Effect of rhIGF-I infusion on whole fetal and fetal skeletal muscle protein metabolism in sheep

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Boyle, David W., Scott C. Denne, Helen Moorehead, Wei-Hua Lee, Ronald R. Bowsher, and Edward A. Liechty. Effect of rhIGF-I infusion on whole fetal and fetal skeletal muscle protein metabolism in sheep. *Am. J. Physiol.* 275 (*Endocrinol. Metab.* 38): E1082–E1091, 1998.—Insulin-like growth factor I (IGF-I) has been shown to have significant anabolic effects in the regulation of fetal protein metabolism. To investigate the tissue-specific effects of IGF-I on fetal skeletal muscle metabolism, we infused recombinant human (rh) IGF-I directly into the hindlimb of nine chronically catheterized, late-gestation fetal sheep. Substrate balance and amino acid kinetics were measured across the hindlimb and were compared with the effects at the whole body level before and during a 3-h infusion of rhIGF-I into the external iliac artery at 150 μg/h. Infusion of rhIGF-I resulted in increases in IGF-I concentrations by 2- to 5.75-fold in the ipsilateral iliac vein and by nearly 3-fold in the abdominal aorta. In the study limb, IGF-I had no effect on protein synthesis (phenylalanine rate of disposal 0.88 ± 0.13 before vs. 0.73 ± 0.19 μmol/min during IGF-I) or breakdown (phenylalanine rate of appearance 0.67 ± 0.13 before vs. 0.60 ± 0.17 μmol/min during IGF-I) and did not alter net phenylalanine balance. IGF-I also did not affect hindlimb oxygen or glucose uptake. In contrast, at the whole body level, the rate of appearance of leucine, indicative of fetal protein breakdown, decreased during IGF-I infusion (rate of appearance of leucine 41.1 ± 3.3 to 37.6 ± 2.7 μmol/min) as did fetal leucine oxidation (8.4 ± 0.8 to 6.8 ± 0.6 μmol/min). There was no change in the umbilical uptake of leucine, and although not statistically significant, fetal leucine accretion increased 2.4-fold. These results provide further evidence that IGF-I promotes fetal protein accretion; however, its site of action is in tissues other than skeletal muscle.

phenylalanine; leucine; amino acid kinetics; hindlimb metabolism; glucose uptake; oxygen uptake; recombinant human insulin-like growth factor I

A GROWING BODY of evidence supports the role of insulin-like growth factor I (IGF-I) as an anabolic hormone in fetal life. IGF-I is widely expressed in almost all fetal tissues (9, 17). Plasma concentrations of IGF-I have been shown to correlate directly with fetal weight in humans (1, 24, 25), and decreased concentrations of IGF-I have been found in association with experimentally induced fetal growth retardation in rats (8, 45), guinea pigs (20), and sheep (19, 36). More direct evidence of IGF-I's importance in regulating fetal growth is provided from studies of transgenic mice whose birth weight is either greater than normal or less than normal depending on whether the IGF-I gene is overexpressed or knocked out (21). In addition, investigators have demonstrated anabolic effects of short-term IGF-I infusion in fetal sheep (18, 27).

Most in vivo studies of this factor have concentrated on the balance between protein synthesis and protein breakdown. Studies in postnatal growing animals and in adult human subjects have suggested that there are tissue-specific effects that may affect conclusions depending on which tissue is studied. This has been especially true when contrasting whole body protein kinetics against those found across an isolated tissue bed such as the forearm (12). Although the magnitude of change has varied among studies, in general, acute recombinant human (rh) IGF-I infusion results in a decrease in whole body proteolysis and an increase in skeletal muscle protein synthesis and amino acid balance (10, 14, 22).

In vitro studies provide further support for IGF-I's anabolic action in skeletal muscle. IGF-I has been shown to stimulate a mitogenic response in skeletal muscle cells and to induce myoblast differentiation (21). In addition, IGF-I has been shown to increase protein synthesis and inhibit proteolysis in rat L6 myoblasts (11).

Limited information is available regarding the site or mode of action of IGF-I in regulating protein metabolism in the fetus. We have previously demonstrated that rhIGF-I infusion decreases fetal whole body proteolysis and amino acid catabolism (27). Tissue-specific effects of IGF-I have not been examined; specifically, the in vivo effect of IGF-I on fetal skeletal muscle protein metabolism has not been investigated previously. The present study was designed to test the hypothesis that a significant portion of the antiproteolytic effect of IGF-I in the fetus is in skeletal muscle. Specifically, we hypothesized that a direct infusion of IGF-I in the external iliac artery in the fetal sheep would result in a significant increase in phenylalanine balance across the hindlimb. To test this hypothesis, we infused rhIGF-I intra-arterially into the hindlimb in late-gestation fetal sheep and measured the rates of appearance (R_a) and disappearance (R_d) and net uptake of phenylalanine across the hindlimb. The tracer model used in this study allowed us to examine the specific effects of IGF-I on fetal skeletal muscle protein synthesis, breakdown, and accretion. We also measured the net hindlimb uptake of tyrosine and the branched-chain amino acids for additional confirmation of IGF-I's potential anabolic action in skeletal muscle. In addition, the effects of IGF-I on fetal skeletal muscle oxygen

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and glucose utilization were measured. The R_a of leucine and phenylalanine in fetal plasma as well as the rate of leucine oxidation by the fetus were also determined to compare the effects of IGF-I on protein metabolism at the whole body level with those in fetal skeletal muscle.

MATERIALS AND METHODS

Surgery and animal care. Nine mixed-breed ewes with time-dated singleton pregnancies were utilized for this study. Animal care was in strict compliance with National Institutes of Health guidelines within an American Association for Laboratory Care-certified facility. The study protocol was approved by the Institutional Animal Care and Use Committee.

