The Effects of Altered Nutritional Status upon Insulin-Like Growth Factors and their Binding Proteins in Neonatal Rats¹

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ABSTRACT. To investigate the role of nutrition in the regulation of IGFs during the perinatal period, 10-d-old rats were infused intravenously with various concentrations of nutrients for 24 h. Breast-fed litter mates served as controls. The effect of caloric intake on concentrations of IGF-I and IGF-II as well as IGF-binding proteins in serum, liver, and brain of neonatal rats was studied. A total of 45 rats from 10 litters was infused with solutions ranging from a caloric intake of 0 (saline) to 75% (glucose, amino acids, and lipids) of the estimated intake of control rats. In serum, both IGF-I and -II concentrations fell markedly in response to fasting. Serum IGF-II levels were linearly related to caloric intake in the pooled data from all groups. Concentrations of IGF-II, but not IGF-I, in liver and brain were depressed by caloric restriction. In contrast to the fall in IGF concentrations, activity of IGF-associated binding proteins rose in serum and in liver cytosol 2- to 4-fold in response to decreased nutrient intake. In serum, but not liver, the rise in binding protein activity was inversely related to caloric intake. In liver cytosol, but not serum, the rise in binding protein activity was inversely related to total serum amino acid concentration. Thus, IGF concentrations in preweanling rats change in response to alterations of nutrient intake. The fasting induced decrements in IGF levels, as well as the elevations in IGF-associated binding protein activity, may serve as a protective mechanism to depress growth in times of caloric restriction. (*Pediatr Res* 26: 128-134, 1989)

Abbreviations

CON, control
SAL, normal saline
GLU, glucose
GA, glucose and amino acids
GAL, glucose, amino acids, and lipid
RRA, radioreceptor assay
HSA, human serum albumin
GH, growth hormone

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Somatomedins, or IGF, comprise a group of peptides having both mitogenic and insulin-like metabolic effects in a variety of mammalian cell types *in vitro* (1, 2). Because of the extremely rapid growth rate of the mammalian fetus and neonate, attention has recently focused upon the somatomedins as possible regulators of fetal somatic and organ growth (3, 4). Little is known, however, about those factors that alter cellular synthesis of somatomedins in the perinatal period. As in the adult (5), *in vitro* evidence suggests that substrate availability may be of importance in regulating somatomedin secretion (4).

During the perinatal period, developmental changes in somatomedins have been studied most extensively in the rat (4, 6). In this species, during fetal life and before weaning (21 days), the predominant somatomedin in blood and tissues is an IGF-II (previously known as multiplication-stimulating activity) which shares close homology with human IGF-II (1, 2, 6). After weaning, the predominant somatomedin species is an IGF-I similar to human IGF-I (3, 4, 7). Unlike the adult, GH does not seem to be of importance in IGF regulation in the preweanling rat (8). For these reasons a study was performed to investigate the role of nutrition in the regulation of IGF-I and -II synthesis during the perinatal period in the rat. In addition, because of the difficulty in measurement of somatomedin activity due to the presence of binding proteins in most biologic fluids (9), care was taken to separate binding proteins from IGF-I and -II before assay.

MATERIALS AND METHODS

Surgical preparation. Male Sprague-Dawley rats from 10 litters between 10 and 12 d postnatal age were studied. Polyethylene chloride catheters were implanted in the right external jugular vein under ether anesthesia. Catheters were secured with a 4-0 silk ligature and a small section of surgical plastic tape. Postoperatively, animals were housed in individual cushioned trays within a humidified Armstrong "Care-Ette" incubator (Ohio Chemical, Madison, WI) at 29°C ambient temperature. Litter mates were obtained as CON and were subjected to similar anesthesia and surgical incisions without catheterization. After recovery these CON animals were then replaced with their respective dams. These studies were approved by the Animal Care Committees of the Karolinska Institute and The University of Connecticut Health Center.

