Glucose and amino acid kinetic response to graded infusion of rhIGF-I in the late gestation ovine fetus

Edward A. Liechty, David W. Boyle, Helen Moorehead, Wei-Hua Lee, Xian-Lin Yang, and Scott C. Denne

Glucose and amino acid kinetic response to graded infusion of rhIGF-I in the late gestation ovine fetus. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E537–E543, 1999.—Insulin-like growth factor I (IGF-I) has anabolic effects and is thought to be important in fetal development. The present study was designed to determine the dose response of recombinant human (rh) IGF-I on ovine fetal glucose and amino acid kinetics. Chronically catheterized fetal lambs were studied at 122–127 days gestation. The kinetics of leucine, phenylalanine, and glucose were measured before and during the infusion of rhIGF-I. rhIGF-I was infused into the fetal inferior vena cava at low, medium, or high rates (9.9, 20.1, or 40.2 nmol/h, respectively). A stepwise increase in serum IGF-I was achieved (164 ± 3, 222 ± 7, and 275 ± 5 ng/ml). Insulin concentrations were decreased at the medium and high rhIGF doses. The rate of appearance (Ra) of leucine and phenylalanine and leucine oxidation decreased. Phenylalanine appearance from protein breakdown was decreased, with a maximal suppression of 30% observed at the highest rate of infusion. Glucose Ra was increased at the medium and high doses; other aspects of glucose metabolism were unchanged. The change in both glucose Ra and suppression of proteolysis was significantly correlated to the rhIGF-I infusion rate. It is concluded that rhIGF-I exerts dose-related effects in the ovine fetus, increasing fetoplacental glucose turnover and causing significant suppression of both proteolysis and amino acid oxidation.

leucine; phenylalanine; tyrosine; stable isotopes

The ovine fetus exhibits high rates of protein breakdown and protein synthesis (21). Protein accretion requires synthesis to exceed breakdown. In postnatal life, this balance is regulated by both substrate supply and circulating hormones, including insulin, growth hormone (24), and the insulin-like growth factors (IGFs; Ref. 2). Insulin appears to be the primary hormonal regulator and does so primarily by suppression of proteolysis (5).

In the fetus, however, the role of insulin in suppression of proteolysis is less clear. Several studies have failed to show a relationship between rates of proteolysis and circulating insulin (18–20). Those studies that have shown insulin to suppress proteolysis have observed less degree of suppression at comparable insulin concentrations than has been seen in mature subjects (23). This has prompted investigators to examine other hormones that may be important regulators of fetal protein kinetics.

IGF-I is an important anabolic hormone with a clear regulatory role in growth and development in postnatal life (27). The regulatory role of IGF-I in prenatal life is less well established, but there is increasing circumstantial evidence that IGF-I is important for fetal growth and development also. For example, IGF-I concentrations are correlated with birth weight (6, 17). However, because IGF-I is also correlated with nutritional status (24), it has not been clear whether the correlation with birth weight simply reflects maternal substrate delivery or plays a primary role in fetal anabolism. We hypothesized that if IGF-I has a primary growth-promoting regulatory role in prenatal life, IGF-I would produce a dose-dependent decrease in fetal proteolysis under constant maternal conditions.

The present study was designed to examine whether IGF-I has a direct, dose-dependent endocrine role in the regulation of fetal proteolysis and, if so, whether IGF-I affects placental substrate transfer rates. Finally, we also examined whether IGF-I has a dose-dependent effect on fetoplacental glucose metabolism.

METHODS

Animals and surgical procedures. Eleven ewes of 115–120 days gestation (0.75 gestation, term = 150 days) were utilized for this study. Animal care was in strict compliance with National Institutes of Health guidelines within an American Association for Accreditation of Laboratory Animal Care certified facility, and the study protocols were approved by the Institutional Animal Care and Use Committee.

Surgical procedures were performed aseptically under general anesthesia. Anesthesia was induced by intravenous ketamine and maintained by isoflurane inhalation. Catheters were placed in the common umbilical vein, fetal aorta, and fetal inferior vena cava and in the maternal aorta and inferior vena cava. Catheters were exteriorized and irrigated daily with 0.9% saline wt/vol, containing 50 U heparin/ml. All ewes consumed ad libitum a diet consisting of hay and pelleted alfalfa and had constant access to water and salt lick.