Ewes were fasted for 48 h before surgery at 115–118 days gestation (term is 150 days). Surgical procedures were performed aseptically under general anesthesia. Anesthesia was induced with intravenous ketamine (350–750 mg) and acepromazine (25 mg) 30 min preoperatively and was maintained with isoflurane inhalation (1.0–1.75%). Maternal catheters were placed in the femoral artery and in the uterine vein of the pregnant horn of the uterus. Fetal catheters were placed in the common umbilical vein and in the inferior vena cava and abdominal aorta by way of the pedal vessels of one hindlimb. The contralateral hindlimb was prepared as described by Wilkening et al. (46) with catheters in the external iliac artery and vein and a 3-mm transit-time blood flow transducer (Transonic Systems, Ithaca, NY) on the external iliac artery for continuous blood flow measurement. This hindlimb was designated as the "study limb." Pancuronium bromide (0.2 mg/kg estimated fetal weight) was administered to the fetus via the inferior vena cava catheter to produce muscle relaxation before catheterization of the study limb. A catheter was also placed in the amniotic cavity for instillation of antibiotic.

The surgical model employed in this experiment is designed to accurately measure blood flow to the fetal hindlimb and sample the venous drainage from this well-defined tissue bed while minimizing interference with the circulation. Wilkening et al. (46) found no difference between the study limb weight and the "nonstudy limb" weight using this model. The catheterization procedures have been described previously and have been used to describe many aspects of fetal hindlimb metabolism (7, 46, 48).

The animals used to generate the data in the present study were also utilized in investigations involving prolonged uterine blood flow reduction. Therefore, in addition to the surgery described above, an adjustable vascular occluder was positioned around the maternal terminal aorta, and vessels contributing to the collateral circulation of the uterus were ligated (33). Finally, a 6-mm transit-time blood flow transducer was positioned around the middle uterine artery of the pregnant horn for continuous monitoring of uterine blood flow during vascular occlusion. All incisions were closed, and the catheters and vascular occluder and flow probe cables were led subcutaneously to an external flank pouch.

After surgery, the animals were maintained in a metabolic cart and were provided water, a salt lick, and a standard alfalfa pellet diet ad libitum. Maternal antibiotics (penicillin 500,000 units im and gentamicin 0.8 mg/kg im) were administered preoperatively and for 5 days postoperatively. Fetal antibiotics (ampicillin 500 mg ia and tobramycin 2.5 mg/kg iv) were administered daily postoperatively. All catheters were irrigated daily with 0.9% saline (wt/vol) containing 50 U of heparin per milliliter.

Study design. The animals were allowed a minimum of 6 days of recovery from operative stress before study (mean 7 days; range 6–13 days). Complete recovery was assessed by monitoring maternal food intake as well as fetal and maternal glucose concentrations and acid-base status.

Animals were studied as illustrated in Fig. 1. On the day of the study, baseline samples were obtained for the tracer-to-tracee ratio of α-ketoisocaproic acid (KIC) and phenylalanine. A primed constant infusion of L-[1-^{13}C]leucine and L-[*ring*-d_5]phenylalanine (Tracer Technologies, Sommerville, MA) was begun in the fetal inferior vena cava. The rates of stable isotope tracer administration were 1.65 and 0.85 μmol/min for leucine and phenylalanine, respectively. L-[1-^{14}C]leucine (Amersham, Arlington Heights, IL) was also infused, specifically to determine the rate of leucine oxidation, at 450,000 disintegrations·min^{-1}·min^{-1}. Ethanol was simultaneously infused at 420 μg/min for determination of umbilical blood flow by the steady-state diffusion method. As suggested by Wilkening et al. (47), the ethanol concentration in the infusate was adjusted, based on estimates of the fetal size and placental clearance of ethanol, to give a fetal arterial ethanol concentration not exceeding 10 mg/dl at the conclusion of the experiment. After a 120-min equilibration period, three or four sets of blood samples were obtained at 20-min intervals, constituting the "before IGF-I" period. rhIGF-I (gift of Eli Lilly Research Laboratories, Indianapolis, IN) was then infused at 150 μg/h in the external iliac artery of the study limb. After a second 120-min equilibration period, blood samples were obtained again at 20-min intervals constituting the "during IGF-I" period. During the rhIGF-I infusion period, fetal whole blood glucose was monitored at 5-min intervals, using a rapid response glucose analyzer (YSI 2300; Yellow Springs Instruments, Yellow Springs, OH), and intravenous glucose was administered to the fetus to maintain glucose concentration constant at the pre-rhIGF-I infusion level. The volume of fetal red blood cells withdrawn was replaced with equal volumes of red blood cells from a donor fetal sheep or from the mother.

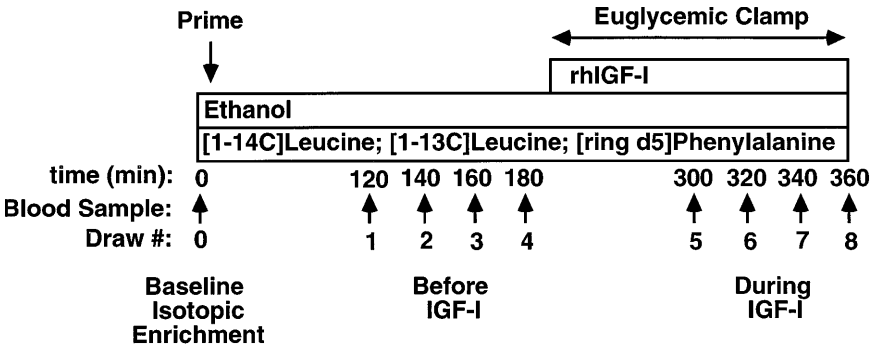

Fig. 1. Schematic diagram of experimental protocol. See text for details. Recombinant human insulin-like growth factor I (rhIGF-I) was infused at 150 μg/h.

At the time of autopsy, animals were killed by a rapid intravenous infusion of a commercially available euthanasia solution. Catheter positions were confirmed, and the fetus, placenta, and fetal organs were weighed. The interval of time between study and autopsy varied among animals from 6 to 15 days (10 ± 3 days, mean ± SD).

Analytical methods. Whole blood oxygen content, oxygen saturation, and hemoglobin concentration were determined immediately in duplicate using an automatic, direct reading photometer (OSM-3; Radiometer, Copenhagen, Denmark). Arterial pH, P_{O_2}, and P_{CO_2} were measured using an Instrumentation Laboratories 1301 Arterial Blood Gas Analyzer corrected to the fetal temperature of 39.5°C.