Experimental protocol. After a 2-h postoperative recovery period, catheterized rats were begun on an iv infusion of one of four nutrient solutions of different composition: SAL, GLU, GA, and GAL. No enteral nutrition was provided. Solutions for infusion were made daily under sterile conditions from the following stock solutions: amino acids as Vamin 14 g N/100 mL

(Kabi Vitrum, Stockholm, Sweden), lipid as Intralipid 10 g/100 mL (Kabi Vitrum), and glucose 50% in sterile water. Final volume of delivery was adjusted to 5.2 mL using sterile water. The infusions were given at rates between 180–230 μ L/h via a precalibrated electric syringe pump (Braun-Melsungen AG, Melsungen, West Germany) to achieve a fluid intake of approximately 200 μ L/g/d. Neonatal rats received tactile stimulation three to four times per day, both to mimic maternal handling and to stimulate micturition. CON litter mates were allowed to nurse *ad libitum* with their dams until the conclusion of the 24-h study period. Thus, for the 24-h study period, infused rats were to receive intakes of nutrients and calories as in Table 1.

At the conclusion of the experimental period, 20 μ L of blood were drawn via tail section in both infused rats and their milk fed controls and analyzed for plasma glucose concentration and hematocrit. All rats were then anesthetized with ether and exsanguinated by cardiac puncture. The blood obtained was allowed to coagulate at room temperature. Serum was removed and stored at -20°C for later assay for IGF and amino acids. Livers and brains were removed, blotted, weighed, and then stored at -80°C for later analysis.

Biochemical Methods. *RIA-IGF-I.* IGF-I RIA was performed using purified human IGF-I and [^{125}I]-IGF-I as standard and tracer-hormone, respectively, as previously described (1, 10). Results are expressed as ng/mL. The standard currently in use in our laboratory is a biosynthetic IGF-I (Kabi Gen AB, Stockholm, Sweden) and has an activity of approximately 180 ng IGF-I/U RIA-IGF-I activity, where 1 U of activity is arbitrarily defined as that activity present in 1 mL of a standard human serum. The term "IGF-I concentration" will be used to denote IGF-I assayed from acidified chromatographed fractions.

RRA-IGF-II. Membranes were prepared using the method of Marshall *et al.* (11) from the livers of 60- to 90-d-old male Sprague-Dawley rats. Studies regarding displacement of [^{125}I]-IGF-II from rat liver membranes were then performed. In comparison to purified human IGF-II (kindly supplied by G. Enberg, M.D., Karolinska Institute, Stockholm, Sweden), purified human IGF-I exhibited approximately 1% cross-reactivity. Human insulin did not cross-react with IGF-II in this assay. Incubation with multiplication-stimulating activity (Collaborative Research, Waltham, MA) produced displacement similar to that of IGF-II. Due to a paucity of purified IGF-II for standards, serial dilutions of an aliquot from a pool of standard human serum were used in the standard curve, and thus, results are expressed as U or mU/mL or g wet tissue wt. The standard serum was calibrated against purified IGF-II and contained 900 ng IGF-II/U IGF-2 activity, where 1 U of activity is again defined as that present in 1 mL of serum. Thus 1 mU/mL represents 0.9 ng/mL IGF-II concentration. Least detectable dose was approximately 20 mU/mL and inter- and intraassay coefficients of variation were 7.6 and 11.6%, respectively. The term "IGF-II concentration" will be used to denote IGF-II assayed from acidified chromatographed fractions and "RRA activity" for that activity assayed using the IGF-II RRA from whole serum, cytosol, or binding protein fractions from column chromatography. In these studies, the majority of RRA activity noted from whole serum and

cytosol specimens was due to binding proteins, and thus represents a crude estimate of binding protein activity.

Somatomedin Separation. *Sera.* All whole, untreated sera specimens were assayed for RRA-activity using the IGF-II RRA. IGF-I and IGF-II in sera specimens were then dissociated from binding proteins using acid gel chromatography. Approximately 300–400 μ L of serum obtained by pooling samples from two to three animals was acidified to 2 M with acetic acid and incubated overnight at $+4^{\circ}\text{C}$. The sample was then chromatographed at $+4^{\circ}\text{C}$ over a 48×1.0 cm Sephadex G-50 (Pharmacia Fine Chemicals, Piscataway, NJ) column equilibrated in 0.1 M acetic acid at a constant flow rate of 2.0 mL/h. Fractions from the column (1.5 mL/fraction) were lyophilized, reconstituted in 50 mM Tris (pH 7.40 at 21°C)–1% HSA buffer, and assayed for RIA-IGF-I and RRA-IGF-II concentrations. When tracer [^{125}I]-IGF-II was preincubated with serum and then acidified using this method, recovery of tracer hormone was 93%. Results are expressed as ng (IGF-I) or mU (IGF-II) per mL serum.