Study design. Animals were allowed to recover from surgery for a minimum of 5 days and were feeding ad libitum before the initial studies were performed and throughout the entire course of study. On the day of the study, baseline samples were obtained for the isotopic enrichment (IE) of leucine, ketosocaproic acid (KIC), phenylalanine, tyrosine, and glucose. A primed constant infusion of L-[1-13C]leucine, L-[1-13C]phenylalanine, L-[ring-2H2]tyrosine, and D-[6,6,2H2]-glucose (Tracer Technologies, Somerville, MA) was begun into the fetal inferior vena cava. The rates of stable isotope tracer administration were 1.6, 0.8, and 0.15 µmol/min for leucine, phenylalanine, and tyrosine, respectively. The glucose tracer...
was infused at 0.181 µmol/min in early studies and increased to 0.33 µmol/min in later studies. [1-14C]leucine (Amersham, Arlington Heights, IL) was also infused, specifically to determine the rate of leucine oxidation, at 450,000 dpm/min. Ethanol was simultaneously infused at 420 µg/min for determination of umbilical blood flow. L-[ring-2H5]phenylalanine was simultaneously infused into the maternal vena cava. After a 120-min equilibration period, four sets of samples were obtained from fetal aorta and umbilical vein and from maternal aorta at 20-min intervals, constituting the control period. An infusion of recombinant human (rh) IGF-I was then begun. Again, a 120-min equilibration period was observed, followed by the drawing of four additional sets of samples that constituted the experimental period. rhIGF-I (gift of Eli Lilly Research Laboratories) was infused at 9.9, 20.1, or 40.2 nmol/h for low, medium, and high-dose studies, respectively. During the rhIGF-I infusion period, fetal whole blood glucose was monitored at 5-min intervals, with a rapid-response glucose analyzer (YSI 2300, Yellow Springs Instruments). The study design allowed for a variable infusion of intravenous glucose to be administered to the fetus to maintain glucose concentration constant at the preinfusion level. It was planned to study each animal at each of the three rhIGF-I infusion rate times; due to technical problems this was not possible. The order of the studies was varied randomly, and the median time between studies was 5 days (range 3–8 days).

Analytical methods. Glucose was analyzed in whole blood by the glucose oxidase method (Boehringer-Mannheim kit no. 189197). Whole blood and tracer infusate leucine, phenylalanine, and tyrosine concentrations were determined by standard ion-exchange chromatography methodology, utilizing a Beckman 6300 automated amino acid analyzer. Intra-assay coefficient of variation was <3%. The whole blood oxygen content was determined in duplicate with an automatic, direct reading by photometer (OSM-3, Radiometer, Copenhagen, Denmark). The within animal coefficient of variation was 13% for arterial or venous oxygen contents.

Leucine and KIC IE ratios were determined as previously described (19). Phenylalanine and tyrosine IE ratios were determined in plasma after derivitization to their tertiary butyldimethylsilyl derivatives (28). The IE of plasma glucose was measured by monitoring ions 98 and 100 after pentacetyl derivatization (16). IE ratios were calculated by the method of Rosenblatt and Wolfe (26).

CO2 content was determined as previously described (18). Intra-assay coefficient of variation was <5%.

Insulin concentrations in plasma were determined in duplicate by a double antibody RIA, with sheep insulin used to construct the standard curve. Intra-assay coefficient of variation is <10%. Total plasma concentrations of IGF-I and II were determined by validated competitive RIAs that employed formic acid-acetone as the IGF extraction procedure (3). RIAs for IGF-II were performed with an anti-IGF-II monoclonal antibody (Amano International Enzyme, Troy, VA; Ref. 1). RIAs for IGF-I were conducted as described previously with a polyclonal rabbit anti-human IGF-I antiserum (1). The ovine IGF-I and IGF-II used for preparing standard curves were kindly provided by Dr. Steve Hodgkinson (Ruakura Agricultural Centre, Hamilton, New Zealand).

