Regression of Fetal Amino Acid Metabolism: Substrate or Hormonal Regulation?1,2

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ABSTRACT Insulin is regarded as the primary fetal growth-promoting hormone, but direct in vivo experimental data supporting this conjecture are sparse. Data obtained from studies in in vivo, chronically catheterized fetal lambs under a variety of experimental circumstances demonstrate that glucose availability is the primary modulator of fetal protein accretion, via its ability to diminish amino acid catabolism. The ovine fetus is shown to be resistant to insulin-induced suppression of proteolysis, relative to the adult. Data from studies in the human premature infant show that the findings in the ovine fetus are similar to those in the ex utero premature human. J. Nutr. 128: 342S–346S, 1998

KEY WORDS: • insulin • ovine fetus • leucine • tracer kinetics • human premature infant

EVIDENCE OF INSULIN ACTION IN THE FETUS

Insulin has long been regarded as the primary fetal growth factor (McCormick et al. 1979, Susa et al. 1979). This belief has been based largely on circumstantial evidence such as large-for-gestational-age, hyperinsulinemic fetuses seen in maternal diabetes and Beckwith-Wiedemann syndrome. In addition, the occasional infant born with pancreatic agenesis and hypoinsulinemia is uniformly small for gestational age (Lemons et al. 1979), and fetal pancreatectomy leads to intrauterine growth retardation (Fowden et al. 1989). Furthermore, growth hormone, a primary regulator of postnatal growth, is not believed to be important, because there is a paucity of growth hormone receptors in the fetus (Arosio et al. 1995, Gluckman 1995).

Despite this circumstantial evidence, investigators have been largely unsuccessful in demonstrating a fetal growth-promoting effect of insulin in animal models. Milley et al. (1986) demonstrated decreased arterial concentration and increased fetal uptake of α-amino nitrogen when the late-gestation ovine fetus was infused with exogenous insulin. The increased fetal amino acid uptake resulted in increased tissue synthesis, expansion of intracellular amino acid pools and increased catabolism, but the partitioning among these fates remains to be determined. They were not, however, able to document increased birth weight in fetal lambs infused chronically with insulin. Similarly, neither Stagenberg et al. (1981) nor Angervall et al. (1981) were able to show any increase in body or protein mass in rat fetuses injected with insulin during late gestation. Fetal pigs failed to show any significant growth response to insulin (Spencer et al. 1983). In contrast, Picon (1967) found a 10% increase in total body nitrogen in insulin-injected rat fetuses. Susa et al. (1979) found that chronic insulin infusion in the fetal rhesus monkey resulted in a significant 33% increase in body mass. Although chemical analysis of the carcasses was not performed, it can be presumed that the total body nitrogen was increased, although not likely to the same degree as body mass. The protein/DNA ratio was not changed, implying that any increase in lean body mass was mainly the result of tissue hyperplasia rather than hypertrophy.

Furthermore, investigators have shown that fetal size is substantially smaller in a diabetic rat model than in controls. Fetal total protein content is decreased in concert with the diminished body size. Fractional synthetic rates are diminished in the rat fetuses of diabetic mothers, whereas fractional breakdown rates are markedly elevated (Canavan and Goldspink 1988). The placentas of these pups have diminished rates of protein breakdown but normal synthetic rates, with placental-meval as the net result (Robinson et al. 1988). These findings are intriguing because it is generally held that fetal tissue responds to hyperinsulinemia by hypertrophy or hyperplasia (or both), whereas the placenta is generally not thought to be an insulin-sensitive organ. Further investigation is warranted to determine if these effects found in the rat can be demonstrated in other species.

1 Presented as part of the symposium “The Roles of Nutrition, Development and Hormone Sensitivity in the Regulation of Protein Metabolism” given at the Experimental Biology 97 meeting, April 7, 1997, New Orleans, LA. This symposium was sponsored by the American Society for Nutritional Sciences and supported in part by educational grants from Diagnostic Systems Laboratories, Inc., Mead Johnson Nutritional Group, Pig Improvement Company USA, Ross Products Division, Abbott Laboratories, Wyeth-Ayerst Laboratories and Zinpro. Guest editor for the symposium publication was Teresa A. Davis, Baylor College of Medicine, Houston, TX 77030.

2 This work was supported by Public Health Service grants RO1-HD19089, RO1-HD29153, K04-HD00865 and PH60-DK-20542 and by the James Whitcomb Riley Memorial Association.