Cytosol. Cytosols from liver and brain specimens were obtained using the method of Sara *et al.* (12). Tissue was minced in 5 mL (per g tissue) of a solution of 50 mM Tris HCl and 0.2 mM phenylmethyl sulfonylfluoride (Boehringer-Mannheim, Inc, Penzberg, W. Germany), using a Polytron tissue homogenizer (Brinkman Instruments, Westbury, NY). The homogenate was then centrifuged at $50\,000 \times g$ for 30 min and the supernatant (cytosol) removed and then stored at -80°C for later assay. All cytosol specimens were assayed prior to chromatography for whole cytosol RRA using the RRA-IGF-II. As with the sera specimens, IGF-I and -II in cytosols were dissociated from binding proteins during the acidification process. Approximately 10 mL of cytosol obtained as a pool of two to three samples was dialyzed (Spectrapor no. 6 dialysis membrane, Spectrum Medical Industries, Los Angeles, CA) against 0.1 M acetic acid for 48 h at $+4^{\circ}\text{C}$. The supernatant was removed after centrifugation and concentrated to 1 mL using a rotary evaporator. The concentrated specimen was then chromatographed using a 40×1 cm Sephadex G-50 column equilibrated with 0.1 M acetic acid. Fractions were subsequently lyophilized, reconstituted in assay buffer, and assayed for both RIA-IGF-I and RRA-IGF-II concentrations. Recovery of a biosynthetic preparation of IGF-I (kindly supplied by L. Fryklund, Kabi Vitrum AB) from acidified cytosol was 72%.

Calculation of Somatomedin Activity (Fig. 1). All Sephadex G-50 columns used were calibrated with Blue Dextran, cobalt chloride, and [^{125}I]-IGF-II. Peak somatomedin activity was de-

Table 1. Constituents of Infusates in 10-d-old rats

	Fluid volume (mL)	Carbohydrate (g)	Lipid (g)	Amino acid (g)	Total cal
CON*	5.2	0.10	0.60	0.35	7.2
SAL	5.2	0.00	0.00	0.00	0.0
GLU	5.2	0.63	0.00	0.00	2.5
GA	5.2	0.10	0.00	0.26	1.4
GAL	5.2	0.10	0.40	0.26	5.2

* Approximate volume and nutrient concentration from Reference 15.

Fig. 1. Typical gel chromatograph of control 10-d-old rat serum. Serum was incubated in 2 M acetic acid and then chromatographed using G-50 Sephadex, 0.1 M acetic acid. Selected fractions were lyophilized, reconstituted in assay buffer, and analyzed for concentrations of RIA-IGF-I (open circles) and RRA-IGF-II (closed circles). The large high mol wt fraction represents IGF binding proteins.

ected at approximately 50% of the column vol with the total peak found between 30 and 70% of the column vol. Therefore, IGF-I and -II concentrations were defined as the total radioimmunoassayable or radioreceptorassayable activity present in these fractions, expressed as either ng (IGF-I) or mU (IGF-II) per g tissue wet wt.