Calculations. Umbilical blood flow was calculated by the steady-state ethanol diffusion method (22). The net umbilical uptakes of oxygen, leucine, phenylalanine, tyrosine, and glucose were calculated as the product of the umbilical arteriovenous concentration difference and the umbilical blood flow.

The rate of appearance (R_a) for glucose and leucine was calculated by the following standard equation

\[ R_a = i[(IE_i/IE_p) - 1] \]

where \( R_a \) is measured in micromoles per minute; \( i \) is the infusion rates of tracer (µmol/min); \( IE_i \) is the infusion IE of glucose or leucine, respectively; and \( IE_p \) is the plasma IE of glucose or KIC, respectively.

Total glucose \( R_a \) was calculated based on a single pool, which includes tracer dilution by glucose appearance from fetal and uteroplacental tissues. Steady-state conditions prevailed, and glucose \( R_a \) was equal to the rate of disappearance (\( R_d \)). The \( R_d \) of glucose includes both fetal utilization and loss to uteroplacental tissues.

Fetoplacental leucine kinetics and fetal leucine oxidation were estimated with the reciprocal pool model we have previously described (19). Neither leucine nor KIC tracer losses to the placenta were determined, and the \( R_d \) therefore reflects the sum of the appearances from the fetus and the placenta.

Model for phenylalanine and tyrosine kinetics. Phenylalanine and tyrosine kinetics was estimated by the method of Clarke and Bier (4), modified for the fetus as we have previously described (18). In this model, fetoplacental phenylalanine \( R_a \) is equal to the sum of phenylalanine derived from protein breakdown and the flux of phenylalanine from the placenta to the fetus.

The flux of maternal phenylalanine from the maternal plasma to fetal plasma \( (F_{Phe}^{fp}) \) was estimated by dilution of the maternal tracer in fetal plasma, according to the following equation

\[ F_{Phe}^{fp} = \frac{IE_{Phe}^{m} · Q_{Phe}^{m}}{IE_{Phe}^{f} · Q_{Phe}^{f}} \]

where \( IE_{Phe}^{m} \) is IE of maternal tracer (D-5-Phe) in fetal plasma; \( IE_{Phe}^{f} \) is IE of fetal tracer ([1-13C]-Phe) in fetal plasma; \( Q_{Phe}^{m} \) is \( R_a \) of phenylalanine in maternal plasma; and \( Q_{Phe}^{f} \) is rate of infusion of maternal tracer.

This estimate assumes that there is no utilization of maternally derived phenylalanine by the placenta. Therefore, this will overestimate \( F_{Phe}^{fp} \) to the extent that maternal tracer is utilized by the placenta for protein synthesis.

Phenylalanine derived from protein breakdown \( (PB_{Phe}) \) was estimated as

\[ PB_{Phe} = Q_{Phe}^{f} - F_{Phe}^{fp} \]

The total \( R_d \) is partitioned into flux rates from the fetus to the placenta, protein synthesis, and irreversible fetal disposal. For phenylalanine, this latter flux represents the conversion of phenylalanine to tyrosine via irreversible hydroxylation.

The rate of phenylalanine hydroxylation calculated as

\[ Q_{Phe → Tyr}^{f} = \frac{IE_{Phe}^{f} Q_{Phe}^{f} + IE_{Phe}^{f} Q_{Phe}^{f}}{Q_{Phe}^{f} Q_{Phe}^{f}} \]

where \( Q_{Phe}^{f} \) is \( R_a \) of tyrosine in fetal plasma; \( Q_{Phe}^{f} \) is \( R_a \) of phenylalanine in fetal plasma; \( IE_{Phe}^{f} Q_{Phe}^{f} + IE_{Phe}^{f} Q_{Phe}^{f} = IE_{Phe}^{f} Q_{Phe}^{f} + IE_{Phe}^{f} Q_{Phe}^{f} \); and \( IE_{Phe}^{f} Q_{Phe}^{f} + IE_{Phe}^{f} Q_{Phe}^{f} \) is IE of phenylalanine, \( m + 1 \).
Phenylalanine used for protein synthesis (PS\textsubscript{Phe}) was estimated as

\[
PS\textsubscript{Phe} = Q\textsubscript{Phe}^i - F\textsubscript{Phe}^f - \bar{Q}^\textsubscript{Phe-Tyr}
\]

