Reduced serum concentrations of insulin-like growth factor-I (IGF-I) in protein-restricted growing rats are accompanied by reduced IGF-I mRNA levels in liver and skeletal muscle


Department of Animal Science, Michigan State University, East Lansing, Michigan 48824, U.S.A.
*Department of Pediatrics, University of North Carolina, Chapel Hill, North Carolina 27599, U.S.A.
†Unité de Diabetologie et Nutrition, University of Louvain School of Medicine, Brussels, Belgium

(Requests for offprints should be addressed to M. J. VandeHaar)

RECEIVED 1 November 1990

ABSTRACT

The serum concentration of insulin-like growth factor-I (IGF-I) is reduced in growing rats fed a low-protein diet, and this decrease is age-dependent, being more pronounced in younger animals. To determine whether this decrease in serum IGF-I is related to a decrease in IGF-I mRNA, growing female rats were given free access to either a 15% protein-sufficient or a 5% protein-deficient diet for 1 week. Protein restriction in 4-week-old rats decreased body weight gain by 44% (P<0.001 compared with 4-week controls), serum IGF-I concentration by 67% (P<0.001) and liver IGF-I mRNA abundance by 51% (P<0.001). During week 6, protein restriction for 1 week resulted in a 20% increase in food intake with no change in weight gain, a 38% reduction in serum IGF-I (P<0.001 compared with 6-week controls) and a 39% decrease in liver IGF-I mRNA (P<0.001). The serum IGF-I concentration was highly correlated (r=0.80; P<0.001) with the hepatic IGF-I mRNA concentration. Skeletal muscle IGF-I mRNA abundance was also decreased significantly by protein restriction (37% at week 4, P<0.001, and 24% at week 6, P<0.01) and was closely correlated (r=0.71; P<0.001) with body weight gain. Liver GH-binding protein and GH receptor mRNA abundance were reduced by 1 week of protein deprivation at week 6 but not at week 4. We conclude that the reduced serum IGF-I of young rats fed a low-protein diet is due, in part, to reduced liver IGF-I mRNA, and that these changes are not dependent on GH binding. Decreased skeletal muscle IGF-I mRNA during protein restriction is consistent with an autocrine/paracrine action of IGF-I in muscle.

Journal of Endocrinology (1991) 130, 305–312

INTRODUCTION

Fasting causes marked reductions in serum concentrations of insulin-like growth factor I (IGF-I) in humans and rats and in the abundance of mRNA for IGF-I in rat liver (Phillips & Young, 1976; Clemmons, Klibanski, Underwood et al. 1981; Merimee, Zapf & Froesch, 1982; Maes, Underwood & Ketelslegers, 1984; Emler & Schalch, 1987). These effects may be mediated by reductions in growth hormone (GH) binding, because liver GH binding and GH receptor mRNA levels are decreased in fasted rats (Maes, Underwood & Ketelslegers, 1983; Maes et al. 1984; Straus & Takemoto, 1990). When dietary protein is restricted, IGF-I in the serum of humans and rats (Shapiro & Pimstone, 1978; Prewitt, D’Ercole, Switzer & Van Wyk, 1982; Isley, Underwood & Clemmons, 1983) and IGF-I mRNA in the liver of rats also decline (Bornfeldt, Anrqvist, Enberg et al. 1989; Moats-Staats, Brady, Underwood & D’Ercole, 1989). This decline in serum IGF-I is age-related, being more marked in young animals (Prewitt et al. 1982; Fliesen, Maiter, Gerard et al. 1989). Protein restriction, unlike fasting, decreases GH binding only modestly, and probably not to an extent sufficient to account for the changes in serum IGF-I. Rather, there is evidence for intracellular, post-receptor resistance to GH action (Maes, Amand, Underwood et al. 1988).
In the present study, we subjected rats to 1 week of dietary protein restriction to determine the relationships between serum IGF-I concentration and the abundance of mRNAs for IGF-I, GH receptor and GH-binding protein in liver. We also examined IGF-I mRNA abundance in skeletal muscle during protein restriction. An important target tissue for growth with major requirements for protein substrates, rat skeletal muscle has been shown to possess IGF-I mRNA, which increases after GH administration (Isgaard, Carlsson, Isaksson & Jansson, 1988) and during regeneration after ischaemic injury (Edwall, Schalling, Jenische & Norstedt, 1989). To determine whether the changes in IGF-I expression induced by protein restriction are age-dependent during early growth, as we have observed for GH binding (Fliesen et al. 1989), rats were studied after 1 week of dietary treatment ending at 4 and 6 weeks of age.