Whole blood ethanol was measured in duplicate using the enzymatic conversion of ethanol to acetaldehyde (Sigma Chemical; see Ref. 5). Whole blood glucose and lactate concentrations were determined in duplicate with a Yellow Springs Instruments model 2300 Stat Glucose/L-Lactate Analyzer. In addition, glucose was analyzed in whole blood by the glucose oxidase method (Boehringer Mannheim kit no. 189197).

Whole blood and tracer infusate amino acid 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 KIC tracer-to-tracee ratio was determined as previously described (28). The phenylalanine tracer-to-tracee ratio was determined in plasma after derivitization to the tertiary butyldimethylsilyl derivative (42). The derivative was injected in a Hewlett Packard 5970 gas chromatography-mass spectrometry with electron impact ionization and selected ion monitoring of ions 234 and 239. The tracer-to-tracee ratio was calculated for phenylalanine ($m + 5$) by the method of Rosenblatt and Wolfe (40).

To determine $^{14}CO_2$ content, ~0.3 ml whole blood were injected in the center well of a previously weighed scintillation vial (Bio-Rad, Melville, NY) containing 1.0 ml Solvable (New England Nuclear). The actual amount of blood injected was then determined gravimetrically. HCl (0.5 ml of 1.0 N) was then added to the center well to liberate the CO_2, which was trapped in the Solvable. After overnight incubation, the center well was removed, 15 ml Econofluor were added to the vial, and the sample radioactivity was counted. Counts per sample were converted to disintegrations per minute, and the sample disintegrations per minute per gram whole blood were converted to disintegrations per minute per milliliter whole blood using the density of whole blood. Intra-assay coefficient of variation was <5%.

Insulin concentrations in plasma were determined in duplicate by a double-antibody radioimmunoassay, with sheep insulin used to construct the standard curve. Intra-assay coefficient of variation was <10%.

Total plasma concentrations of IGF-I and -II were determined by validated competitive radioimmunoassays that employed formic acid-acetone as the IGF extraction procedure (6). Briefly, 100 µl of plasma were acidified with 50 µl of 8.0 M formic acid containing 0.5% Tween 20. After the addition of 350 µl of acetone, the samples were mixed thoroughly by vortexing and centrifuged at ~4°C for 20 min at 3,500 *g*. Supernatants were diluted in assay buffer that consisted of 0.1 M sodium phosphate-0.1% NaCl-0.1% EDTA-0.1% sodium azide-0.2% protamine sulfate-0.05% Tween 20. Radioimmunoassays for IGF-II were performed using an anti-IGF-II monoclonal antibody (Amano International Enzyme Company, Troy, VA; see Ref. 6). Radioimmunoassays for IGF-I were conducted as described previously using a polyclonal rabbit anti-human IGF-I antiserum (3). 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. Uterine and umbilical blood flows were calculated by application of the Fick principle to the steady-state transplacental diffusion of ethanol (32).

Hindlimb blood flow was measured continuously using the 3-mm transit-time flow transducer on the external iliac artery of the study limb. Data were digitized at 100 Hz, using a 12-bit analog-to-digital board, with analysis and storage performed using LabView 2 software (National Instruments, Austin, TX). Average blood flow was determined over 5 min during blood sampling periods. Fetal heart rate was counted from the arterial pulse frequency.

The net fluxes of oxygen, glucose, lactate, and amino acids from placenta to the fetus were calculated as the product of the umbilical arteriovenous concentration difference and the umbilical blood flow. The uptakes of oxygen, glucose, lactate, and amino acids by the fetal hindlimb were calculated by application of the Fick principle to the hindlimb circulation.

Fetal oxygen extraction was calculated from the following formula

$$\frac{[O_2]_\gamma - [O_2]_\alpha}{[O_2]_\gamma} \times 100$$

where $[O_2]_\gamma$ is the oxygen content in the umbilical vein and $[O_2]_\alpha$ is the oxygen content in the descending aorta. Similarly, hindlimb oxygen extraction was calculated as

$$\frac{[O_2]_\alpha - [O_2]_\phi}{[O_2]_\alpha} \times 100$$

where $[O_2]_\phi$ is the oxygen content in the external iliac vein.

Model for amino acid kinetics. The total R_a for leucine and phenylalanine were calculated by the following standard equation

$$R_a = i[(r_i/r_p) - 1]$$

where units for R_a are µmol/min, i is the infusion rate of tracer (µmol/min), r_i is the infusate tracer-to-tracee ratio of leucine or phenylalanine, and r_p is the plasma tracer-to-tracee ratio of KIC or phenylalanine.

Leucine oxidation rate was determined from the product of the oxidation fraction of the L-[1-^{14}C]leucine infusion and the leucine R_a. The following equations were used

$$\text{OxFx} = \frac{([^{14}C]CO_{2\alpha} - [^{14}C]CO_{2\gamma}) \times \text{umbilical blood flow}}{i}$$

$$\text{Leu Ox} = \text{OxFx} \times \text{Leu } R_a$$

where OxFx is the leucine oxidation fraction, $[^{14}C]CO_{2\alpha}$ and $[^{14}C]CO_{2\gamma}$ are the concentrations in disintegrations per minute per milliliter of $[^{14}C]CO_2$ in the umbilical arterial and venous blood, respectively, i is the infusion rate of L-[1-^{14}C]leucine in disintegrations per minute per minute, Leu Ox is the leucine oxidation rate in micromoles per minute, and Leu R_a is the leucine R_a in micromoles per minute. No correction for CO_2 fixation was made, as Van Veen et al. (44) have demonstrated no appreciable label fixation during fetal infusion of $NaH^{14}CO_3$.

Leucine and phenylalanine kinetics were calculated on the basis of a single pool that includes tracer dilution by leucine or phenylalanine appearance from fetal and uteroplacental tissues. Likewise, the rate of leucine or phenylalanine disap-

pearance includes both fetal utilization and loss to uteroplacental tissues.

