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Insulin-Like Growth Factor I in Substrate-Deprived, Growth-Retarded Fetal Rats

IRA M. BERNSTEIN, MARY M. DeSOUZA, AND KENNETH C. COPELAND

Departments of Obstetrics and Gynecology and Pediatrics, University of Vermont College of Medicine, Burlington, Vermont 05401

ABSTRACT. Plasma, amniotic fluid, and tissue concentrations of IGF-I were examined in nutritionally deprived, growth-retarded fetal rats to determine whether IGF-I concentration serves as a marker for nutritional status. Growth retardation was induced by 72 h of maternal fasting. Twenty-three control and 17 growth-retarded fetuses were individually analyzed and compared. Plasma IGF-I concentrations were significantly lower in test compared with control animals (test 56.8 ± 14.9, control 87.4 ± 17.5 ng/mL, $p < 0.01$). Amniotic fluid IGF-I concentrations were not different (test 14.0 ± 8.7, control 12.2 ± 2.6 ng/mL). IGF-I concentrations obtained from both placental and hepatic tissues were lower in test compared with control animals [placenta: test 293 ± 25 *versus* control 655 ± 114 ng/g ($p < 0.001$); hepatic: test 173 ± 38 *versus* control 230 ± 51 ng/g ($p < 0.01$)]. Reductions in fetal, placental, and hepatic weights in test animals were more closely related to changes in placental IGF-I concentration than to either plasma or hepatic IGF-I concentrations. We conclude that fetal plasma IGF-I is a valuable marker for intrauterine substrate deprivation and that the growth-retarded rat fetus is accurately identified and specifically characterized by a low placental concentration of extractable IGF-I. (*Pediatr Res* 30: 154–157, 1991)

Circulating concentrations of IGF-I are influenced by both growth hormone and nutritional status (1–10). Although the importance of IGF-I in the regulation of fetal growth has been explored, a central role for IGF-I in fetal growth has not been firmly established (4). Both IGF-I and its receptor have been found in multiple fetal tissues, suggesting an autocrine and/or paracrine mechanism of biologic action (5, 11–14). Based on its role as a marker of nutritional status, we hypothesized that fetal plasma and amniotic fluid concentrations of IGF-I would be depressed in nutritionally deprived, growth-retarded fetal rats. We also hypothesized that tissue concentrations of IGF-I would correlate best with the weights of the respective tissues of origin, consistent with an autocrine or paracrine mode of action.

MATERIALS AND METHODS

Animals. A maternal substrate deprivation model of fetal growth retardation was used (10, 15). Virgin Sprague Dawley rats (240–280 g) were used as experimental animals. Seven control and six test maternal animals were studied. Pregnancies were dated, with d 1 representing the day on which vaginal sperm were identified after overnight mating. Test animals were fasted between d 18 and 21 of gestation, but had full access to water. Control animals were allowed free access to both food and water. Sampling was performed on d 21 in all animals, after intraperitoneal pentobarbital (45 mg/kg) administration. Midline laparotomy incision was used to expose the uterine horns. Fetuses in each horn were counted, and the two inferior fetuses of each horn were sampled to match the control and test group with regard to intrauterine location. A maximum of four fetuses per dam were analyzed to insure matching of uterine location between groups and to minimize any metabolic impact of the surgical procedure. Only those fetuses in whom results from all specimens were available were included in the analyses. The sampling sequence was initiated by the removal of amniotic fluid with a 19- or 20-gauge 1-mL syringe. The fetus was then removed from its location in the uterine horn, and an axillary cutdown was performed (15). Fetal blood was collected by heparinized capillary tube, placed on ice, and centrifuged for 15 min at 12 000 × *g*. The fetus was separated from the placenta, and the fetus, placenta, and then fetal liver were weighed. The placenta and liver were quickfrozen in liquid nitrogen. All samples were stored at −80°C until analysis. After fetal sampling, a maternal venous plasma sample was obtained from the inferior vena cava just below the renal vein. Euthanasia was performed by means of exsanguination after all samples had been obtained. Individual fetal sex was identified and recorded. This study was approved by the University of Vermont Animal Care and Use Committee in accordance with N.I.H. guidelines.