Binding Protein Activity. That portion of the column fractions from 0–30% exhibited a peak of RRA activity in serum and cytosol, presumably related to the ability of binding proteins to compete with receptor for [125 I]-IGF-II binding. To test this assumption, liver cytosol was preincubated with [125 I]-IGF-II for 2 h at +4°C and then chromatographed over a 40 × 1 cm G-50 Sephadex column equilibrated with 50 mM Tris–0.2% HSA. Flow rate and fraction volume were as in the previous studies. Fractions were then counted in a γ -counter and the results plotted as fraction vol *versus* cpm and compared to the elution pattern of [125 I]-IGF-2 alone. Similar chromatograms were performed on a lyophilized aliquot of the cytosol reconstituted in Tris–0.2% HSA and also on another aliquot dialyzed against 0.1 M acetic acid, lyophilized, and then reconstituted in buffer. In further studies characterizing liver cytosol and serum binding proteins, similar schemata were used with chromatography performed on a 90 × 1.9 cm G-200 Sephadex column with 50 mM Tris–0.2% HSA. To compare chromatographs, results were converted to cpm per fraction as a percent of total counts added to the column (% cpm).

Miscellaneous. Glucose concentrations in plasma were measured using a glucose oxidase method (Beckman). Hb concentration was measured colorimetrically and protein concentration was determined using the method of Lowry *et al.* (13). The formula derived by D'Ercole *et al.* (14) was used to estimate the percentage blood concentration in cytosol samples of liver and brain. Some blood contamination of liver (but not brain) cytosol specimens was observed and could theoretically have accounted for up to 14% of the RRA activity observed in control livers. However, because of the relatively larger increase in RRA activity in liver cytosol *versus* serum in the experimental groups, maximal contamination from serum represented only 5% of the total RRA activity. In selected cases, plasma samples from four to six animals from each group were assayed individually for the con-

centrations of 20 amino acids using a Waters Liquid Chromatography Amino Acid Analyzer (Waters Associates, Milford, MA).

Statistical analysis. All results are expressed as mean $\pm$ SEM. Statistical significance was assessed using ANOVA and the unpaired Student's *t* test. Linear regression analysis was performed using the least squares method.

RESULTS

General metabolic parameters. A total of 45 rats was infused during the 24-h study period (Table 2). A total of 17 wt- and sex-matched litter mates served as the CON. No differences were observed in age or wt at the time of study (Table 2). At the conclusion of the study, all infused rats appeared vigorous and active. Plasma glucose concentration in the SAL infused group was 3.78 ± 0.44 mM ($54 \pm 6\%$ of the CON concentration, $p < 0.05$) and in the GLU was 8.22 ± 0.89 mM ($118 \pm 13\%$ of control, $p < 0.05$). In GA- or GAL-infused rats, plasma glucose concentrations were no different from the CON value of 6.94 ± 0.32 mM. In addition, packed red blood cell volumes in all five groups were similar, suggesting adequate hydration in the experimental groups. Caloric intakes in the four infused groups are also shown in Table 2. Caloric intakes of GLU and GA rats were not statistically different from one another, but were different from those of SAL or GAL rats. Results were not contrasted with those of CON rats because only estimates of caloric intake of breast-fed rat pups of similar age were available (15) for comparison.

IGF concentrations. When sera and cytosols from CON rats were subjected to acidification, column chromatography, and subsequent IGF-I-RIA and IGF-II-RRA concentration determinations, significant activities of both IGF were present. A representative chromatogram from CON acidified serum is shown in Figure 1 where the low mol wt portion (IGF, fractions 32–42) represents approximately 10% of the total RRA activity assayed, the remainder consisting of a higher mol wt binding protein portion (fractions 20–31).

After a 24-h fast (SAL group), IGF-I and IGF-II concentrations in serum fell to 12 and 24%, respectively, of control values (Table

Table 2. Selected biochemical parameters in 5 groups of 10-d-old rats

	(n)	Wt (g)	Age (d)	Caloric intake (kcal/g/d)	Final plasma glucose (mmol/L)	Final packed cell volume (%)	Total serum amino acid concentration (mM)
CON	(15)	21.7 ± 1.1	10.6 ± 0.2	0.4	6.94 ± 0.32	36 ± 1	4.16 ± 0.35
SAL	(7)	21.6 ± 1.0	10.9 ± 0.5	0	$3.78 \pm 0.44^*$	39 ± 1	3.17 ± 0.51
GLU	(8)	23.9 ± 1.1	10.9 ± 0.5	$0.09 \pm 0.01^\dagger$	$8.22 \pm 0.89^*$	37 ± 1	$2.25 \pm 0.33^*$
GA	(12)	22.2 ± 0.5	10.6 ± 0.5	$0.06 \pm 0.01^\dagger$	6.22 ± 0.28	37 ± 2	$8.12 \pm 1.81^*$
GAL	(16)	22.1 ± 0.6	10.3 ± 0.5	$0.25 \pm 0.01^\ddagger$	6.53 ± 0.18	34 ± 2	5.86 ± 0.70

* $p < 0.05$ from CON.