The unidirectional flux of phenylalanine from plasma to the placenta (F\textsubscript{Phe}^f) was estimated by the product of the percentage of phenylalanine tracer loss to the placenta, determined by the Fick principle, and total phenylalanine \(R_p\).

Statistics. Eleven animals were used in the present study. However, due to factors such as catheter failure or premature fetal delivery or demise, only six of the six fetuses had complete studies performed at all three dosages. Three animals had two of three studies performed, and two animals had a single study performed. In addition, in some studies, umbilical blood flows, and thus parameters that are products of umbilical blood flow, were not obtained. Because of this, data from unequal numbers of animals were used for each of the three infusion rates and/or experimental parameters. Three-way ANOVA was used for primary data analysis across the three infusion rates. The experimental factors were low, medium, or high dose and pre- or during rhIGF-I infusion; interanimal variation was controlled for as a random factor. Significant differences between infusion rates were identified as a statistically significant interaction effect between low, medium, or high dose and pre- or during rhIGF-I infusion. In effect, this identified differing response slopes among the different doses. A priori hypotheses were tested by three-way ANOVA and Student-Newman-Keuls multiple comparison technique, with a P < 0.05 as level of significance (JMP, SAS Institute, Cary, NC). Differences before and during a single infusion rate category were analyzed by paired t-test. Where the initial ANOVA indicated significant effects of rhIGF-I but no significant differences between infusion rates, differences between the pooled means before and during the infusion are reported.

RESULTS

Mean fetal mass at time of autopsy was 3,862 ± 648 g. The mean fetal-to-placental ratio was 9.2 ± 2.0. On the basis of the fetal weights obtained at autopsy, the actual infusion rates of rhIGF-I normalized for body mass were 2.1 ± 0.7, 5.1 ± 0.3, and 10.6 ± 0.7 nmol·kg\(^{-1}\)·h\(^{-1}\) for the low-, medium-, and high-dose studies, respectively. Table 1 shows the changes in IGF-I, IGF-II, and insulin. IGF-I increased in a step-wise manner, as expected. Insulin decreased to nearly identical concentrations in all studies. The decrease was 50% during medium and high infusions. IGF-II concentrations were not changed during IGF-I infusion.

Glucose metabolism. Table 2 contains the parameters of fetal glucose metabolism. Arterial glucose concentrations were held constant by a variable glucose infusion into the fetal inferior vena cava. No animal required glucose infusion during the low- or medium-dose studies. During the high-infusion studies, three animals required no exogenous glucose, whereas six animals did receive exogenous glucose. These six animals required 23.7 ± 9.2 µmol/min of exogenous glucose to maintain plasma glucose concentrations constant during rhIGF-I infusion. This resulted in the total exogenous glucose intake (net umbilical glucose uptake + during the high-dose studies to exceed the net umbilical uptake; 190 ± 23 vs. 166 ± 13 µmol/min). There were increases in glucose Ra (and R\textsubscript{p}) during the medium- and high-dose studies. The percent change in glucose Ra during rhIGF-I infusion was significantly correlated to the infusion rate of rhIGF-I (Fig. 1), although the correlation was not strong. Glucose net umbilical uptake did not change during the low and medium studies; it decreased during the high-dose studies, but the decrease was counterbalanced by the exogenous glucose infusion.

Leucine metabolism. Major parameters of leucine metabolism are depicted in Table 3. Whole blood leucine concentrations decreased during the medium and high rhIGF-I infusion. There were no statistically significant differences in the net umbilical uptakes of leucine during IGF-I infusion. Total fetoplacental leucine R\textsubscript{p} was significantly decreased only during the high-infusion rate. Leucine oxidation and the percentage of leucine R\textsubscript{p} oxidized were decreased at both the medium and high infusion rates.