Analyses. IGF-I concentrations in maternal and fetal plasma and amniotic fluid were determined by RIA [National Institute of Diabetes and Digestive and Kidney Diseases somatomedin-C antiserum (polyclonal) code no. UBK487 was a gift of Drs. Underwood and Van Wyck, University of North Carolina] after acid-chromatography (16). Fetal plasma (0.025 mL) was incubated with 0.2 M glycine HCl (0.1 mL, pH 2.0) at 37°C for 1 h. After incubation, samples were added to a G-50 Sephadex column (1.6 × 41.0 cm) and eluted at 25°C with 1 M acetic acid (pH 2.5) at a flow rate of 60 mL/h. Two-mL fractions were collected, and absorbance at 280 nm was determined. The tubes corresponding to the free IGF-I fraction (k_d = 0.3–0.8) were pooled. A 1-mL aliquot of each pooled group was lyophilized to dryness. Aliquots were then washed with ammonium carbonate (1 mL, 0.025 M, pH 7.8) and relyophilized. The dried sample was then reconstituted in 0.15 mL of RIA buffer (without protamine) and frozen at −80°C until assay.

Hepatic and placental IGF-I extractions were performed according to the method of D'Ercole and Underwood (17), with modifications as noted. Frozen tissue samples were placed in a porcelain mortar, covered with liquid nitrogen, and pulverized with a precooled pestle. The tissue powder was transferred to a tared disposable polystyrene centrifuge tube, and sample weight was recorded. Acetic acid (1.0M) was added (5 mL/g tissue), and the tubes were shaken for 15 s, incubated on ice for 2 h, and then centrifuged at 1000 × *g* for 10 min at 4°C. A 1.3-mL aliquot was taken from each tube, neutralized with sodium hydroxide (5 M pH 6–8) and frozen at −80°C until assay. The contribution

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Reprint requests: Ira M. Bernstein M.D., Department of Obstetrics and Gynecology, Shepardson 419 M.C.H.V., Burlington, VT 05401.

of tissue blood IGF-I in the hepatic and placental samples was calculated to represent less than 2% of the total IGF-I concentration in these exsanguinated and blotted tissue samples. This contribution was not corrected for in the analysis (17).

IGF-I concentrations in plasma and amniotic fluid were expressed as ng/mL and tissue concentrations in ng/g tissue dry weight after comparison with a pooled human serum standard. Aliquots of placental and liver extracts, as well as fetal and maternal sera, all displaced IGF-I tracer in parallel fashion to that observed with pure IGF-I standard (Imcera, Terre Haute, IN). Displacement of tracer binding by amniotic fluid could be achieved only at very high volumes, and the displacement curves showed slightly less parallelity to the pure standard.

Recovery of added cold IGF-I (Imcera) from placental extract, liver extract, fetal serum, maternal serum, and amniotic fluid, over a wide range of doses, were 59, 79, 66, 67, and 72%, respectively.

Statistics. Mean somatic and tissue weights, amniotic fluid IGF-I concentrations, and both maternal and fetal plasma concentrations of IGF-I were compared by two-tailed unpaired *t* test. Results are expressed as the mean ± SD. p values ≤ 0.05 were accepted for significance.

RESULTS

Complete data sets were available on 23 control fetuses and 17 test fetuses. Growth retardation was successfully achieved with a significant reduction in hepatic, placental, and fetal weights of test animals compared with controls (Table 1). These differences could not be accounted for by a difference in prefast maternal weights, mean litter size, or the ratio of male to female fetuses within the groups (Table 2). The impact of 72 h of maternal fasting on IGF-I concentrations in the maternal plasma, fetal plasma, amniotic fluid, and fetal tissues is outlined in Table 3. Plasma IGF-I concentrations were reduced in both fasted dams and corresponding fetuses. Amniotic fluid IGF-I concentrations, however, were not different between test and control animals. Placental and hepatic tissue concentrations of IGF-I were lower in nutritionally deprived test fetuses.

The relationship between fetal weight and fetal plasma IGF-I is illustrated in Figure 1. Maternal starvation resulted in a marked reduction in both fetal weight and fetal plasma IGF-I, although approximately 40% of the treated fetuses had plasma IGF-I levels overlapping with the controls. Figures 2 and 3 illustrate the same reduction in fetal weight relative to hepatic and placental tissue concentrations of IGF-I. As with the fetal plasma IGF-I concentrations, significant overlap was observed in hepatic IGF-I concentrations between control and test groups (Fig. 2). Figure 3 shows the relationship between fetal weights and placental IGF-

Table 1. *Fetal morphometry*

	Control (mean ± SD)	Test (mean ± SD)	p
Fetal wt (g)	3.68 ± 0.24	2.76 ± 0.19	<0.001
Hepatic wt (g)	0.29 ± 0.04	0.17 ± 0.03	<0.001
Placental wt (g)	0.54 ± 0.10	0.43 ± 0.07	<0.01
Hepatic wt/Fetal wt	0.08 ± 0.01	0.06 ± 0.01	<0.05