† $p < 0.05$ from SAL or GAL.

‡ $p < 0.05$ from SAL, GLU, or GA.

Table 3. Concentrations of IGF-I and IGF-II in acid gel chromatographed specimens

Serum				Liver			Brain		
	n	IGF-I (ng/mL)	IGF-II (mU/mL)	n	IGF-I (ng/g)	IGF-II (mU/g)	n	IGF-I (ng/g)	IGF-II (mU/g)
CON	5	301 ± 45	230 ± 16	4	23 ± 6	143 ± 31	3	7 ± 2	127 ± 32
SAL	3	$35 \pm 23^*$	$56 \pm 31^*$	3	20 ± 3	$60 \pm 13^*$	3	5 ± 1	76 ± 7
GLU	4	$80 \pm 34^*$	$121 \pm 25^*$	3	12 ± 1	$33 \pm 10^*$	3	9 ± 5	$37 \pm 5^*$
GA	5	$50 \pm 35^*$	$139 \pm 10^*\dagger$	4	17 ± 4	$25 \pm 7^*$	3	3 ± 1	$55 \pm 18^*$
GAL	6	$30 \pm 6^*$	$161 \pm 17^*\dagger$	4	12 ± 2	$16 \pm 3^*$	5	3 ± 1	71 ± 21

* $p < 0.05$ from CON.

† $p < 0.05$ from SAL.

3). Intravenous nutrition did not prevent the fall in serum IGF-I or IGF-II concentrations. However, serum concentrations of IGF-II were 70% of control in GAL rats ($p < 0.02$ different from SAL rats). When IGF-II concentrations in serum were plotted against caloric intake (Fig. 2A), a significant linear relationship was observed. However, no relationship between caloric intake and serum IGF-I concentration was noted (Fig. 2B).

In liver cytosols, fasting (SAL group) induced a decrease in IGF-2 but not IGF-I concentrations (Table 3). No significant differences were noted, however, in IGF-II concentrations between any of the four infusion groups. In brain cytosols, changes similar to those in liver were observed, with decreases in IGF-II but not IGF-I concentration. However, because of interanimal variability, significant changes in IGF-II concentration in brain were noted only in the GLU and GA groups as compared to CON.

IGF-binding protein activity. RRA activity for untreated whole sera and livers and brain cytosols are shown in Table 4. Despite the decreases in IGF-I and -II concentration observed in chromatographed specimens, significant increases in RRA activity were present in both whole sera and liver cytosol samples of all four infused groups when compared to CON rats. However, RRA activities in sera and liver cytosol of GAL rats were significantly decreased from those of SAL rats. No differences in brain cytosol RRA activity were observed among the five groups. When RRA activities in whole sera (Fig. 3) were contrasted with rat caloric intake (assuming a mean control caloric intake of 0.4 cal/g/d) (15), a significant inverse relationship was present ($r = 0.67$, $p < 0.01$). No relationship was apparent, however, between RRA

activity in liver cytosol and caloric intake, but the data suggested a depression of RRA activity in liver cytosol by infusates containing amino acids, irrespective of caloric intake, *i.e.* RRA activities in livers of GA or GAL rats were significantly different from those in GLU or SAL rats.