MATERIALS AND METHODS

Experimental design

Female Sprague–Dawley rats (Charles River, Wilmington, MA, U.S.A.) were weaned at day 20, housed individually, and given free access to water and a pelleted diet containing 15% protein (control diet). Each kg of this diet contained 173 g casein, 3 g methionine, 529 g sucrose, 150 g corn starch, 50 g corn oil, 50 g cellulose and 45 g minerals and vitamins (diet no. 87448; Teklad, Madison, WI, U.S.A.). The low-protein diet contained 5% protein, and each kg contained 58 g casein, 1 g methionine, 629 g sucrose, 51 g corn oil, 65 g cellulose and 46 g minerals and vitamins (Teklad diet no. 87449). These diets were isocaloric (15.7 KJ/g) and contained similar amounts of fat (5.2%) and all essential minerals and vitamins (0–68% calcium and 0–54% phosphorus).

On day 22, rats weighed 55 ± 5 (s.e.m.) g and were randomly assigned to one of five groups with ten rats per group. One group was killed on day 23 (week-3 controls), two groups were fed the control diet until they were killed (week-4 and -6 controls), and two groups were switched to the low-protein diet 1 week before being killed (week-4 and -6 low-protein). Control rats were killed on day 29 (4 week) or on day 43 (6 week), 1 day before rats fed the low-protein diet. Diet intake and body weight gain were determined from data collected twice weekly. Rats were anaesthetized with pentobarbital (10 mg/100 g body weight), bled by cardiac puncture and killed by decapitation. Serum was stored at −20 °C. Liver and longissimus muscle were removed immediately, blotted, frozen in liquid nitrogen, weighed and stored at −70 °C.

Laboratory methods

Serum IGF-I concentrations were measured after removal of IGF-binding proteins (greater than 99% removal) by C-18 silica cartridge chromatography (Sep-Pak; Waters Associates, Milford, MA, U.S.A.) (Maiter, Underwood, Maes et al. 1988b). Recovery of IGF-I by this extraction method was 68%, and IGF-I concentrations were corrected for this recovery. The radioimmunoassay (RIA) for IGF-I used a purified IGF-I standard from human plasma (Svoboda & Van Wyk, 1985) and a polyclonal antiserum (No. 805) that has <0.5% cross-reactivity with IGF-II. This assay has been described and validated previously (Copeland, Underwood & Van Wyk, 1980).

Measurements of hepatic somatogenic GH binding were performed with 125I-labelled bovine GH as described previously (Maiter, Underwood, Maes & Ketelslegers, 1988c), with the exception that homogenates were prepared from fresh rather than fresh liver. Frozen tissue was briefly homogenized with an Ultra-Turrax T25 blender (low speed, 10 s; Janke & Kunkel, IKA, Labor Technik, Staufen, Germany), and this suspension was used in further homogenization and processing. The GH-binding capacities of homogenates prepared from fresh and frozen liver were essentially the same. Water-treated homogenates were used to determine free GH-binding sites and MgCl2-treated pellets were used to determine total binding sites. Pellets were resuspended in water for measurement of protein content (Lowry, Rosebrough, Farr & Randall, 1951).