Phenylalanine metabolism. There were significant changes in fetal concentration of phenylalanine during the medium infusion only. There were no changes in its net umbilical uptake (Table 4). Total fetoplacental phenylalanine R\textsubscript{p} was decreased during the high infu-
sion protocol. In contrast to leucine oxidation (a reflection of irreversible leucine catabolism), the irreversible conversion of phenylalanine to tyrosine was not significantly decreased by IGF-I infusion. The unidirectional flux of phenylalanine from the maternal to the fetal plasma ($F_{fp}$Phe) was significantly increased by IGF-I infusion from $4.00 \pm 0.2$ to $4.6 \pm 0.2 \mu$mol/min, $P < 0.03$). The loss of phenylalanine tracer from fetal plasma to the placenta averaged $6.01 \pm 1.01\%$; the tracer loss was normally distributed, and there were no significant effects due to rhIGF-I infusion. With the use of these data, the rate of disposal of fetal phenylalanine to the placenta, $F_{pf}$Phe, was calculated. This rate decreased from $1.1 \pm 0.2$ to $0.46 \pm 0.2 \mu$mol/min, $P < 0.04$; no dose effect was seen.

Phenylalanine appearance from protein breakdown decreased significantly during the high infusion protocol, where the decrease was from 11 to 9.17 µmol/min. (Fig. 2). Figure 2 also shows the stepwise decrease in percent suppression of fetal proteolysis. There was a significant relationship between the percent suppression of fetal proteolysis and recombinant human insulin-like growth factor I (rhIGF-I) infusion rate. An extreme outlier, depicted by ©, was not included in the regression analysis.

### Table 3. Fetal arterial leucine concentration, net leucine umbilical uptake, leucine $R_a$, and leucine oxidation rates

<table>
<thead>
<tr>
<th>rhIGF-I, nmol/h</th>
<th>Fetal Arterial Concentration, µM</th>
<th>Net Umbilical Uptake, µmol/min</th>
<th>$R_a$, µmol/min</th>
<th>Oxidation, µmol/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>0 8</td>
<td>$110 \pm 1.4$</td>
<td>$7.58 \pm 1.6$ (7)</td>
<td>$42.5 \pm 1.5$ (7)</td>
</tr>
<tr>
<td></td>
<td>9.9 8</td>
<td>$111 \pm 1.4$</td>
<td>$6.89 \pm 1.9$</td>
<td>$40.2 \pm 1.3$</td>
</tr>
<tr>
<td>Med</td>
<td>0 5</td>
<td>$126 \pm 3.2$</td>
<td>$11.5 \pm 1.7$</td>
<td>$36.3 \pm 1.6$</td>
</tr>
<tr>
<td></td>
<td>20.1 5</td>
<td>$113 \pm 3.2^*$</td>
<td>$11.1 \pm 1.7$</td>
<td>$39.9 \pm 1.8$</td>
</tr>
<tr>
<td>High</td>
<td>0 9</td>
<td>$130 \pm 2.1$</td>
<td>$7.02 \pm 2.0$ (8)</td>
<td>$53.4 \pm 7.3$ (8)</td>
</tr>
<tr>
<td></td>
<td>40.2 9</td>
<td>$116 \pm 2.0^*$</td>
<td>$10.5 \pm 2.0$</td>
<td>$41.4 \pm 3.2^*$</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n =$ no. of animals, except where noted by no. in parentheses. *During infusion differs from preinfusion, $P < 0.05$. 