Assay of serum amino acids was performed from selected samples in each of the five groups of neonatal rats (Table 2). As can be seen, SAL or GLU rats had total amino acid concentrations 50–75% of control animals. Decreases in concentration of the majority of amino acids assayed were noted. Total amino acid concentrations in amino acid infused groups (GA or GAL) were at or above control levels. To explore whether or not the RRA activity in neonatal rat serum or liver cytosol was dependent upon circulating amino acid concentration, regression analyses were performed. Although no relationship between total serum amino acid concentrations and serum RRA activity was noted, a highly significant relationship between log total serum amino acid concentration and liver cytosol RRA activity was observed (Fig. 4). In addition, the relationship was similar when the serum concentrations of individual amino acids were studied, specifically those of lysine, serine, arginine, valine, leucine, isoleucine, threonine, and tyrosine.

Characterization of binding protein activity changes. The apparent increase in binding protein activity during fasting was explored further using specimens from rats in control and saline-

Fig. 2. Concentrations of RRA-IGF-II (A) and RIA-IGF-I (B) in treated sera from all groups related to caloric intake. Caloric intake in CON rats (CON IGF-II) was estimated from Reference 15.

Table 4. RRA-IGF-II activity-untreated specimens

	<i>n</i>	Whole sera (U/mL)	Whole liver cytosol (U/g)	Whole brain cytosol (U/g)
CON	15	2.0 ± 0.2	0.7 ± 0.1	0.7 ± 0.0
SAL	7	4.2 ± 0.8*	4.3 ± 0.9*	0.7 ± 0.0
GLU	8	3.3 ± 0.6*	3.4 ± 0.2*	0.7 ± 0.1
GA	12	3.3 ± 0.3*	2.0 ± 0.3*†	0.7 ± 0.1
GAL	16	2.6 ± 0.2*†	1.3 ± 0.2*†	0.7 ± 0.1

* $p < 0.05$ from CON.

† $p < 0.05$ from SAL.

Fig. 3. Whole serum RRA-IGF-II activity related to caloric intake in the five groups of 10-d-old rats.

Fig. 4. Whole liver cytosol RRA-IGF-II activity related to serum total amino acid concentration in the pooled data from the five experimental groups.

treated groups. Using the RRA-IGF-II assay, RRA activity of control serum or liver cytosol was not changed by the acidification process. In contrast, RRA activity of serum and liver cytosol of saline treated rats was diminished by 50–90% by the acid treatment. Chromatography of pre- and postacid-treated serum and liver cytosol of saline-treated rats was then performed to test the hypothesis that the apparent rise in binding protein activity during starvation was due to the presence of binding proteins that were either precipitated or destroyed by acid treatment.

Incubation of an aliquot of pooled SAL liver cytosol with purified [125 I]-IGF-II caused a migration of the IGF-II peak to a large mol wt fraction and accounted for 100% of added radioactivity, consistent with the activity of a binding protein. Prior lyophilization of another aliquot caused only a small decrease in RRA-activity (92% of tracer in large mol wt peak), but acidification of a third aliquot, with subsequent lyophilization, caused a marked (50%) reduction in tracer bound to the binding protein peak.

Fig. 5. *A*, gel chromatographs of pooled serum from 10-d-old rats given 24-h saline infusions. Chromatograph in *open boxes* represents pattern of binding of [125 I]-IGF-I to untreated serum. Chromatograph in *closed diamonds* represents pattern of binding of [125 I]-IGF-I to serum previously acidified to 2 M, lyophilized, and reconstituted in 50 mM Tris-0.2% HSA. *B*, gel chromatographs of pooled liver cytosol from 10-d-old rats given 24-h saline infusions. Chromatograph in *open boxes* represents pattern of binding of [125 I]-IGF-I to untreated liver cytosol. Chromatograph in *closed diamonds* represents pattern of binding of [125 I]-IGF-I to liver cytosol previously acidified to 0.1 M acetic acid, lyophilized, and reconstituted in 50 mM Tris. All chromatographs were performed using G-200 Sephadex with 50 mM Tris-0.2% HSA.

To characterize the binding protein changes further, G-200 Sephadex chromatography was applied to SAL rat serum and liver cytosol before and after acidification (as in "Materials and Methods") using two aliquots of each pooled sample. Incubation with [125 I]-IGF-II and subsequent chromatography of the untreated serum sample (Fig. 5*A*) showed three peaks of binding protein activity, corresponding to mol wt of approximately 40, 150, and 250 kD, respectively. Acidification of SAL serum led to a relative decrease in binding activity of the 150 and 250 kD peaks. In liver cytosol of SAL rats (Fig. 5*B*), only two peaks at 40 and 250 kD were noted before acid treatment. Acidification caused a shift of radioactivity to the 40 kD peak with a decrease in the 250 kD peak.