The abundance of hepatic IGF-I, GH receptor and GH-binding protein mRNAs were determined by Northern blot analysis. To reduce the number of samples processed, five pools of liver (two rats/pooled sample) were formed for each diet by age group. Liver poly(A)+ RNA was isolated (Aviv & Leder, 1972; Ulrich, Shine, Chirgwin et al. 1977; Chirgwin, Przybyla, MacDonald & Rutter, 1979), and then samples in duplicate were size-fractionated (Thomas, 1980) and transferred to nylon membranes (GeneScreen, New England Nuclear, Cambridge, MA, U.S.A.) as described previously (Lund, Moats-Staats, Hynes et al. 1986). For analysis of IGF-I, blots contained 20 µg of poly(A)+ RNA per lane and, for analysis of the GH receptor, blots contained 10 µg of poly(A)+ RNA per lane. The amount of RNA per lane was verified by ethidium bromide staining. For IGF-I, a 786 bp rat IGF-IA cDNA that includes the entire coding sequence (Casella, Smith, Van Wyk et al. 1987) was labelled with 32P by nick translation (Rigby, Diekmann, Rhodes & Berg, 1977). For the GH receptor, a 951 bp rat cDNA (gift of L. S. Mathews, Salk Institute, La Jolla, CA, U.S.A.) was used to prepare a 32P-labelled antisense riboprobe (Cox, Journal of Endocrinology (1991) 130, 305–312
DeLeon, Angerer & Angerer, 1984) with homology to the extracellular, transmembrane and intracellular domains of the GH receptor (Mathews, Enberg & Norstedt, 1989). Labelled blots were washed and exposed to autoradiography film (Kodak XRP; Eastman Kodak Company, Rochester, NY, U.S.A.) for 3 and 5 days to ensure a linear densitometric response. Hybridization signal intensity was quantified by densitometry (Ultrascan SL; LKB Instruments, Piscataway, NJ, U.S.A.), with each lane scanned along its longitudinal axis to quantify all IGF-I mRNA species. The relative abundances of the GH receptor mRNA (4.3 kb) and the GH-binding protein mRNA (1.3 kb) were determined at 4 and 6 weeks.

Pools of muscle (0.2 g/rat; two rats/sample) were formed as for the liver, and total nucleic acids (TNA) were isolated (Durnam & Palmiter, 1983). The abundance of IGF-I mRNA in TNA samples (30–80 μg) was determined by a ribonuclease protection–solution hybridization assay using 9000 c.p.m. 32P-labelled antisense rat IGF-I cRNA/tube (Davenport, D’Ercole & Underwood, 1990). The amount of rat IGF-I mRNA was quantified by comparing the radioactivity of the protected probe in the sample with that of a standard curve constructed from incubations with known amounts of the sense strand of rat IGF-I mRNA. Solution hybridization was used for muscle samples because adequate sensitivity could not be achieved using Northern hybridization. To validate the solution hybridization assay, liver TNA samples were prepared from week-4 rats for comparison with results of Northern blot analysis. We determined that the relative abundance of IGF-I mRNA in the livers of control versus protein-restricted rats measured by solution hybridization were the same as those measured by Northern hybridization.

The relative abundance of each mRNA species per μg poly(A)+ RNA or per μg TNA was calculated as a percentage of the respective mRNA abundance in week-4 control rats. Statistically significant differences among groups were detected by analysis of variance (ANOVA; SAS Institute, Cary, NC, U.S.A.). Specifically, contrast statements in ANOVA were made to test for effects of dietary protein at 4 and at 6 weeks of age, to test for an interaction of age with dietary protein, and to test for an effect of age in rats fed control diets. Results are reported as group means with pooled S.E.M. Pooled S.E.M. was calculated from the s.d. determined by ANOVA and was justified because the assumption of homogeneous variance was judged tenable.