### Table 4. Fetal arterial phenylalanine concentrations, net umbilical uptakes, Phe $R_a$, and Phe hydroxylation rates

<table>
<thead>
<tr>
<th>rhIGF-I, nmol/h</th>
<th>Fetal Arterial Concentration, µM</th>
<th>Net Umbilical Uptake, µmol/min</th>
<th>Phe $R_a$, µmol/min</th>
<th>$Q_{Phe\rightarrow Tyr}$, µmol/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>0 8</td>
<td>$86.07 \pm 4.46$</td>
<td>$3.05 \pm 1.2$ (7)</td>
<td>$18.9 \pm 2.3$</td>
</tr>
<tr>
<td></td>
<td>9.9 8</td>
<td>$83.07 \pm 3.91$</td>
<td>$2.62 \pm 1.4$</td>
<td>$16.5 \pm 2.3$</td>
</tr>
<tr>
<td>Med</td>
<td>0 5</td>
<td>$80.49 \pm 4.82$</td>
<td>$4.46 \pm 0.84$</td>
<td>$14.0 \pm 1.0$</td>
</tr>
<tr>
<td></td>
<td>20.1 5</td>
<td>$70.26 \pm 3.23^*$</td>
<td>$4.23 \pm 0.84$</td>
<td>$14.7 \pm 0.9$</td>
</tr>
<tr>
<td>High</td>
<td>0 9</td>
<td>$81.3 \pm 2.95$</td>
<td>$1.72 \pm 1.2$</td>
<td>$14.6 \pm 2.0$</td>
</tr>
<tr>
<td></td>
<td>40.2 9</td>
<td>$77.82 \pm 3.89$</td>
<td>$2.87 \pm 1.2$</td>
<td>$12.5 \pm 1.4^*$</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n =$ no. of animals, except where noted by no. in parentheses. $Q_{Phe\rightarrow Tyr}$, Phe hydroxylation rates. *During infusion differs from preinfusion, $P < 0.05$. There were no significant differences in net umbilical uptakes. 

---

**Fig. 1.** Relationship between the change in glucose rate of appearance ($R_a$) and recombinant human insulin-like growth factor I (rhIGF-I) infusion rate. An extreme outlier, depicted by ©, was not included in the regression analysis.

**Fig. 2.** A: percent change in fetal proteolysis during rhIGF-I infusion. Data are means ± SE. #differs from low dose; *differs from medium dose, $P < 0.05$, 2-way ANOVA. B: rate of fetal proteolysis; $Phe_{f, PB} = Q_{Phe} - Phe_{f, Pf}$, where $PB$ is protein breakdown; $Q_{Phe}$ is $R_a$ of Phe in fetal plasma; and $Phe_{f, Pf}$ is flux of Phe plasma to placenta. Basal observations are depicted by solid bars, and observations during rhIGF-I infusion are depicted by open bars. Data are means ± SE; $n =$ 8 for low, 5 for medium, and 9 for high. *During infusion differs from basal, $P < 0.05$ by paired t-test.
sion of proteolysis and the rate of rhIGF-I infusion normalized per fetal mass (Fig. 3). The $r^2$ for this relationship was 0.59; the maximum suppression observed was 58% at an infusion rate of 14.3 nmol·kg$^{-1}$·h$^{-1}$.

In contrast to the changes in phenylalanine derived from protein breakdown, there were no significant changes in phenylalanine for protein synthesis (9.8 ± 1.3 to 11.5 ± 1.2 µmol/min, $P > 0.05$). However, this may have been due to a lack of sufficient statistical power. Leucine accretion, the difference between net umbilical uptake and leucine oxidation, increased from 2.0 ± 0.8 to 3.9 ± 1.0 µmol·kg$^{-1}$·min$^{-1}$ ($P < 0.05$, paired t-test). There were no significant differences in the extent to which leucine accretion increased among the different rhIGF-I doses nor was there a significant relationship between the infusion rate and leucine accretion. Phenylalanine accretion was not affected by rhIGF-I infusion (1.9 ± 0.9 to 1.4 ± 0.9 µmol·kg$^{-1}$·min$^{-1}$, $P > 0.05$).

**DISCUSSION**

The present study examines the role of IGF-I as a potential fetal growth factor by assessing ovine fetal protein and glucose metabolism in response to three levels of IGF-I administration to the fetus. The data demonstrate a reduction of fetal proteolysis in response to the highest rate of IGF-I infusion. In addition, leucine oxidation was significantly reduced at the two highest IGF-I infusion rates, and overall fetal leucine balance was improved during IGF-I infusion. Other protein anabolic effects during IGF-I infusion included increased unidirectional transfer of phenylalanine from maternal to fetal plasma and decreased transfer of fetal phenylalanine to the placenta. IGF-I infusion also produced increases in fetoplacental glucose $R_g$ and $R_p$; however, the fetal glucose utilization rate (as reflected by umbilical glucose uptake) was unchanged during IGF-I infusion.