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Contrasted with the effect in fetuses of well-fed ewes. After obtaining basal kinetic measurements, glucose was infused into the fetus at 15 mg/min, a rate chosen to duplicate normal fetal umbilical glucose uptake. In the fed animals, glucose concentration increased by 30%, insulin by 22% and total glucose intake (umbilical + infused) by 30%. There was no effect on leucine Ra or on leucine oxidation. In the fasted state, glucose concentration doubled, and insulin increased by 72%, but total glucose intake was not significantly increased. Again, no change in leucine Ra was seen, but leucine oxidation, which was 1.3-fold higher in the fasted basal state compared with the fed state, decreased by 50%, returning to a rate similar to the fed basal state. These data are depicted in Figure 2A. This decline in oxidation resulted in a change from net catabolism to net accretion of leucine during the glucose infusion.

EFFECT OF GLUCOSE INDEPENDENT OF INSULIN

In the first study, the insulin response to altered glucose was variable. Because the insulin and glucose concentrations were highly correlated, it was not possible to delineate effects as specific to glucose or insulin. Therefore, two additional studies were performed. In the first, insulin was “clamped” by fetal somatostatin infusion while glucose concentration was varied (Liechty et al. 1993), whereas in the second, euglycemia was maintained while hyperinsulinemia was induced by fetal insulin infusion (Liechty et al. 1992).

When insulin was clamped, infusion of glucose resulted in stepwise increases in glucose concentration without an increase in glucose utilization rate (GUR). As in the previous study, no change in leucine Ra was observed, but a significant stepwise decrease in leucine oxidation was seen in both the fed and fasted states, as shown in Figure 2B. There was a significant linear relationship between fetal glucose concentration and leucine oxidation rate; after controlling for interanimal variability, the $\tau = 0.85$. There was no correlation of leucine oxidation with insulin concentration.

EFFECT OF INSULIN INDEPENDENT OF GLUCOSE

In the converse study, clamping glucose at basal levels while infusing insulin resulted in 44 and 100% increases of GUR (fed and fasted, respectively), with no change in glucose concentration. Insulin concentration was increased four- and sevenfold, respectively. Again, no change in leucine Ra was observed, but a large decrease in fetal leucine oxidation was observed in the fasted state only (Fig. 2C).

SUMMARY OF STUDIES IN EWES

These three studies can be summarized as follows:

1. Fetal proteolysis is relatively resistant to suppression by insulin. Despite large variations in insulin and glucose concentrations, leucine Ra did not decrease, suggesting that fetal protein breakdown is remarkably resistant to suppression by insulin. However, this conclusion must be accepted with the caveat that these studies were performed without accounting for the bidirectional flux of tracee at the fetal-placental interface. Fetal protein breakdown was estimated simply as total leucine Ra minus umbilical uptake. Therefore protein breakdown was overestimated to the extent that fetal tracee exits to the placenta. This seems to be ~20% of total Ra for leucine (Milley 1994).

GLUCOSE EFFECTS ON FETAL AMINO ACID KINETICS

The first study (Liechty et al. 1991) was designed to study the effect of substrate replacement in the fasted catabolic fetus, contrasted with the effect in fetuses of well-fed ewes. After obtaining basal kinetic measurements, glucose was infused into the fetus at 15 mg/min, a rate chosen to duplicate normal fetal umbilical glucose uptake. In the fed animals, glucose concentration increased by 30%, insulin by 22% and total glucose intake (umbilical + infused) by 30%. There was no effect on leucine Ra or on leucine oxidation. In the fasted state, glucose concentration doubled, and insulin increased by 72%, but total glucose intake was not significantly increased. Again, no change in leucine Ra was seen, but leucine oxidation, which was 1.3-fold higher in the fasted basal state compared with the fed state, decreased by 50%, returning to a rate similar to the fed basal state. These data are depicted in Figure 2A. This decline in oxidation resulted in a change from net catabolism to net accretion of leucine during the glucose infusion.

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TABLE 1

Experimental conditions of studies to delineate the independent effects of insulin and glucose on ovine fetal amino acid kinetics

<table>
<thead>
<tr>
<th>Study</th>
<th>Insulin</th>
<th>Glucose</th>
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<tbody>
<tr>
<td>Liechty et al. 1991</td>
<td>Varied by fetal glycemic state</td>
<td>Fetal glucose infusion, 15 mg/min</td>
</tr>
<tr>
<td>Liechty et al. 1993</td>
<td>Somatostatin insulin clamp</td>
<td>Medium and high dose glucose infusion</td>
</tr>
<tr>
<td>Liechty et al. 1992</td>
<td>Hyperinsulinemia by fetal insulin infusion, 1.4 mU/min</td>
<td>Clamped at basal concentration</td>
</tr>
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1 All studies were performed in fetuses of well-fed and fasted ewes.