RESULTS

Dietary protein restriction for 1 week produced a 44% reduction in the rate of body weight gain during week 4 (P<0.001 vs controls) but had no effect on weight gain during week 6 (Table 1). Intake of the low-protein diet was not significantly different from intake of the control diet during week 4, but was increased during week 6 (P<0.001 vs week-6 controls). Taking both weight gain and food intake into account, food efficiency (g weight gain/g food eaten) was less for restricted rats than for controls at both 4 and 6 weeks of age (P<0.05).

Protein restriction for 1 week caused the expected reduction in serum IGF-I concentrations during week 4 (67% reduction; P<0.001 vs week-4 controls) and during week 6 (38% reduction; P<0.001 vs week-6 controls; Fig. 1a). The reduction observed at week 4, however, was not significantly greater than that at week 6. Serum IGF-I increased with age in control (15% protein diet) rats as reported previously (Handelsman, Spaliviero, Scott & Baxter, 1987).

The abundance of IGF-I mRNA in liver was reduced by 51% by protein restriction during week 4 (P<0.001 vs week-4 controls; Fig. 1b) and by 39% during week 6 (P<0.001 vs week-6 controls).

**TABLE 1. Growth and food intake of rats fed control or low-protein diets for 1 week before being killed at 3, 4 and 6 weeks of age. Values are group means for ten rats per group, with the pooled S.E.M. determined from analysis of variance (ANOVA)**

| Week 3 | | Week 4 | | Low protein | | Week 6 | | Low protein | | Pooled S.E.M. |
|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
|        | Control | Control |        |        |        |        |        |        |        |
| Body weight (g) | 60 | 96 | 85** |        |        |        |        |        |        |
| Weight gain (g/day) | 4-1 | 6-5 | 3-7*** |        |        |        |        |        |        |
| Food intake (g/day) | 9-8 | 14-4 | 15-7 |        |        |        |        |        |        |
| Food efficiency (%) | 43 | 45 | 23*** |        |        |        |        |        |        |
| Liver weight (g) | 2-8 | 5-3 | 5-3 |        |        |        |        |        |        |
| Control | 172 | 5-8 | 21-0 | 28 | 9-4 | 10-7** |        |        |        |
| Low protein | 175 | 5-6 | 25-4*** | 22* | 10-7 | 9-4 |        |        |        |
| Pooled S.E.M. | 3 | 0-4 | 0-5 | 2 | 0-3 |        |        |        |        |

*P<0.05, **P<0.01, ***P<0.001 for the effect of dietary protein at week 4 or week 6 using contrast statements in ANOVA.

Age significantly (P<0.001 using contrasts in ANOVA) altered body weight, food intake, food efficiency and liver weight in control animals. The interaction between age and diet was significant (P<0.01 using contrasts in ANOVA) for weight gain, food intake and food efficiency.

1Weight gain was calculated for the last 3 days before rats were killed.

2Food efficiency was calculated as g weight gain/g food intake.

Journal of Endocrinology (1991) 130,305-312
during week 6 \( (P < 0.001 \text{ vs week-6 controls}) \). The abundance of IGF-I mRNA increased significantly with age, but the difference in the effect of dietary protein restriction at weeks 4 and 6 was not statistically different. Protein restriction reduced the intensity of the 7.5 kb IGF-I mRNA species more than the other IGF-I mRNA size classes (Fig. 2). At 4 weeks, the 7.5 kb band in protein-restricted rats was 5.3% of total IGF-I mRNA vs 6.9% for controls, and at 6 weeks it was 4.1% vs 7.6% for controls. Using data from control rats at 3 weeks of age and control and protein-restricted rats at 4 and 6 weeks, liver IGF-I mRNA abundance correlated closely with serum IGF-I concentration \( (r = 0.80; P < 0.001) \).