DISCUSSION

IGF (somatomedins) form a class of small mol wt polypeptide hormones with amino acid sequences and cellular metabolic actions similar to those of insulin (1, 2). In contrast to insulin, however, at physiologic concentrations, IGF exert potent mitogenic effects on a wide range of cell types such as myoblasts (16), fibroblasts (16), erythroid progenitor cells (17), chondrocytes (18), and oligodendrocytes (19) when tested *in vitro*. The IGF are ubiquitous, with a large variety of tissue types demonstrated to have the capacity for IGF synthesis and release (1, 2). In the adult, control of IGF synthesis, particularly IGF-I, appears due to changes in secretion of GH (1, 2) and, during pregnancy, to changes in the circulating concentrations of the GH homologue, human placental lactogen (1, 4). However, information also exists to suggest that other factors, particularly nutritional intake, may be important in regulating IGF synthesis (20–22).

IGF have recently been shown to be synthesized by a number of cell types in the fetus (23), raising the possibility that IGF may be important in growth regulation during this period of rapid cell division and development. During fetal and early neonatal life, IGF synthesis appears not to be regulated by GH (3, 8). Studies in neonatal rats have also suggested little relationship between somatic growth and GH secretion until after weaning (approximately 21 days) (4). Although not clearly defined in the human, information in laboratory animals such as the rat suggests that the predominant fetal IGF is IGF-II, and that IGF-I concentrations in blood rise only after GH-dependent mechanisms come into play near time of weaning (3, 4). Because of adult studies suggesting some relationships between nutrition and IGF synthesis, the current series of experiments were performed in preweanling rats to assess the relationship between nutrition and IGF concentration in perinatal life.

Our studies document that in 10-d-old rats, fasting of 24-h duration caused marked decrements in serum and liver IGF concentrations. Of particular interest is the demonstration that serum IGF-II concentrations were linearly related to caloric intake. However, intravenous nutrition did not raise the concentrations of IGF-I in serum, nor IGF-I or -II in liver or brain cytosols. Only limited information is available regarding the effects of altered nutrition on IGF concentrations during the perinatal period. The results of such studies suggest that nutritional intake is of paramount importance in regulation of IGF-I synthesis. For example, fetal rats made growth retarded via maternal uterine artery ligation have low levels of serum somatomedin activity (24) and of serum and liver RIA-IGF-I concentrations (25) when compared to controls. In addition, RIA-IGF-I concentrations were linearly related to fetal wt and fetal serum glucose concentration. The authors could only speculate, however, that deficient substrate transfer to the fetus might be responsible for both depressed IGF synthesis and subsequent growth retardation. No information has previously been available regarding the effects of changes in substrate availability on IGF-2 concentrations in the perinatal period. Inasmuch as the predominant fetal and neonatal somatomedin in the rat is IGF-II, the current data provided further evidence for an important role

for IGF-II in the control of perinatal growth. Synthesis of IGF-I may be more sensitive to sufficiency and quality of caloric intake than IGF-II (20, 21), but data from the present study also do not rule out the possibility that stress or hormonal factors related to enteral nutrition may influence IGF-I or -II synthesis, particularly in the liver.

Unlike many classical endocrine hormones, IGF are found in blood and tissue cytosol linked to high affinity binding proteins (1, 2). Sites of binding protein synthesis are presently unknown but appear to include a number of cell types including liver (26). In the rat and human, at least several mol wt species of carrier proteins are present in serum. In both adult rat and human, the predominant carrier protein has a mol wt of approximately 150 kD, is altered by acidification, and is under GH regulation (1, 2, 26, 27). Hypopituitary dwarfs and hypophysectomized rats have deficient circulating levels of this carrier protein that can be altered with GH injection (26, 28). The 150 kD binding protein fraction is present in significant quantities in late gestation rat and mouse fetuses. However, in the human and rat fetus, the predominant carrier protein species is a 35–40 kD mol wt protein that is not under GH regulation and appears immunologically distinct from acid generated proteins from the adult 150 kD mol wt species (28, 29). A 250 kD mol wt species has also been identified and, in the rat, may be identical to the type II IGF receptor (30). However, regulation of synthesis and release of this protein are not understood.