One study has demonstrated a small suppressive effect of insulin on fetal proteolysis (Milley 1994). The kinetic model employed accounted for disposal of leucine tracer from the fetus to the placenta, which was not done in the previously mentioned experiments. This investigator was able to demonstrate a small decrease in leucine appearance from protein breakdown, in spite of a lack of significant change in total plasma leucine Ra. These data are contrasted with those from our comparable study in Figure 3. It is unclear whether the differences in the conclusions between these studies are the result of the model for analysis or differences in experimental protocols, or simply lack of adequate statistical power in our study. However, it is clear that any suppressive effect insulin may have on fetal proteolysis is not of the magnitude of that seen in postnatal subjects, in which suppression of proteolysis by 30% is commonly demonstrated.

2. Leucine oxidation is decreased by increased glucose availability, as reflected by fetal plasma glucose concentration. Leucine oxidation is highly correlated with plasma glucose concentration, whereas it has no correlation with insulin concentration. This is depicted in Figure 4 in which the mean glucose insulin and leucine oxidation rates from each period have been plotted and regression analysis applied. It can be seen that there is a significant relationship between fetal glucose concentration and leucine oxidation.

In addition, the clear stepwise decrease in leucine oxidation seen in the second experiment, in which glucose concentration was increased without an increase in GUR, implies that glucose concentration rather than GUR is the defining parameter of glucose availability.

3. Leucine accretion is enhanced by either glucose or insulin. Our data imply that enhanced accretion is secondary to the decreased oxidation of leucine, because insulin did not alter total Ra, net umbilical uptake or protein breakdown. Although a direct effect of insulin on protein synthesis cannot be ruled out by our data, changes seen in protein synthesis are likely to be secondary to decreased leucine oxidation, leading to increased availability of leucine for protein synthesis.

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**FIGURE 2** Composite of data from three experiments in fetal lambs. Leucine rate of appearance (Ra) is depicted on the left and leucine oxidation is depicted on the right. The basal conditions are shown by open bars, experimental by solid bars (panel B, medium glucose infusion by hatched bars, high glucose infusion by solid bars).

**FIGURE 3** Comparison of data from experiments utilizing the eu-glycemic hyperinsulinemia protocol in ovine fetal lambs. The data of Milley (1994) were converted from $\mu$mol/kg·min to $\mu$mol/min by multiplying by the mean fetal weight reported in that study.
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HUMAN STUDIES

We have also compared protein metabolism in the ovine fetus with that in human premature infants by conducting a similar set of studies examining the effect of glucose and insulin on proteolysis in extremely premature newborns. Clinically stable, minimally stressed, extremely premature infants (approximately 26 wk gestation) were studied by using two glucose infusions (6.2 and 9.5 mg/(kg·min)) to determine whether an increase in glucose delivery suppresses proteolysis (as reflected by the rates of appearance of the essential amino acids phenylalanine and leucine) (Hertz et al. 1993). Although increases in glucose delivery resulted in clear increases in glucose utilization, substantial reductions in endogenous glucose production and threefold increases in insulin concentration, there was no change in whole-body proteolysis in these extremely premature infants (Fig. 5). Additional studies conducted in full-term newborns in the first few days of life have demonstrated that two- to threefold increases in insulin concentration produced by intravenous infusions of glucose and/or lipid result in no change in whole-body proteolysis (Denne et al. 1995). Changes in insulin concentration of this magnitude have consistently produced reductions in whole-body proteolysis in adult subjects (Flakoll et al. 1989, Shangraw et al. 1988, Tessari et al. 1986). Thus, human premature and term newborns, like the ovine fetus, appear to be resistant to the antiproteolytic effects of insulin, at least at low-to-moderate insulin concentrations.

We have conducted an additional study to determine whether proteolysis might be suppressed by high concentrations of insulin in extremely premature infants (Poindexter et al. 1996). The rates of appearance of leucine and phenylalanine were measured in premature infants (26-wk gestation) before and during euglycemic hyperinsulinemia. Insulin concentrations were increased 11-fold during exogenous insulin infusion, and whole-body proteolysis was reduced by 18%. Thus, as in the ovine fetus, supraphysiologic concentrations of insulin may reduce proteolysis in extremely premature infants, although perhaps not to the same degree as that observed in adult subjects (Tessari et al. 1986).

CONCLUSIONS

In summary, the results of our investigations suggest that proteolysis in the ovine fetus is remarkably resistant to suppres-
sion by insulin. This conclusion is supported by data from other investigators. In addition, this conclusion appears to be compatible with data obtained in postnatal premature human infants of similar gestational age, suggesting that this phenomenon is not unique to ruminants, nor simply a feature of in utero life, but a developmental feature of immature, growing organisms.

Substrate supply, especially glucose, the major fetal fuel, appears to have a substantial influence on fetal amino acid catabolism and hence, protein accretion. Insulin likely has a role in partitioning substrates into tissue accretion or catabolism, although available data suggest that for the amino acids, glucose concentration may be more important than either insulin or the rate of glucose utilization in the regulation of such partitioning.

LITERATURE CITED