The abundance of IGF-I mRNA per µg TNA of muscle was about 5% that of liver. As observed with liver, the abundance of muscle IGF-I mRNA was decreased by protein restriction at both week 4 (37% decrease, \( P < 0.001 \text{ vs week-4 controls} \)) and week 6 (24% decrease, \( P < 0.01 \text{ vs week-6 controls} \), Fig. 3).
TABLE 2. GH binding and the abundance of GH receptor mRNA in liver of rats fed control or low-protein diets for 1 week before being killed at 3, 4 and 6 weeks of age. Values for GH binding are group means for ten rats per group, with the pooled s.e.m. determined from analysis of variance (ANOVA). Values for mRNA abundance are from five pooled samples per group.

<table>
<thead>
<tr>
<th></th>
<th>Week 3</th>
<th>Week 4</th>
<th>Week 6</th>
<th>Pooled S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Low protein</td>
<td>Control</td>
<td>Low protein</td>
</tr>
<tr>
<td>Liver GH binding†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free</td>
<td>4.9</td>
<td>4.8</td>
<td>3.9</td>
<td>4.9*</td>
</tr>
<tr>
<td>Total</td>
<td>8.4</td>
<td>10.7</td>
<td>10.7</td>
<td>12.7</td>
</tr>
<tr>
<td>mRNA abundance‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GH receptor</td>
<td>100</td>
<td>83</td>
<td>147</td>
<td>69**</td>
</tr>
<tr>
<td>GH-binding protein</td>
<td>100</td>
<td>94</td>
<td>176</td>
<td>113**</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01 for the effect of dietary protein at week 4 or week 6 using contrast statements in ANOVA.

The effect of age was significant (P < 0.05 using contrasts in ANOVA) in control rats for all parameters listed in the table.

†GH binding is expressed as % of total labelled bovine GH specifically bound/mg protein.

‡MRNA data are expressed relative to week-4 controls as abundance/μg poly(A)⁺ RNA.

The abundance of muscle IGF-I mRNA correlated more closely with body weight gain (r = 0.71, P < 0.001) than did serum IGF-I concentration (r = 0.56) or liver IGF-I mRNA abundance (r = 0.61).

Although the free GH binding by liver homogenates (water treated) was decreased at week 6 but not at week 4, the decline in the more relevant total GH binding (MgCl₂ treated) did not reach statistical significance with protein restriction at either week 4 or 6 (Table 2). Concordant with the GH-binding data, dietary protein restriction during week 4 did not change the abundance of GH receptor or GH-binding protein mRNAs. At week 6, however, protein restriction significantly reduced the abundance of both the GH receptor mRNA (4.3 kb size class; 53% decrease, P < 0.01 vs week-6 controls) and the GH-binding protein mRNA (1.3 kb; 36% decrease, P < 0.01; Fig. 4).

DISCUSSION

Our results confirm that dietary protein restriction reduces serum IGF-I concentrations (Shapiro & Pimstone, 1978; Prewitt et al. 1982; Isley et al. 1983; Maes et al. 1988; Fliesen et al. 1989) and indicate that there is a close relationship (r = 0.8) between the decline in serum IGF-I and the steady-state levels of IGF-I mRNA in liver. These results are consistent with an earlier observation that liver IGF-I mRNA abundance and serum IGF-I concentrations are highly correlated (r = 0.9) in young rats during fasting and refeeding (Emler & Schalch, 1987). Straus & Takemoto (1990) found that decreased rates of IGF-I transcription did not account for this reduced
abundance of IGF-I mRNA in the livers of fasted rats, suggesting post-transcriptional regulation of mRNA stability. The half-life for the 7.5 kb IGF-I mRNA species is much shorter than that of the other IGF-I mRNA size classes (Hepler, Van Wyk & Lund, 1990). Thus, our finding that the 7.5 kb IGF-I mRNA was more sensitive to protein deprivation than the smaller IGF-I mRNA size classes is consistent with the idea that decreased mRNA stability may be partly responsible for reduced levels of IGF-I mRNA during protein restriction.

