Decreased serum insulin-like growth factor I response to growth hormone in hypophysectomized rats fed a low protein diet: evidence for a postreceptor defect

M. Maes, Y. Amand, L. E. Underwood, D. Maiter 
and J.-M. Ketelslegers

Unité de Diabète et Croissance, School of Medicine, University of Louvain, Brussels, Belgium, 
and the Department of Pediatrics, Division of Pediatric Endocrinology (L.E.U.), 
University of North Carolina Chapel Hill, North Carolina, USA

Abstract. In protein-calorie malnutrition, serum IGF-I concentrations might be low despite high GH. This GH resistance might be due to a reduced number of liver GH binding sites as suggested by studies performed in fasted rats that were refed a low protein diet. To determine whether a postreceptor defect in GH action might also contribute to the GH resistance, we measured the number and the affinity constant of the liver GH binding sites and the serum IGF-I responses to injections of recombinant bGH in hypophysectomized female rats, fed a standard (15% protein) diet (N = 25) or a low (5%) protein diet (N = 25) for 8 days. There were no significant differences in the liver GH binding capacities between the 15% and the 5% protein-fed rats, whether expressed as pmol per liver (20.6 ± 3.5 vs 14.4 ± 1.3; mean ± SEM; P < 0.2; N = 5, respectively), pmol per mg DNA (1.08 ± 0.16 vs 0.84 ± 0.07; P < 0.4) or fmol per mg of protein (28.98 ± 5.04 vs 30.26 ± 2.00; P > 0.5). Likewise, the affinity constants of the GH binding sites of the 15% and the 5% protein-fed rats were not significantly different (0.78 ± 0.05 vs 0.78 ± 0.07 × 10⁹ l/mol; P > 0.5). Despite these non-significant reductions in liver GH binding sites, the IGF-I responses 24 h after sc injections of increasing doses of bovine GH were blunted in the rats fed the 5% protein diet. The maximal IGF-I response in the rats with the normal protein intake was 360 ± 30 U/l, but only 130 ± 40 U/l in the 5% protein-fed animals (P < 0.001). The blunted serum IGF-I responses to GH, together with decreased maximal stimulation in the 5% protein-fed hypophysectomized rats, support the possibility that a postreceptor defect in GH action contributes to the GH resistance in protein-calorie malnutrition.

Children with protein-calorie malnutrition have low serum IGF concentrations despite high basal GH (Grant et al. 1973; Hintz et al. 1978) suggesting that they are resistant to the biological effects of GH. Furthermore, the production of IGF by the livers of protein-deprived animals is reduced and cannot be restored to normal by GH added in vitro or given in vivo (Shapiro & Pimstone 1978; Shapiro et al. 1978). These observations suggest that the liver in protein-calorie malnutrition is resistant to GH action. One possible mechanism for this resistance is a reduction in the number of somatogenic binding sites in the liver. In this regard, liver GH binding and serum IGF-I concentrations are reduced in 3-day fasted rats that have been refed a low protein diet (Maes et al. 1984). Studies in animals subjected to such stringent dietary manipulations, however, may not be appropriate for understanding the changes in GH binding induced by more moderate degrees of undernutrition. Indeed, we have observed that feeding 4-week-old rats a low protein diet for 12 h causes a dramatic decline in serum IGF-I at a time
when liver GH binding sites are decreased only modestly (submitted for publication). This observation caused us to explore in the present study the possibility that postreceptor defects in GH action on the liver also might be involved in the GH resistance that occurs in protein-calorie malnutrition.

Materials and Methods

Animals

Fifty female Wistar rats (IFAA Credo, Lyon, France) were hypophysectomized during the fifth week of life and were delivered to our animal quarters 1 week after surgery. They were kept 5 to a cage under conditions of controlled lighting (12 h-light and 12 h-dark cycle) and constant temperature (22 ± 2°C). The animals had free access to tap water supplemented with 0.9% NaCl and were fed ad libitum a powdered diet (325 Cal/100 g) containing 15% casein, 45% carbohydrates and 5% lipids (UAR, Villemoisson-sur-Orge, France). Body weights were recorded daily for the 2 weeks preceding the experiment. During this observation period, none of the animals gained more than 5 g.