Table 2. *Population characteristics*

	Control (mean ± SD)	Test (mean ± SD)	p
Maternal wt (g)			
Day 1	261.2 ± 13.2	261.5 ± 19.2	NS
Day 18	368.5 ± 41.3	345.0 ± 27.0	NS
Day 21	407.0 ± 43.0	309.7 ± 22.4	<0.001
Litter size	14.9 ± 4.5	14.0 ± 1.3	NS
Fetal sex ratio (M/F)	0.87	0.85	NS

Table 3. *IGF-I concentrations*

	Control (mean ± SD)	Test (mean ± SD)	p
Materal plasma IGF-I (ng/mL)	345 ± 100	171 ± 100	<0.01
Fetal plasma IGF-I (ng/mL)	87.4 ± 17.5	56.8 ± 14.9	<0.01
Amniotic fluid IGF-I (ng/mL)	12.2 ± 2.6	14.0 ± 8.7	NS
Hepatic IGF-I (ng/g)	230 ± 51	173 ± 38	<0.01
Placental IGF-I (ng/g)	655 ± 114	293 ± 25	<0.001

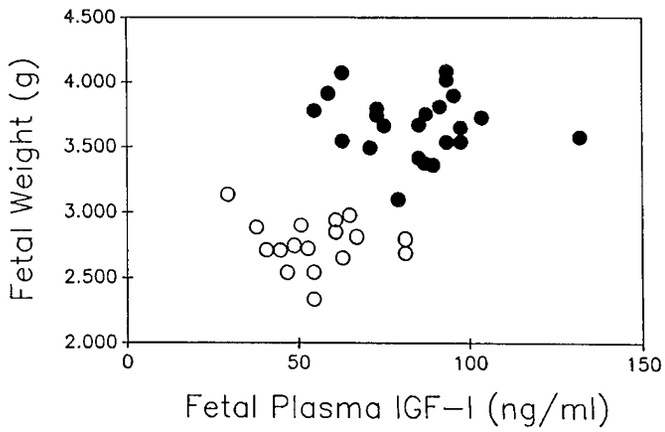

Fig. 1. Fetal plasma IGF-I concentration plotted against fetal weight for normally grown control (*filled circles*) and growth-retarded (*open circles*) fetal rat pups.

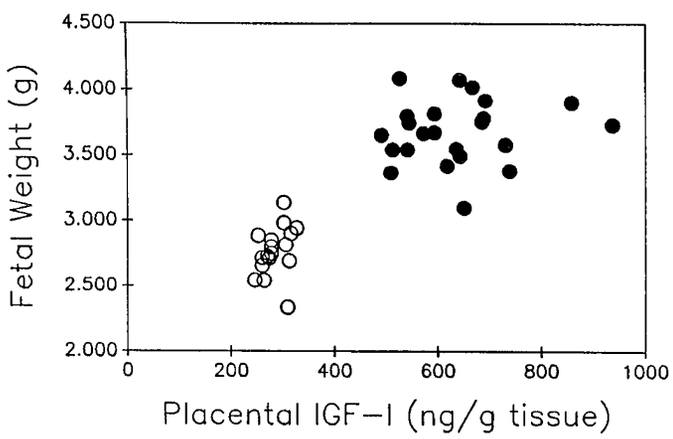

Fig. 2. Placental IGF-I concentration plotted against fetal weight for normally grown control (*filled circles*) and growth-retarded (*open circles*) fetal rat pups.

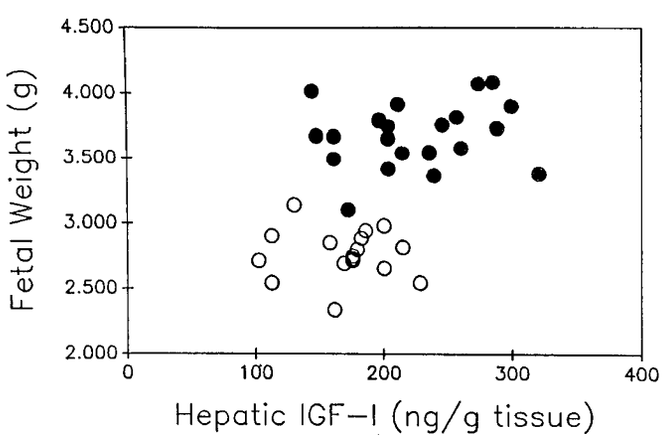

Fig. 3. Hepatic IGF-I concentration plotted against fetal weight for normally grown control (*filled circles*) and growth-retarded (*open circles*) fetal rat pups.