We have previously demonstrated suppression of proteolysis when IGF-I is administered to the fetus. In this previous study, a single rate of infusion, 6.7 nmol·fetus$^{-1}$·h$^{-1}$, was used (18); the infusion was based on estimated fetal weight and resulted in a great degree of variability in actual IGF-I concentrations. Proteolysis was decreased by ~10% in this previous study. The present study design extended the range of rhIGF-I infusion rates from 9.9 to 40.2 nmol/h. This resulted in a wide range of achieved IGF-I concentrations, with median concentrations of 165, 210, and 270 ng/ml at the low, intermediate, and high infusion rates, respectively. A clear effect was seen on fetal proteolysis; at the lowest rates of infusion, no change in proteolysis was seen, whereas at the highest infusion rate a 30% reduction in proteolysis was observed.

Proteolysis estimations in the present study differ from estimations in our previous studies, as we accounted for the unidirectional flux of phenylalanine from the placenta to the fetus. Fetoplacental phenylalanine $R_g$ is the sum of this unidirectional flux and the contribution of phenylalanine derived from proteolysis. Subtraction of the net umbilical uptake of an amino acid from its fetoplacental $R_g$ yields a reasonable approximation to proteolysis. However, it will be an overestimation to the extent that the unidirectional flux, $F_{pf}^{phe}$, exceeds the unidirectional backflux $F_{fp}^{phe}$.

The range of response was seen most clearly when the reduction in proteolysis was correlated to the rate of rhIGF-I infusion, normalized for fetal mass. Indeed, no significant correlation was observed between suppression of proteolysis and IGF-I concentration. The reasons for this are unclear. However, we have observed, both in the present study and in our previous study, that there is a great degree of variability in the IGF-I concentration achieved during rhIGF-I infusion. Only a small percentage of the total circulating concentration may be free and biologically active, resulting in a poor correlation between concentration and biological effects such as suppression of proteolysis. Indeed, in the existing studies in the literature where a dose-response effect of IGF-I was sought, none have presented IGF-I concentration as an independent variable correlated with a biological effect (2, 29, 30). The infusion rate may be more closely related to either the free IGF-I or the IGF-I metabolic clearance rate than is the total IGF-I concentration. Assuming that IGF-I is cleared from the circulation by receptor interaction and internalization, it is logical that the infusion rate would correlate closely with biological effect. We speculate that this is the reason we observed an excellent correlation between the rhIGF-I infusion rate and suppression of proteolysis, whereas we observed no correlation with circulating IGF-I concentration. This speculation must be presented with caution, however, as we did not measure free IGF-I concentration.

It must be pointed out that the reduction in proteolysis produced by IGF-I occurred despite significant decreases in insulin and amino acid concentrations.
Although we have previously been unable to demonstrate an antiproteolytic effect of insulin in the ovine fetus (19), other investigators have observed at least some suppression of proteolysis in the fetus and in postnatal individuals (5, 23). Similarly, concentrations of amino acids also appear to exert an antiproteolytic effect (7, 25). Therefore, the decreases in proteolysis produced by IGF-I in the present study are most likely underestimates; maintaining basal insulin and amino acid concentrations during IGF-I infusion may well have produced greater suppression of fetal proteolysis. This may also partially explain the discrepancy between the present study and our previous study, where an rhIGF-I infusion rate similar to the medium infusion rate effected suppression of proteolysis in fasted animals, whereas not in the present study. Circulating amino acid concentrations were considerably higher in the previous study, whereas glucose and insulin concentrations were lower. It is likely that the prevailing nutritional state of the fetus has an impact on the metabolic response to rhIGF-I.