Model for hindlimb phenylalanine kinetics. Hindlimb phenylalanine kinetics were calculated using the equations derived by Barrett and co-workers (2). The R_a and R_d of phenylalanine across the hindlimb were determined as follows

$$\text{HL Phe } R_a = BF \times [Phe]_\alpha \times \left(\frac{r_p\alpha}{r_p\phi} - 1\right)$$

where HL Phe R_a is hindlimb phenylalanine R_a in micromoles per minute, BF is hindlimb blood flow in milliliters per minute, $[Phe]_\alpha$ is fetal arterial phenylalanine concentration (μM), and $r_p\alpha$ and $r_p\phi$ are the plasma tracer-to-tracee ratios of phenylalanine in arterial and femoral venous blood, respectively. Net phenylalanine balance across the hindlimb was determined by the Fick principle

$$\text{Net balance Phe} = BF \times ([Phe]_\alpha - [Phe]_\phi)$$

where $[Phe]_\phi$ is the study limb femoral venous phenylalanine concentration (μM). Because

$$\text{Net balance} = \text{Phe } R_d - \text{Phe } R_a$$

Therefore

$$\text{HL Phe } R_d = \text{net balance} + \text{Phe } R_a$$

where HL Phe R_d is hindlimb phenylalanine R_d in micromoles per minute.

Phenylalanine is not catabolized within skeletal muscle; therefore, HL Phe R_d is essentially equal to phenylalanine used for protein synthesis. Likewise, there is no endogenous production of phenylalanine within the hindlimb. Therefore, HL Phe R_a is essentially equal to phenylalanine derived from protein breakdown.

Gelfand and Barrett (15) have discussed the use of phenylalanine in examining muscle protein turnover in vivo. Direct measurement of incorporation of tracer amino acid into protein would require tissue biopsy, which is not practical in the study of fetal amino acid kinetics. In fact, determination of fractional protein synthetic rates via muscle biopsy and the tracer dilution technique have yielded reasonably consistent results, both qualitatively and quantitatively (34, 37). In addition, there is no viable method to measure tissue proteolysis in vivo. Therefore, the tracer dilution method is the best option for studying protein synthesis and breakdown in fetal skeletal muscle.

The kinetics model employed, however, does have potential limitations. It relies on the dilution of labeled phenylalanine by unlabeled phenylalanine in the deep venous drainage of the hindlimb to determine the R_a of the tracee. Likewise, the determination of whole body leucine or phenylalanine R_a relies on dilution of tracer by unlabeled tracee. Recent evidence suggests that, for leucine, there exist within skeletal muscle several functionally heterogeneous pools (29). These pools do not contribute to plasma equally. The evidence suggests that muscle protein breakdown may contribute preferentially to protein synthesis, whereas plasma-derived leucine may contribute preferentially to the transamination pool. Thus, in experiments in which rates of skeletal muscle protein breakdown change, the magnitude of the change may be significantly underestimated. It is unknown whether these same caveats apply to phenylalanine pools. However, it is possible that the hindlimb kinetic model employed underestimates hindlimb protein breakdown to a greater degree than does the whole body kinetic model.

Statistical analysis. Two-factor ANOVA was used to compare physiological measurements obtained before and during rhIGF-I infusion. The experimental factor was before/during; interanimal variation was controlled for as a random factor. A priori hypotheses were tested by two-factor ANOVA followed by the Fisher's protected least significant difference procedure comparing mean data from the before IGF-I and during IGF-I study periods, with a $P < 0.05$ as the level of significance. Correlations between variables were examined using linear and exponential univariate regression, with a $P < 0.05$ as the level of significance. All analyses were performed using SuperANOVA or StatView software (Abacus Concepts, Berkeley, CA).

Comparisons between before and during rhIGF-I infusion are for $n = 9$ with the exception that the hindlimb flow transducer failed in one animal; therefore, calculations involving hindlimb blood flow are based on $n = 8$. In addition, the lactate analyzer did not work for three studies; therefore, lactate concentration is presented for $n = 6$. Data are expressed as means ± SE unless noted otherwise. Data are not normalized to fetal weight, since the fetal weight was unknown at the time of the study.

RESULTS

The gestational age of the nine animals at the time of study was 124 ± 3 days (mean ± SD). At the time of autopsy, fetal weight was 3,243 ± 492 g (n =8) and study limb weight was 329 ± 70 g (n = 7). The study limb was 10% of the fetal weight, which is consistent with a previous report (46). Autopsy occurred, on average, 10 days after study (range 6–15 days). Six of the nine animals were further studied before autopsy. There were no differences in fetal or study limb weight between these six sheep and the other three by unpaired *t*-test.

In Table 1, fetal plasma concentrations of IGF-I, IGF-II, and insulin are shown. Infusion of rhIGF-I in the external iliac artery of the study limb resulted in a greater than threefold increase in the level of IGF-I in the ipsilateral external iliac vein (range 2 times to 5.7 times). Likewise, recirculation of IGF-I led to a threefold rise in arterial IGF-I concentration. rhIGF-I infusion did not effect IGF-II concentration in the venous drainage of the study limb or in the arterial circulation. Plasma insulin concentration was significantly decreased during the IGF-I infusion period. As illustrated in Fig. 2, a significant negative correlation was ob-

Table 1. *Fetal plasma concentrations of insulin-like growth factors and insulin*

	Before IGF-I	During IGF-I	*P*
Arterial IGF-I, ng/ml	60.47 ± 3.86	169.47 ± 9.41	<0.01
Venous IGF-I, ng/ml	57.55 ± 4.27	184.70 ± 9.78	<0.01
Arterial IGF-II, ng/ml	462.19 ± 24.70	424.57 ± 22.53	NS
Venous IGF-II, ng/ml	442.00 ± 23.90	435.54 ± 19.01	NS
Arterial insulin, μU/ml	14.81 ± 1.70	7.39 ± 1.17	<0.01

Data are means ± SE. Venous insulin-like growth factors (IGF) I and II are from the external iliac vein draining the study limb. *P* determined by ANOVA. NS, not significant.