Hormone preparations

Recombinant bGH (G11010, lot E) was a generous gift of Genentech, Inc, (South San Francisco, CA). L-T4 sodium salt (T4, 500-9) was purchased from Aldrich Co (Milwaukee, MI). Bovine GH, used as tracer in the binding studies, was generously provided by A. C. Paladin (Buenos Aires, Argentina), whereas bGH (B18) was obtained through the NIADDK hormone distribution program.

Experimental design

After 2 weeks of observation, 25 hypophysectomized (Hypox) rats were distributed randomly to each of 2 experimental groups: 1) the control rats (body weight: 103 ± 5 g; mean ± SD) were fed a powdered (15% protein) diet with the same caloric content and composition as the diet provided during the observation period; 2) the low protein-fed rats (body weight: 102 ± 5 g) were fed a powdered 5% protein diet that was isocaloric to the control diet. This was achieved by increasing the carbohydrate content to 55% while maintaining the lipid content unchanged. Both diets were supplemented with vitamins and minerals.

Starting on the day of assignment to the test diets, (experimental day 0), each animal received the first of seven daily injections of 1 µg of T4, dissolved in 0.2 ml of isotonic saline made alkaline to pH 9.4 with 1 mol/l NaOH. T4 solutions were prepared daily before use. Twenty-four h after the seventh injection (day 7 of the experiment), control and low protein-fed rats were injected sc with 0.9% NaCl or with recombinant bGH in doses of 250, 500, 1000 and 5000 µg, dissolved in 0.2 ml saline (5 rats per dose). To avoid bias due to differences in nutritional status, rats of comparable weights were chosen from different cages and allocated to the various injection groups. Animals were sacrificed 24 h after GH or saline injections. Blood was allowed to clot at room temperature, centrifuged and the serum was stored at −20°C for measurement of IGF-I. The livers of the saline-treated animals were removed and homogenized immediately in ice-cold 0.3 mol/l sucrose (10% w/v) as previously described (Maes et al. 1983). The homogenates were kept frozen at −20°C until thawed for binding studies.

Radioimmunoassays

The RIA for IGF-I was carried out on unextracted serum using a non-equilibrium technique (Hurley et al. 1977). The standard was a pool of serum from 10 adult male rats and was assigned arbitrarily a potency of 1000 units/l. As reported previously (Maes et al. 1986), a good correlation (r = 0.89) is observed between IGF-I values of unextracted serum and those obtained after exposure to acid for 24 h at 37°C (0.1 mol/l glycine, pH 3.6).

Binding assays

Binding studies for determining the numbers and the affinity constants of the specific GH binding sites were performed as reported previously (Maes et al. 1983). In brief, 100 µl of 125I-bGH (20 000 cpm, SA: 80 µCi/µg) were incubated in triplicate with 100 µl of homogenate (1.6–2.1 mg protein) and 100 µl of increasing concentrations of unlabelled bGH (0, 0.5, 1, 2, 5, 10, 20, 50, 100, 200 and 500 ng).

Incubations were performed at 22°C for 2 h under continuous shaking in 25 mmol/l Tris-HCl, pH 7.4; 10 mmol/l CaCl2; and 0.1% BSA (w/v). At the end of the incubation, after addition of 3 ml of ice-cold Tris buffer, the tubes were centrifuged at 1500 × g for 30 min at 4°C. After aspiration of the supernatants, this procedure was repeated and the radioactivity of the pellets was counted in a gamma-spectrometer. The binding-inhibition experiments were expressed as bound vs free hormone concentrations. The analysis of these curves was done by use of a nonlinear curve-fitting programme (Knott 1979). The binding data were compatible with a three parameter model describing the binding of the ligand to one class of independent sites of high affinity and low capacity (specific binding), and a nonsaturable compartment of low affinity (non-specific binding). The protein and DNA contents of the homogenates were determined as described previously (Maes et al. 1984).
Statistical analysis

Statistical differences among experimental groups were determined by Student's unpaired two-tailed *t*-test (Snedecor & Cochran 1980). *P*-values less than 0.05 were considered to be significant. Values are expressed as mean ± SEM unless otherwise stated.

Results

By the end of the seventh day of diet and T4 therapy, the body weights of the control, 15% protein-fed hypox rats had increased by 5.6 ± 0.6 g (Fig. 1), a weight gain consistent with previous observations in T4-treated hypox rats (Maes et al. 1986). By contrast, the rats fed the 5% protein diet lost 2.8 ± 0.7 g (*P* < 0.001; 15 vs 5% protein-fed rats). The 5% protein-fed rats had lower liver wet weight (Table 1) and liver protein concentrations, but higher DNA concentrations per g of liver wet weight.