In our experiments, acute fasting in 10-d-old rats induced marked increases in binding activity, as crudely measured by competition for binding in RRA-IGF-II. Increased binding protein activity using this assay could be due to increases in actual concentration of binding protein(s) but might also be due to changes in binding protein-IGF affinity or receptor site occupancy. The elevations in binding protein activity observed occurred in the face of decreased concentrations of IGF when they were dissociated from their carrier proteins after acidification and gel chromatography. Gel chromatography with G-200 Sephadex demonstrated that the majority of the increased binding activity in the serum and liver cytosol of fasted rats eluted in the fraction of low mol wt IGF binding protein. Interestingly, Sara *et al.* (31) found paradoxically elevated RIA-IGF-I levels in untreated serum from preweanling rats exposed to pre- and postnatal malnutrition. It is likely that changes in RIA-IGF-I concentration represented similar increases in binding protein activity due to caloric deprivation. Powell *et al.* (32) have observed increases in IGF binding protein activity in children with chronic renal failure. Increases in concentrations of low mol wt IGF binding proteins have been found in the serum of anorexia nervosa patients (33) and in those with insulin-dependent diabetes mellitus (34), suggesting that intracellular malnutrition may indeed be one causative factor in the generation of IGF binding proteins.

Fasting in 10-d-old rats also induced a marked increase in binding protein activity in liver cytosol. A significant portion of this activity was diminished by acid treatment. Using G-200 Sephadex gel chromatography, an apparent decrease in activity at 250 kD and, perhaps 40 kD, mol wt was observed after acid treatment. Whether these changes are due to destruction by acid or precipitation of specific binding proteins is unclear. Of particular interest is the demonstration that binding protein activity in liver cytosol but not serum was inversely related to circulating amino acid concentrations. It is tempting to speculate that such changes in IGF binding protein concentrations may have been modulated by circulating substrate concentrations, specifically amino acids or by hormonal factors (*i.e.* insulin) (35) as yet undefined.

The physiologic significance of IGF binding proteins in biologic fluids is not known at present (9). Some investigators have suggested a role for binding proteins as transport proteins to provide dissemination of IGF to sites distant from those of secretion. Because of the high affinity of binding proteins relative

to cellular IGF receptors, it has also been suggested that carrier proteins represent either a storage form of IGF or a mechanism for depressing IGF receptor interaction. The current data may be interpreted to suggest that depression of IGF synthesis coupled with the fasting-induced increases in binding protein activity constitute a protective mechanism to inhibit growth during times of caloric deprivation. Furthermore, these studies suggest that nutritional intake is an important factor influencing IGF synthesis in the postnatal period.

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Announcement

NIH CONSENSUS DEVELOPMENT CONFERENCE: TREATMENT OF DESTRUCTIVE BEHAVIORS IN PERSONS WITH DEVELOPMENTAL DISABILITIES

A Consensus Development Conference on Treatment of Destructive Behaviors in Persons with Developmental Disabilities will be held in Masur Auditorium in the Warren Grant Magnuson Clinical Center, National Institutes of Health, Bethesda, MD, September 11-13, 1989. This open forum will focus on the various treatments and approaches used to modify or eliminate destructive behaviors in persons with developmental disabilities.

On the first 2 days, experts will present current scientific thinking about the diagnosis, management, and prevention of destructive behaviors in persons with developmental disabilities, and concerned voluntary organizations will be invited to make statements. On the 3rd day, after considering the scientific evidence, the consensus panel will present its draft report and invite comments from the audience. Dr. R. Rodney Howell, Professor and Chairman, Department of Pediatrics at the University of Miami School of Medicine, will chair the panel.

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