and thymus may be due to the different forms of available IGF-I. In contrast to IGF-I, GH infusions increased the weight of the gastrocnemius and soleus muscles. Thus, effects of GH on skeletal muscle are probably direct and not mediated by IGF-I. rhIGF-I, unlike GH, decreased the weight of the fat pads.

IGF-I injected intravenously as a bolus in large doses in humans induces hypoglycemia (24). This effect is not observed after intravenous administration of GH. The anabolic effects of rhIGF-I described here occurred at hormone concentrations that are not hypoglycemic. Our observations on bone marrow, thymus, spleen, and kidneys suggest new therapeutic roles of rhIGF-I.

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