Therefore, the total liver DNA content of the rats fed the 5% protein diet was not significantly different from that of controls (17.1 ± 0.7 vs 18.6 ± 0.7 mg/liver; *P* < 0.2), whereas their protein content was significantly lower (473.5 ± 16.9 vs 709.9 ± 18.8 mg/liver; *P* < 0.001).

There was a non-significant decrease in the number of the specific hepatic GH binding sites in the protein-malnourished rats when expressed per liver (30% decrease) or per mg of DNA (22% decrease; Fig. 2). No decrease was observed, however, when binding was expressed per mg of protein. No significant difference between the affinity constants of the GH binding sites of control and protein-deprived rats was present (0.78 ± 0.05 vs 0.78 ± 0.07 × 10^9 l/mol).

Although basal serum IGF-I concentrations of control (15% protein) and protein-deficient (5%) rats were not significantly different (40 ± 10 U/l; *N* = 10), the IGF-I responses to injection of GH in the protein-malnourished rats were severely blunted when compared with the responses of the controls (Fig. 3). Whereas the mean IGF-I concentration of the 15% protein-fed animals injected with the 5 mg dose increased 9-fold over basal values, only a 3-fold increase was observed in the

![Graph](chart.png)

**Fig. 1.**

Cumulative body weight changes of hypophysectomized rats fed a 15% protein diet (●; *N* = 25) or a 5% protein diet (○; *N* = 25). Starting on the first day of the experiment (day 0), each rat received a daily sc injection of 1 μg of T4 for 7 days until day 6. Data are represented as means ± SEM. *P* < 0.001 15 vs 5% protein-fed rats from day 1 until day 7.
5% protein-fed rats (360 ± 30 vs 130 ± 40 U/l; P < 0.001). Also their mean serum IGF-I responses to the lower doses of GH were decreased, but only significantly so for the 1 mg dose.

Twenty-four hours after GH injections, there was a increase in the body weights of the control hypox rats (GH-treated: 6.4 ± 0.5 g; N = 20 vs saline-treated: 0.5 ± 1.3 g; N = 5; P < 0.05). By contrast, the body weights of the 5% protein-fed rats showed no or little increase after GH injections (GH-treated: 1.0 ± 0.6; N = 20). The body weight changes of the GH-treated rats fed the low protein diet were significantly (P < 0.05) lower than those of the controls, except for the 5 mg-injected rats.

Table 1.
Liver wet weight, protein and DNA concentrations of 15 and 5% protein-fed hypophysectomized rats, injected with saline and treated for 7 days with daily injections of 1 µg of T₄.

<table>
<thead>
<tr>
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<th>15% protein (N = 5)</th>
<th>5% protein (N = 5)</th>
</tr>
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<tbody>
<tr>
<td>Liver wet weight (g)</td>
<td>3.65 ± 0.17</td>
<td>2.82 ± 0.21*</td>
</tr>
<tr>
<td>Liver protein (mg/g wet weight)</td>
<td>195.9 ± 8.0</td>
<td>170.6 ± 8.3 NS</td>
</tr>
<tr>
<td>Liver DNA (mg/g wet weight)</td>
<td>5.2 ± 0.2</td>
<td>6.1 ± 0.3*</td>
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* P < 0.025 and NS: not significant vs 15% protein-fed rats, mean ± SEM.

Discussion
The blunted serum IGF-I responses to GH, together with decreased maximal stimulation in the 5% protein-fed hypox rats support the possibility that a postreceptor defect in GH action contributes to the GH resistance in protein-calorie malnutrition. Our findings underscore the importance of nutritional status in conditioning the biological responses of peripheral tissues to GH.

Because GH binding was assessed only in the saline-treated animals, we cannot exclude the possibility of up-regulation of GH binding sites in the 15% protein-fed rats after GH treatment, thereby accounting for the difference in the IGF-I dose-

Fig. 2.
Liver GH binding capacities of hypophysectomized rats fed a 15% protein (■; N = 5) or a 5% protein diet (□; N = 5). Livers were obtained 24 h after sc injection of saline given on day 7 of the experiment. The number of the binding sites was determined on homogenates from binding-inhibition curves as described in Materials and Methods. GH binding capacities are expressed as pmol per liver, pmol per mg DNA or fmol per mg of protein. The data are represented as means ± SEM.
response curves. Such induction of GH binding sites 24 h after a single injection of GH is unlikely, however, because up-regulation of hepatic GH receptors in hypox rats has been observed only after one week of continuous infusion of GH (Baxter & Zaltsman 1984). In addition, after single injections of GH (10 to 500 µg), liver GH receptors are acutely down-regulated and return after 24 h to values similar to those measured in the livers of saline-treated animals (Maiter et al., in press).