I concentrations. As illustrated, a potent treatment effect was seen in both fetal weight and placental IGF-I concentration. Mean fetal weight was reduced by 25%, whereas mean placental IGF-I concentration was reduced by 55%. No overlap in IGF-I concentrations was observed among treated and control animals, even though one control fetus had a weight that overlapped with those of the treated fetuses.

Figure 4 demonstrates the relationship between hepatic weight and placental tissue concentrations of IGF-I. As shown, hepatic weights were markedly reduced in treated animals, although one control fetus had a hepatic weight falling within the range of those of the treated fetuses. The clear treatment effect on placental IGF-I is again apparent, with little overlap among hepatic weights. Once again, data from a single control fetus overlapped with those of the treated animals. The hepatic size of this fetus fell outside the lower range of those of the remaining control fetuses and close to the mean of the test animals. The hepatic and placental tissue concentrations of IGF-I for this animal, however, fell near to the mean for the control animals and outside the range of the test animals.

Figure 5 demonstrates the relationship between placental weight and placental tissue concentrations of IGF-I. Although the mean placental weight of treated animals was 20% less than that of the controls (Table 1), there was marked overlap of placental weight between control and test groups. And although the mean placental weight of treated animals was reduced by only 20% compared with that of controls, mean placental IGF-I tissue concentration was reduced by 55% (Table 3), with no overlap seen in individual IGF-I values between treated and control animals.

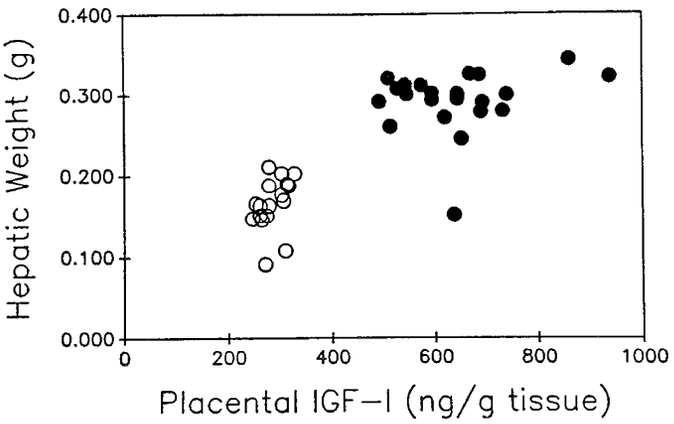

Fig. 4. Placental IGF-I concentration plotted against hepatic weight for normally grown control (*filled circles*) and growth-retarded (*open circles*) fetal rat pups.

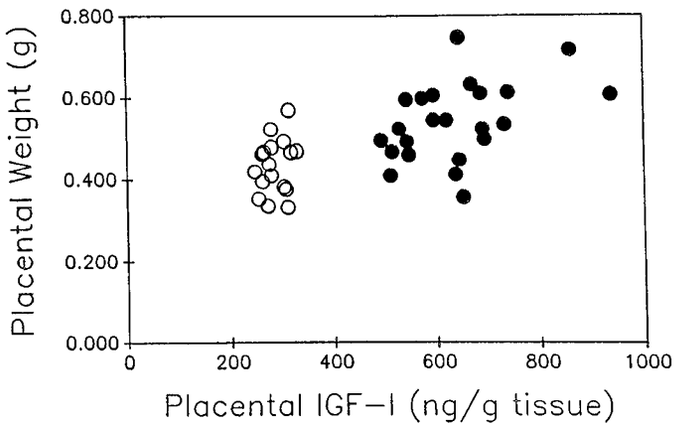

Fig. 5. Placental IGF-I concentration plotted against placental weight for normally grown control (*filled circles*) and growth-retarded (*open circles*) fetal rat pups.