Other investigators have speculated that suppression of proteolysis observed during high rates of rhIGF-I infusion are due to IGF-I acting through the insulin receptor (8). This does not seem a likely explanation for our results for two reasons. First, we have previously demonstrated that insulin infusion, resulting in fetal insulin concentrations of 80 µU/ml, does not suppress proteolysis (19). It does, however, markedly stimulate glucose utilization by 73%, which was not seen in the present study. Therefore, although we cannot rule out a small insulin-receptor effect, we do not believe it is the major mechanism by which IGF-I acts in the fetus.

In addition to reducing fetal proteolysis, IGF-I acts in other ways to promote fetal protein anabolism. Fetal leucine catabolism (via oxidation) is diminished during IGF-I infusion, resulting in improved fetal leucine balance. IGF-I also appears to influence the transfer of amino acids to the fetus, as well as the partitioning of amino acids between the fetus and placenta. With the use of a three-compartment model, we have demonstrated for the first time an increase in the unidirectional flux of phenylalanine from the mother to the fetus during fetal IGF-I infusion. In addition, the present study measured a 50% decrease in the loss of fetal phenylalanine to the placenta during fetal IGF-I infusion. This finding, combined with the indirect evidence obtained by other investigators (10), adds substantial support for the hypothesis that IGF-I has a role in regulating placental fetal amino acid exchange to promote fetal protein anabolism.

There are limitations to the methods used in this study. In particular, the parameters that utilize the Fick principle exhibit significant experimental error, which limits the ability to obtain statistical significance. In addition, the enrichments and specific activities of substrates in the precursor pools for protein synthesis, leucine oxidation, and phenylalanine hydroxylation were not known precisely but rather estimated from plasma values. These model uncertainties may underlie the discrepancies between the findings with phenylalanine (decreased Ra but no change in catabolism) and leucine (decreased Ra and catabolism).

In contrast to fetal protein metabolism, the effect of IGF-I on fetal glucose metabolism is more equivocal. Regulation of fetal glucose kinetics has been shown to be primarily by fetal insulin concentration and maternal glucose concentration (11–13). The ewes and fetuses in the present study were normoglycemic. Fetal IGF-I infusion resulted in no change umbilical glucose uptake. Because it is unlikely that significant fetal glucose production occurs under these physiological conditions (14), this suggests that IGF-I, over a wide range of concentrations and infusion rates, does not increase fetal glucose utilization. However, there was a clear correlation between IGF-I infusion rate and fetal-placental glucose Ra and Rg. These combined observations would suggest that IGF-I acts to increase maternal transfer of glucose to the fetoplacental unit and to increase placental but not fetal glucose utilization. Thus it is intriguing to hypothesize that the glucose metabolic effects of IGF-I are directed primarily toward the placenta, whereas the protein metabolic effects are directed primarily toward the fetus.

It is open to question whether the findings of this study represent physiology or pharmacology. Certainly, the highest concentrations of IGF-I that were achieved are outside of the normal range found in the ovine fetus. However, shortly after birth concentrations rise and have been found to exceed those of the present study by 20 days of age (9). In addition, fetal nephrectomy also increases circulating fetal IGF-I concentrations to within the range found in the high-dose experiments (15).

In summary, acute fetal infusions of IGF-I produce a wide range of actions on fetal protein anabolism, including a sparing of fetal protein stores, reduced essential amino acid loss from the fetus to the placenta, and increased transfer of essential amino acids from the maternal to the fetal compartment. These findings support the notion that IGF-I acts in a complicated but coordinated fashion to promote fetal growth.

We gratefully acknowledge the assistance of Sara Lecklitner, William Zwyers, and Larry Auble in the performance of these studies and Nancy Chapman for the manuscript preparation.

This work was supported by National Institutes of Health Grants R01-HD-19089, R01-HD-29153, KO8-HD-1048, and PH60-DK-20542 and the James Whitcomb Riley Memorial Association.

Address for reprint requests and other correspondence: E. A. Liechty, Riley R-208, 699 West Drive, Indianapolis, IN 46202-5210 (E-mail: eliechty@upiui.edu).

Received 9 September 1998; accepted in final form 22 April 1999.

REFERENCES