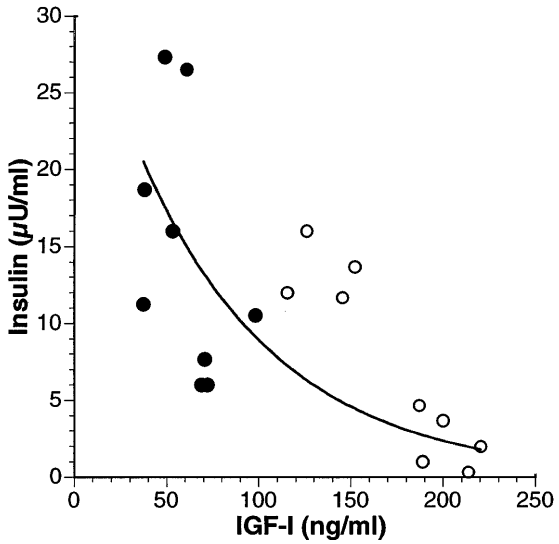

Fig. 2. Correlation between fetal plasma concentration of insulin and IGF-I [$y = 33.64 \times \exp(-0.013x)$, $r^2 = 0.548$]. ●, Before IGF-I infusion; ○, during IGF-I infusion.

served between the arterial concentrations of insulin and IGF-I.

Hindlimb blood flow increased during rhIGF-I infusion from 46.9 ± 1.7 to 52.8 ± 1.4 ml/min ($P < 0.01$). Associated with the increase in hindlimb blood flow, there was a decrease in umbilical blood flow from 549.4 ± 19.5 to 492.9 ± 22.8 ml/min ($P < 0.01$) and an increase in fetal heart rate from 178 ± 3 to 197 ± 4 beats/min ($P < 0.01$). There was, however, no correlation between hindlimb blood flow and IGF-I concentration ($R = 0.27$ for hindlimb blood flow vs. arterial IGF-I; $R = 0.17$ for hindlimb blood flow vs. venous IGF-I). During the rhIGF-I infusion period, arterial hemoglobin concentration fell from 9.21 ± 0.16 to 8.67 ± 0.15 g/dl ($P < 0.01$). Thus, in spite of attempts to maintain red blood cell mass throughout the study, blood sampling resulted in a significant fall in fetal hemoglobin. When hemoglobin concentration was controlled for as a covariate, there was no significant difference in hindlimb blood flow between the before and during IGF-I infusion states ($F = 1.1214$, $P = 0.3022$).

In Table 2, fetal arterial concentrations for oxygen, glucose, and lactate, as well as net umbilical and study limb uptakes for oxygen and glucose are shown. Arterial oxygen concentration decreased by nearly 15% during the rhIGF-I infusion. This fall is most likely due to the combination of the decrease in fetal hemoglobin concentration, as previously noted, and a statistically significant fall in the percent saturation of hemoglobin from 60.62 ± 1.12 to 54.72 ± 1.42% ($P < 0.01$). Despite the fall in arterial oxygen content and the previously noted decrease in umbilical blood flow, there was no change in net umbilical oxygen uptake. Umbilical oxygen uptake was maintained by an increase in fetal oxygen extraction from 31.91 ± 0.01 to 37.01 ± 0.01% ($P < 0.01$). There was also no change in net study limb oxygen uptake or hindlimb oxygen extraction (29.90 ± 0.01% before IGF-I, 30.33 ± 0.01% during IGF-I). Although the study was designed to maintain whole blood glucose concentration constant during the IGF-I infusion, no hypoglycemic effect was observed, and no exogenous glucose was infused in any animal. In fact, there was a statistically significant increase in glucose concentration during IGF-I infusion. Neither net umbilical glucose uptake nor net study limb glucose uptake was affected by IGF-I infusion. There was a small but significant increase in arterial lactate concentration during the rhIGF-I infusion period. This increase in lactate concentration was associated with a decrease in arterial pH from 7.33 ± 0.01 to 7.31 ± 0.01 ($P < 0.01$). There were no changes in fetal arterial P_{O_2} or P_{CO_2} during the study.

Fetal arterial concentrations and net umbilical and study limb uptakes of the branched-chain and aromatic amino acids studied are shown in Table 3. With the exception of isoleucine, amino acid concentrations decreased by as much as 11% during IGF-I infusion. There were, however, no changes in either umbilical or study limb uptake for any of the amino acids studied. The umbilical and study limb uptakes were within the ranges reported in previous studies (26, 49).

The steady-state tracer-to-tracee ratios of phenylalanine and KIC are shown in Fig. 3. Fetal leucine kinetics are depicted in Fig. 4. rhIGF-I infusion significantly decreased leucine R_a from 41.1 ± 3.3 to 37.6 ± 2.7

Table 2. *Fetal arterial concentrations of oxygen, glucose, and lactate and net umbilical and study limb uptakes of oxygen and glucose*

	Before IGF-I	During IGF-I	*P*
[O_2], mM	3.22 ± 0.10	2.75 ± 0.09	<0.01
[Glucose], mM	1.03 ± 0.06	1.14 ± 0.04	<0.01
[Lactate], mM	1.13 ± 0.03	1.38 ± 0.07	<0.01
Φ O_2, μmol/min	833.97 ± 37.49	778.93 ± 34.48	NS
Φ Glucose, μmol/min	101.30 ± 11.14	103.93 ± 11.70	NS
HL Upt O_2, μmol/min	44.54 ± 2.65	43.85 ± 2.66	NS
HL Upt Glucose, μmol/min	5.37 ± 1.23	5.06 ± 1.12	NS

Data are means ± SE; $n = 8$ ewes. Φ, net umbilical uptake; HL Upt, study limb uptake. Brackets denote concentration. *P* determined by ANOVA.

Table 3. *Fetal arterial concentrations and net umbilical and study limb uptakes of amino acids*

	Before IGF-I	During IGF-I	*P*
[Ile], μmol/ml	0.111 ± 0.006	0.105 ± 0.007	NS
[Leu], μmol/ml	0.200 ± 0.011	0.183 ± 0.011	<0.01
[Val], μmol/ml	0.485 ± 0.028	0.437 ± 0.027	<0.01
[Tyr], μmol/ml	0.089 ± 0.006	0.079 ± 0.005	<0.01
[Phe], μmol/ml	0.096 ± 0.004	0.091 ± 0.003	<0.01
Φ Ile, μmol/min	5.47 ± 1.02	5.05 ± 0.49	NS
Φ Leu, μmol/min	9.11 ± 0.77	8.81 ± 0.77	NS
Φ Val, μmol/min	5.69 ± 2.71	8.58 ± 1.84	NS
Φ Tyr, μmol/min	1.98 ± 0.70	2.64 ± 0.37	NS
Φ Phe, μmol/min	2.83 ± 0.32	2.75 ± 0.35	NS
HL Upt Ile, μmol/min	0.31 ± 0.07	0.36 ± 0.07	NS
HL Upt Leu, μmol/min	0.80 ± 0.09	0.71 ± 0.12	NS
HL Upt Val, μmol/min	1.25 ± 0.24	0.88 ± 0.20	NS
HL Upt Tyr, μmol/min	0.12 ± 0.04	0.08 ± 0.04	NS
HL Upt Phe, μmol/min	0.21 ± 0.04	0.13 ± 0.04	NS

Data are means ± SE; $n = 8$ ewes. *P* determined by ANOVA.