DISCUSSION

Although IGF-I is felt to be a primary regulator of growth in postnatal life, its role during fetal life is unclear. Although IGF-I concentrations in postnatal life are directly influenced by growth hormone, other factors, including human placental lactogen and prolactin, may be operative *in utero* (7, 18). IGF-I levels in blood have been shown to reflect changes in nutritional balance and have been proposed as markers for nutritional status (19). In this study, we used a rat maternal starvation model, both to examine the utility of plasma and amniotic fluid IGF-I concentrations as markers of suboptimal nutrition and to evaluate the relationship between IGF-I concentrations and fetal somatic and tissue growth. The model used effectively resulted in a reduction in hepatic, placental, and overall fetal size. Analysis of the hepatic weight/fetal weight ratio revealed an asymmetric form of growth retardation; that is, the reduction in hepatic weights was proportionately greater than the overall reductions in fetal weights (Table 1). Significant reductions in IGF-I concentrations were noted in all compartments studied with the exception of amniotic fluid. It is unclear why amniotic fluid IGF-I concentrations failed to coincide with the plasma and tissue levels in this model. Assuming that amniotic fluid IGF-I is of fetal origin, then the lack of responsiveness of IGF-I to acute maternal substrate deprivation may reflect a pool that is slowly responsive to acute perturbations of fetal nutritional status. Alternatively, it may be that G-50 acid chromatography does not adequately separate IGF-I from the amniotic fluid binding protein, and thus does not accurately reflect the state of IGF-I in the amniotic fluid compartment under conditions of suboptimal nutrition (20, 21).

The changes observed in IGF-I in this study are consistent with previous data evaluating changes in IGF-I in the growth-retarded fetus and in early postnatal life (8–10, 22). Fetal rat pups rendered growth-retarded by uterine artery ligation were found to have depressed serum and tissue levels of IGF-I when compared with controls (9). Similarly, 10-d-old rat pups deprived of calories for 24 h showed significant reductions in serum levels of IGF-I, although no significant effects on hepatic IGF-I concentrations were found (8). Our results show absolute plasma IGF-I values in control and intrauterine growth-retarded animals similar to those reported previously, although the hepatic concentrations were approximately 10-fold higher than those previously reported using similar methodology (9). This discrepancy may reflect inherent differences in hepatic IGF-I concentration induced by uterine artery ligation compared with maternal substrate deprivation.

Associations between placental IGF-I concentrations and indices of fetal growth retardation have not been previously reported. It should be noted that most placental tissue samples contain a small amount of maternal decidua. Although IGF-I is extractable from maternal decidua (unpublished observation), the relative contribution of maternal tissue to the samples obtained is small. It therefore seems unlikely that maternal IGF-I contributed significantly to the IGF-I concentrations obtained from the placental tissue samples.

In our current study, IGF-I levels from growth-retarded fetuses were not only depressed in blood, but also in various tissue compartments. Some observations, however, were unanticipated. Previous studies have suggested that the majority of circulating IGF-I is of hepatic origin (23). In the present study, the absolute levels of IGF-I in the placenta were more than 2.5-fold higher than hepatic levels in the fed state and approximately 75% higher than hepatic levels in the substrate-deprived state. The significance of this observation is unclear. Fant *et al.* (13, 24) suggested that persistent placental IGF-I production through term lends support for an autocrine/paracrine mode of IGF-I action. However, inasmuch as previous studies have demonstrated that both DNA expansion and placental growth are complete well before term (25, 26), the relatively high concentrations of IGF-I in placental tissue (as noted in the control animals) suggest a

discordance between placental growth and placental IGF-I production. Furthermore, we observed that in test animals a reduced placental IGF-I concentration was closely associated with fetal and hepatic growth retardation, independent of the size of the corresponding placenta. Together these observations suggest that, if placental IGF-I is involved in fetal growth, then it is functioning at least in part in classic endocrine as opposed to autocrine or paracrine fashion.

IGF-I appears to be involved in the growth and differentiation of fetal tissues (4). Recent evidence, however, suggests that the biologic action of IGF-I is strongly modified by the relative concentrations of its binding proteins in plasma, amniotic fluid, and a variety of tissues (27–32). Thus, a measurement in blood or tissue of the IGF-I concentration after removal of the binding proteins gives only one index of the biologic action of this growth factor. Nevertheless, the strong associations between IGF-I and fetal and tissue size observed in this study suggest that IGF-I may indeed be involved in fetal growth.

Together, these data suggest that IGF-I concentrations in fetal plasma, as well as those obtained from hepatic and placental tissue, serve as valuable markers for fetal growth retardation induced by maternal substrate deprivation. In contrast to IGF-I obtained from plasma or hepatic tissue, however, IGF-I obtained from placental tissue appears to reflect fetal and hepatic growth with high degrees of accuracy and specificity. Because placental size is not associated with placental IGF-I concentration, whereas fetal size and placental IGF-I appear closely associated, one can speculate that the placenta is involved in the regulation of fetal growth, in part through the production and dispersion of IGF-I in classic endocrine fashion.

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