Parallel decreases in hepatic GH binding sites and serum IGF-I concentrations have been described previously in fasted (Baxter et al. 1981; Postel-Vinay et al. 1982) and 5% protein-refed rats (Maes et al. 1984), suggesting a possible regulatory role of somatogenic binding sites on IGF-I secretion. These findings and those reported in this study suggest that the mechanisms involved in the GH resistance might be dependent upon the severity of the nutritional insult. Accordingly, stringent dietary restrictions such as fasting may produce GH resistance by reducing GH binding sites, whereas less severe nutritional deficiency such as short-term protein deprivation may cause GH resistance through postreceptor mechanisms. The relative contributions of these defects in the decline of IGF-I in non-hypophysectomized protein-malnourished animals as well as their possible interrelationships remains to be determined.

It is possible that the GH resistance present in protein-calorie malnutrition is reversible after prolonged exposure to GH, because Pilistine et al. (1984) have reported that serum IGF-I and IGF-II concentrations decrease after severe protein deprivation in pregnant rats, and that this effect is reversed by continuous infusion of human GH.

The increases observed in serum IGF-I and body weights following GH injections in hypox rats consuming the 15% protein diet confirm our previous observations (Maes et al. 1986). However, the magnitude of these changes is less than described previously. This apparent discrepancy might be due to differences in diet formulations, in ages of the hypox animals or duration of hypophysectomy before injecting GH. Such variable responses to GH are in accordance with previous studies (Phillips et al. 1974).

As in protein-calorie malnutrition, hepatic resistance to GH is present in poorly controlled insulin-dependent diabetic children (Winter et al.

**Fig. 3.**

Serum IGF-I concentrations of 15% protein-fed (●) and 5% protein-fed (○) hypophysectomized rats 24 h after sc injections of saline or graded doses of recombinant bGH. GH was injected on day 7 of the experiment after seven daily sc injections of 1 µg of T₄ per rat. Each value represents the mean of 5 determinations from individual animals, the brackets represent 1 SEM. P < 0.025; **P < 0.001; NS, not significant: 15% vs 5% protein-fed rats.
1980; Lanes et al. 1985) and in diabetic rats (Phillips & Young 1976), since injection of GH does not produce any significant increase in their serum IGF concentrations. As with protein deprivation, alterations in GH binding and events that follow binding have been proposed as possible mechanisms for the GH resistance in diabetes. In particular, rats with severe insulin-dependent diabetes have decreased numbers of somatogenic liver binding sites (Baxter et al. 1980), whereas rats with moderately severe diabetes have unaltered GH binding (Maes et al. 1983). Supporting the existence of a postreceptor defect, we have reported that the increase of serum IGF-I concentrations of diabetic hypox rats in response to GH injections was severely attenuated, whereas no significant changes in hepatic GH binding occurred (Maes et al. 1986).

The in vitro studies of Shapiro et al. (1978) suggest that the reduced serum IGF-I concentrations in protein deficiency might be due to the decreased hepatic synthesis and/or release of this growth factor. It is likely that this decreased IGF-I release/synthesis is due to a generalized depression of hepatic protein synthesis. It is not known, however, whether the synthesis of hepatic secretory proteins such as IGF-I is affected more than the synthesis of intracellular proteins. Such differential effects on liver protein synthesis has been observed in insulin-dependent diabetic rats in which the synthesis rate of albumin was relatively more reduced than the rate of total protein synthesis (Peavy et al. 1978). Decreased synthesis might result from alterations in transcription or/and translation of IGF. The finding that changes in serum IGF-I during fasting and refeeding in rats are paralleled by changes in the liver mRNAs for IGF-I (Emler & Schalch 1987) suggests that the GH postreceptor defect observed in our study may be related to impaired IGF-I transcription. Whether these intracellular disturbances are related directly to limited nutrient availability or hormonal changes induced by malnutrition needs further study.

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Dr M. Maes,
Unité de Diabète et Croissance, UCL 5474,
Avenue Hippocrate 54
B-1200 Brussels,
Belgium.