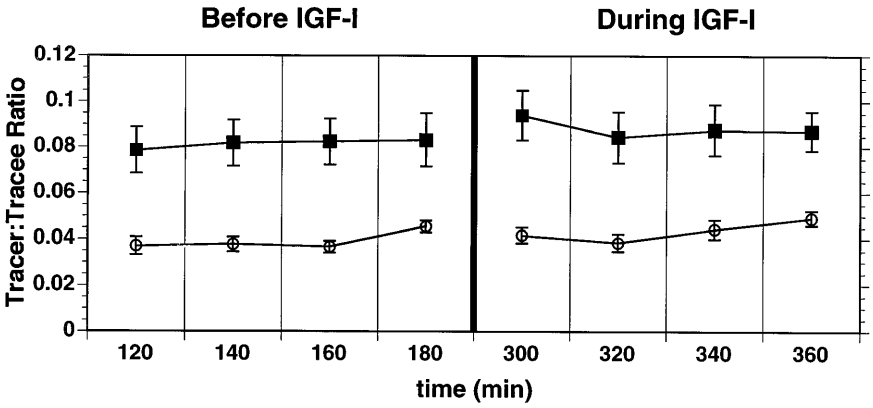

Fig. 3. Time-dependent plasma tracer-to-tracee ratios for phenylalanine (■) and ketoisocaproic acid (○) before and during rhIGF-I infusion. Time of sampling is noted in minutes along *x*-axis after isotopic tracer infusion was begun (not drawn to scale).

μmol/min. As noted above, leucine umbilical uptake was not affected by IGF-I infusion. Even though the specific R_a of leucine from uteroplacental tissues was not measured, the decrease in leucine R_a in this study is most consistent with a decrease in the R_a of leucine from endogenous protein stores, i.e., a decrease in whole body proteolysis. Fetal leucine oxidation also decreased significantly during IGF-I infusion from 8.4 ± 0.8 to 6.8 ± 0.6 μmol/min. Fetal leucine accretion, calculated as the difference between net umbilical uptake and leucine oxidation, increased by 2.4-fold from 1.0 ± 0.8 to 2.4 ± 0.8 μmol/min; however, this increase was not statistically significant. Total phenylalanine R_a was not affected by rhIGF-I infusion (9.2 ± 0.5 μmol/min before IGF-I vs. 8.6 ± 0.6 μmol/min during IGF-I, $P = 0.1059$).

In Fig. 5, fetal hindlimb phenylalanine kinetics are depicted. There was no difference in the R_a of phenylalanine across the hindlimb (0.67 ± 0.13 μmol/min before IGF-I vs. 0.60 ± 0.17 μmol/min during IGF-I), and there was no difference in hindlimb phenylalanine uptake. Similarly, rhIGF-I infusion had no effect on hindlimb phenylalanine R_d (0.88 ± 0.13 μmol/min before IGF-I vs. 0.73 ± 0.19 μmol/min during IGF-I).

DISCUSSION

IGF-I is an important factor regulating intermediary metabolism in the mammalian organism. It has been found to have significant anabolic effects, even in a nongrowing organism. However, its relationship to the pituitary growth hormone endocrine system has prompted interest in its anabolic effects in growing organisms, including the mammalian fetus. Both circumstantial and direct evidence now support its anabolic role in fetal life (1, 8, 19–21, 24, 25, 27, 36, 39, 45). This fact, coupled with its availability through recombinant DNA technology, make it a potential therapeutic tool in fetal growth restriction.

Before any consideration can be given to IGF-I treatment of the fetus, the tissue-specific effects of IGF-I must be examined. Studies in postnatal animals and adult human volunteers have consistently demonstrated significant anabolic changes in skeletal muscle during acute infusions of IGF-I. Fryburg (12) infused IGF-I intra-arterially in the forearm at three different doses in postabsorptive adult human subjects. IGF-I concentrations in the forearm vein increased ~50, 150, and 300%; the net phenylalanine balance was significantly improved at all three levels. In a follow-up study using a similar design, an intra-arterial infusion of IGF-I also substantially improved skeletal muscle anabolism during hyperaminoacidemia produced by intravenous infusion of amino acids (14). Analogous with these studies in humans, Oddy and Owens (35) infused IGF-I in the femoral artery of 5-mo-old lambs, resulting in an approximately threefold increase in

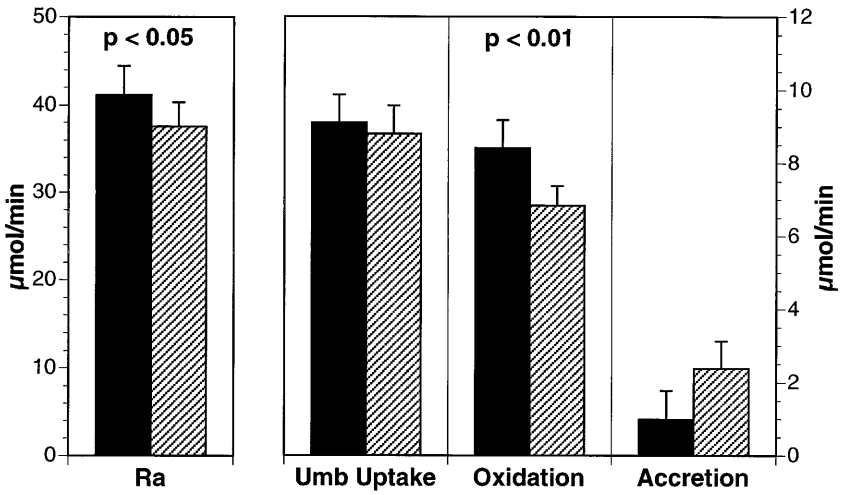

Fig. 4. Fetal leucine rate of appearance (R_a), umbilical (Umb) uptake, oxidation, and accretion. Filled bars, before rhIGF-I infusion; hatched bars, during rhIGF-I infusion. Significant differences between before and during IGF-I infusion by 2-factor ANOVA.