In addition to decreased abundance of IGF-I mRNA, other mechanisms may also contribute to the reduced serum IGF-I concentration in dietary restriction. Dietary protein restriction causes a decrease in the serum concentration of IGF binding protein-3 (IGFBP-3), the principal carrier of IGF-I in serum (Clemmons, Thissen, Maes et al. 1989). Reduced IGFBP-3 may be responsible for increased clearance of serum IGF-I, as observed in pregnant rats having low IGFBP-3 (Davenport, Clemmons, Miles et al. 1990).

Our results confirm that changes in binding of GH to its receptors are not critical for the reductions in serum IGF-I concentration during protein restriction. Although GH binding by liver homogenates is decreased by fasting (Maes et al. 1983), protein restriction for 1 week decreased GH binding only in rats killed at 5 and 7 weeks, but decreased serum IGF-I in both younger and older rats (Fliesen et al. 1989). We have also reported that serum IGF-I concentrations fall more rapidly than GH-binding activity after the initiation of protein restriction (Maiter, Maes, Underwood et al. 1988a). In addition, protein-restricted hypophysecotomized rats have post-receptor resistance to GH, as indicated by impaired ability of GH injections to stimulate IGF-I concentrations despite normal GH binding (Maes et al. 1988). In the present experiment, concentrations of liver IGF-I mRNA and serum IGF-I protein were markedly reduced in 4-week-old animals, in which no significant reduction in GH binding was observed. Consistent with this minimal effect on GH binding was the absence of a significant change in mRNA abundance for the liver GH receptor and GH-binding protein at 4 weeks. At 6 weeks, levels of the GH receptor and binding protein mRNA were reduced, but to a greater extent than the changes in GH binding, suggesting that post-mRNA events may be involved in the regulation of GH binding.

We and others have reported that young growing rats have significantly greater reductions in growth and serum IGF-I during protein restriction than older rats (Prewitt et al. 1982; Fliesen et al. 1989; Phillips, Drakenberg, Persson et al. 1989). In the current study, we observed a significant effect of age on the growth response to protein restriction, but not on the serum IGF-I response. Protein restriction for 1 week during week 4 decreased weight gain, but had no such effect during week 6, reflecting the greater dependency that young animals have on dietary protein. While week-6 protein-restricted rats ate more food and gained weight normally, their concentrations of serum IGF-I and liver IGF-I mRNA were reduced significantly, suggesting that IGF-I expression is a more sensitive index of the adverse effects of protein restriction than is weight gain. We assume that more prolonged protein restriction would have produced greater effects on the growth of these animals.

Previous studies in hypophysecotomized rats have shown that the concentrations of IGF-I and its mRNA in muscle are increased by injection of GH (D'Ercole, Stiles & Underwood, 1984; Isgaard et al. 1988) and during post-ischaemic muscle regeneration (Edwall et al. 1989). Whereas Bornfeldt et al. (1989) found no change in IGF-I mRNA abundance in heart or diaphragm muscle during protein restriction, our study shows that the abundance of IGF-I mRNA in skeletal muscle is influenced by protein nutritional status. Protein restriction during both weeks 4 and 6 caused significant decreases in IGF-I mRNA levels in longissimus muscle. Furthermore, body weight gain was more highly correlated with skeletal muscle IGF-I mRNA levels than with liver IGF-I mRNA or serum IGF-I concentrations. Muscle growth comprises a major component of body weight gain in young growing animals. Thus, this finding suggests that locally produced IGF-I may be an important regulator of muscle growth and further supports the hypothesis of an autocrine/paracrine mode of action for IGF-I in muscle.

ACKNOWLEDGEMENTS

This work was supported by NIH Research Grants HD-08299 and AM-01022, NIH Training Grant AM-07129 and NIH Clinical Investigator Award HD-00857. Support was also provided by the Michigan Agriculture Experiment Station and the Fonds National de la Recherche Scientifique Medicale (3.4538.80) and Fonds National de la Recherche Scientifique (1.5.333.86F), Brussels, Belgium.

REFERENCES