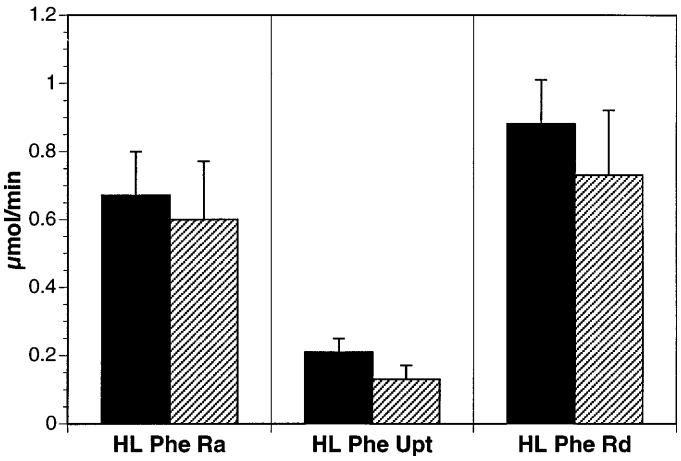

Fig. 5. Fetal hindlimb (HL) phenylalanine R_a, uptake (Upt), and rate of disappearance (R_d). Filled bars, before rhIGF-I infusion; hatched bars, during rhIGF-I infusion; $n = 8$ ewes.

IGF-I concentrations in the femoral vein and producing significant improvements in phenylalanine balance across the hindlimb. This anabolic response was measured in these lambs during fasting, feed restriction, and normal feeding. Thus, under different nutritional conditions and in different species, acute infusion of IGF-I results in skeletal muscle anabolism in postnatal life. In contrast, we did not observe a significant improvement in hindlimb phenylalanine, tyrosine, isoleucine, leucine, or valine balance during an external iliac arterial infusion of IGF-I in fetal sheep. It must be noted that no change in fetal skeletal muscle anabolism was measured despite a threefold increase in the concentrations of IGF-I in the femoral vein using an IGF-I infusion rate greater than delivered in the previous postnatal investigations.

Most studies carried out in postnatal life have suggested that the acute anabolic effect of IGF-I on skeletal muscle occurs by stimulating protein synthesis. In the human studies cited above, intra-arterial IGF-I infusion resulted in significant increases in muscle protein synthesis (12, 14); reductions in skeletal muscle proteolysis were occasionally, but less consistently, observed. In fasted lambs, systemic IGF-I infusion produced significant increases in skeletal muscle fractional synthetic rates at a dose comparable to that used in the present study (10, 22). In contrast, Oddy and Owens (35) measured a decrease in skeletal muscle protein synthesis in lambs during a hindlimb IGF-I infusion; however, this change in protein synthesis was accompanied by an even greater reduction in muscle proteolysis, leading to a net anabolic effect of IGF-I in the hindlimb. In the present study, neither fetal skeletal muscle protein synthesis nor proteolysis was altered by external iliac artery infusion of IGF-I.

Despite the lack of anabolic effect in fetal skeletal muscle, IGF-I infusion did produce significant anabolic changes in the whole fetus. Leucine R_a, reflecting whole body proteolysis, was significantly reduced by 8.5% during the IGF-I infusion; phenylalanine R_a similarly declined by 6.5% but did not reach statistical significance ($P = 0.1$). Examination of the individual experiments showed that fetoplacental Phe R_a decreased by an average of 13% in five of the nine animals studied, increased by 8.5% in just two of the nine, and did not change in two others. Therefore, the apparent difference between leucine and phenylalanine kinetics at the whole body level appears to be related more to a lack of statistical power rather than to an intrinsic difference between the two tracer amino acids. In a previously published, independent study from our laboratory, systemic infusion of rhIGF-I in the fetus, at the same dose as in the present study, resulted in statistically significant decreases in the endogenous R_a of both leucine and phenylalanine (27). In our previous study, rhIGF-I was infused through a central venous catheter and resulted in a 37% greater systemic IGF-I concentration compared with the present study. This difference in IGF-I concentration is likely due in large part to extraction of rhIGF-I by the hindlimb tissues.

IGF-I infusion also resulted in a significant decrease in fetal leucine oxidation, reflecting a decrease in amino acid catabolism. This decrease in leucine oxidation without a change in umbilical uptake supports the anabolic role of IGF-I in the fetus, even though the 2.4-fold increase in leucine accretion was not significant. These results are consistent with previous studies carried out by ourselves and others examining acute systemic infusions of IGF-I in fetal sheep (18, 27) and are qualitatively similar to those found in postnatal animals and adult humans (10, 14, 22, 24).

Based on the findings in the present study, it appears that IGF-I exerts its anabolic effects in the fetus in tissues other than skeletal muscle. There is at least one previous study that would support this concept. Lok et al. (30) administered IGF-I for 10 days to late-gestation fetal sheep; the IGF-I infusion resulted in demonstrable increases in the weight of selected fetal organs, such as liver, lung, heart, and kidney. However, skeletal muscle weight was not increased in this study. Thus both the chronic IGF-I infusions of Lok et al. and the acute intra-arterial IGF-I infusions of the present study clearly demonstrated a significant anabolic effect in the fetus but failed to produce a specific anabolic effect in skeletal muscle. Therefore, the available evidence in postnatal and fetal animals strongly suggests that the anabolic actions of IGF-I on skeletal muscle are developmentally regulated, with increasing anabolic action on muscle tissue as the organism matures.

A further difference between the present study and those in postnatal subjects is the effect of IGF-I on glucose metabolism. In nearly all studies in adults, significant alterations in glucose kinetics are produced by rhIGF-I infusion. Glucose utilization has been found to increase dramatically, and hepatic glucose output decreases significantly during IGF-I infusion (23, 41, 43). The net result of these changes is a significant hypoglycemic effect if glucose supplementation is not provided. Skeletal muscle appears to be the major tissue in which glucose utilization is increased. Both Oddy and Owens (35) in postnatal lambs as well as Fryburg et al. (12, 14) in adult human volunteers have shown an increase in skeletal muscle glucose uptake in

response to an intra-arterial IGF-I infusion. In contrast, the lamb fetuses in the present study exhibited no change in hindlimb glucose uptake during IGF-I infusion nor was there a change in net umbilical glucose uptake. Fetal glucose concentration increased slightly, presumably due to decreased glucose utilization as a result of a decrease in fetal insulin concentration. Thus, as with protein metabolism, there appear to be important developmental differences in the effect of IGF-I on fetal skeletal muscle glucose utilization.

A potential limitation of the present study is the decrease in amino acid concentrations that occurred during IGF-I infusion. This decrease in amino acid concentrations during IGF-I infusion has been previously described in fetal sheep (18, 27) and is most likely due to inhibition of whole body proteolysis. One might speculate that the fall in plasma amino acid concentrations blunted any change in protein synthesis and possibly also in proteolysis. Fryburg et al. (14) demonstrated that hyperaminoacidemia leads to stimulation of muscle protein synthesis, an effect that was enhanced by IGF-I infusion. However, we are not aware of any studies in fetal or postnatal subjects in which amino acid kinetics have been examined during IGF-I infusion while maintaining amino acid concentrations constant.

Another potential limitation of the present study is the decrease in insulin concentration during IGF-I infusion. This decrease in insulin has been described in nearly all studies in which rhIGF-I has been infused and is consistent with our previous finding in fetal sheep (12–14, 18, 22, 23, 27, 30, 35, 43). This decrease in insulin concentration has been attributed to an inhibition of pancreatic insulin secretion during IGF-I infusion (16, 23, 50). Although it is possible that the fall in insulin concentration in the present study may have influenced the results, it should be noted that, in all of the postnatal studies previously cited, there was a similar and proportional fall in insulin concentration (12, 13, 35). Furthermore, in these studies, there were also significant decreases in amino acid concentrations. Nonetheless, in spite of the fall in insulin and amino acids, IGF-I infusion produced significant effects on skeletal muscle protein and glucose metabolism. Further studies are needed to delineate the effects of IGF-I on fetal amino acid kinetics independent of insulin and amino acid concentration. These studies are currently in progress in our laboratory.

The kinetic model that we employed would have detected changes in either synthesis or proteolysis. Therefore, the lack of response suggests that the fetal skeletal muscle is not responsive to excess circulating IGF-I. There are several possible explanations for this finding. First, it is possible that fetal skeletal muscle lacks IGF-I receptors. To our knowledge, there are no data on IGF-I receptor content in ovine fetal skeletal muscle. However, these receptors are present in skeletal muscle in fetal pigs at 75 days of gestation (31, 38), as well as in the fetal calf (4), and thus are likely present in the ovine fetus also. Therefore, reduced receptor numbers cannot explain the lack of effect of IGF-I on fetal hindlimb protein metabolism. Second, it is possible that IGF binding proteins limit access of the infused hormone to the receptor. Finally, it is possible that there are alterations in postreceptor signaling that cause fetal skeletal muscle to be resistant to IGF-I. Further studies will be required to elucidate these issues.

Others have argued that, at low doses of rhIGF-I, growth hormone-like effects, including stimulation of protein synthesis, are observed, whereas, at higher doses, insulin-like effects, such as an increase in glucose utilization and decrease in proteolysis, are observed (12). Although the exact receptors mediating these effects are not known, these investigators have speculated that, at low doses, rhIGF-I acts through the IGF-I receptor, whereas, at the higher doses, activation of the insulin receptor occurs. We feel that, at least in the fetus, this is unlikely. We have previously documented a dissociation of the insulin and IGF-I effects in the ovine fetus at the whole body level (27, 28). The present study provides further evidence, in a specific, insulin-sensitive tissue, that IGF-I does not stimulate fetal glucose utilization. However, the fetal hindlimb clearly responds to insulin infusion, with a 63% increase in hindlimb glucose uptake (48). The lack of an increase in hindlimb glucose uptake in the present study, despite IGF-I concentrations similar to those of other investigators, provides evidence that, in the fetus, IGF-I does not simply act through the insulin receptor. The same logic applies at the whole body level. We have demonstrated that rhIGF-I infusion causes a decrease in whole body proteolysis and amino acid catabolism, without a significant increase in fetal glucose utilization. Insulin infusion, on the other hand, stimulates fetal glucose utilization without effecting a decrease in proteolysis (28).

We did, in agreement with other investigators, demonstrate an increase in hindlimb blood flow during the IGF-I infusion (12). Interestingly, in the study of Oddy and Owens (35) in lambs, such an increase was not demonstrated. However, our changes in blood flow were highly correlated with simultaneous changes in fetal arterial hemoglobin concentration. The changes in blood flow were not significant if hemoglobin concentration was used as a covariate. This calls into question whether the increase in blood flow was real or an artifact of the study design. However, in adult human volunteers, IGF-I clearly increases skeletal muscle blood flow and does so though a nitric oxide-dependent mechanism (13).

In summary, this study provides further evidence that rhIGF-I significantly decreases fetal proteolysis. Taken together with the evidence that net umbilical uptake of amino acids does not change while amino acid catabolism decreases, it is clear that IGF-I promotes fetal protein accretion. However, its site of action is predominately in tissues other than skeletal muscle, most likely in visceral organs. In addition, the present study provides further evidence in a specific tissue that IGF-I does not simply act as an insulin substitute but has distinct actions of its own.

We gratefully acknowledge the assistance of Sara Lecklitner, Kathleen Cooper, Brenda McKinley, Angela Murff-Maxey, and Brian Sloan in the performance of these studies. We also acknowledge Nancy Chapman and Fran Kandrac for assistance in preparing the manuscript.

This work was supported by National Institute of Child Health and Human Development Grants KO8-HD-1048 and RO1-HD-19089 and by grants from the James Whitcomb Riley Memorial Association and from Bristol-Meyers Squibb.

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Received 9 February 1998; accepted in final form 11 August 1998.

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