Effect of IGF-I on serine metabolism in fetal sheep

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Abstract

Acute infusion of IGF-I to the fetus has been shown to inhibit amino acid oxidation and appears to increase fetoplacental amino acid uptake. This study was designed to investigate further the effects of IGF-I on fetal amino acid metabolism. Radiolabeled serine was used to test the hypothesis that fetal IGF-I infusion enhances serine uptake into the fetus and/or placenta and inhibits serine oxidation.

Eight fetal sheep were studied at 127 days of gestation before and during a 4-h infusion of IGF-I (50 µg/h per kg). During the infusion there was no change in uptake of serine or its oxidation by fetus or placenta. However, both uptake and oxidation of serine and glycine decreased in the fetal carcass. There was also a decrease in fetal blood serine and glycine concentrations which could indicate a decrease in protein breakdown, although reduced amino acid synthesis cannot be excluded. Thus IGF-I appeared to influence the distribution of these amino acids as oxidative substrates between different fetal tissues. In addition, fetal IGF-I infusion increased the conversion of serine to glycine which is likely to have increased the availability of one-carbon groups for biosynthesis. Our data provide further evidence that IGF-I plays a role in the regulation of fetoplacental amino acid metabolism.

Introduction

Amino acids are important substrates for the growing fetus. Their uptake by the fetus exceeds their rate of accretion into protein. In addition to protein synthesis, amino acids contribute to fetal carbon and nitrogen requirements (Lemons et al. 1976, Battaglia 1992) and are also used as oxidative metabolic fuels (Van Veen et al. 1987, Battaglia & Meschia 1988).

Placental amino acid and nitrogen balance involves a unique cycling of particular amino acids. One pathway reported in fetal sheep is the fetal uptake of glycine which is then converted to serine in the fetal liver, whereas fetal serine is taken up by the placenta where it can be converted to glycine or used in other metabolic pathways (Cetin et al. 1991, 1992). The exact purpose of this pathway remains unclear, but it is likely that it is an important part of the fetoplacental interactions necessary for the maintenance of fetal growth.

There is indirect evidence that insulin-like growth factor I (IGF-I) has anabolic and antica tabolic effects on amino acid metabolism in fetal life. It increases fetoplacental amino acid uptake in late gestation fetal sheep (Harding et al. 1994) and decreases net amino acid oxidation (Harding et al. 1994, Liechty et al. 1996, Boyle et al. 1998). In addition, there is evidence to suggest that IGF-I decreases fetal protein breakdown (Liechty et al. 1996, Boyle et al. 1998). However, the effects of IGF-I on the metabolism of specific amino acids and the site of these effects at tissue level remain unknown.

The present study was designed to investigate further the effects of fetal IGF-I infusion on fetal and placental amino acid metabolism. Specifically, we wished to determine the effect of IGF-I on serine uptake by the fetus and/or placenta and on serine oxidation.

Materials and Methods

Animals used for this study were the same as those reported in the control group in our previous study of the effects of acute IGF-I infusion in the growth-retarded fetus (Jensen et al. 1999). Details of the animal preparation and experimental procedure were described in that report. Briefly, eight pregnant ewes carrying singleton fetuses were prepared under general anesthesia at 113 ± 0.8 days gestation (term = 145 days). Catheters were placed in both fetal femoral arteries and veins, the fetal common umbilical vein, the common uterine vein, and the maternal femoral artery and vein and carotid artery and jugular vein. Ewes were allowed at least 10 days to recover after surgery.

At 127 ± 0.3 days gestation an IGF-I challenge was performed on each animal. After a 5 ml loading bolus, a tracer solution comprising 250 mg antipyrine, 250 µCi [14C]urea, 1.5 mCi 3-O-[methyl-3H]d-glucose and 500 µCi [U-14C]serine in 34 ml saline was infused into.
the fetal femoral vein at 3 ml/h for 8 h. A separate infusion of saline, also at 3 ml/h, was connected to the same catheter and then changed to IGF-I (50 µg/h per kg estimated fetal weight) after 3.5 h of infusion. Five sets of blood samples were taken at 15-min intervals beginning after tracer steady state was reached (3 h). Each set consisted of blood taken simultaneously from the fetal femoral artery (2.5 ml) and matching fetal femoral vein (1.5 ml), umbilical vein (1.5 ml), uterine vein (5.5 ml) and maternal artery (6.5 ml). A further five sets of samples at 15-min intervals were taken 3 h later during the last 1 h of the 4-h IGF-I infusion. Immediately after IGF-I infusion, the ewe was killed with an overdose of phenobarbitone. The positions of all the catheters were checked, and the fetus and placenta dissected and weighed.

Sample handling
Blood was collected in sterile heparinized syringes and aliquots put into separate tubes for later measurement of radioactivity, antipyrine, metabolites and amino acids. Additional aliquots were centrifuged and the plasma collected for later IGF-I assay. All samples were then frozen at −80 °C.

Measurement of 14CO2
A separate aliquot of blood was held on ice for measurement of blood gases (Chiron M845 blood gas analyser, Chiron Corporation, Emeryville, CA, USA) and 14CO2 radioactivity. The oxidation of labeled serine to carbon dioxide was determined by a method based on that of Hay et al. (1983). Each blood sample (100 µl fetal and 500 µl maternal) was injected in duplicate anaerobically into a polypropylene vial, glued to the inside wall of a Poly-Q scintillation vial (Beckman Instruments Inc., Fullerton, CA, USA) containing 500 µl Soluene-350 (Canberra-Packard, Meriden, CT, USA). A volume of 1 M HCl equal to that of blood (100 µl or 500 µl) was similarly injected to release the CO2 from the blood. The vials were shaken gently overnight. The following morning the inserts containing blood and acid were discarded. Ten milliliters Hionic Fluor (Canberra-Packard) was added to each vial and the radioactivity measured (Rack-Beta model 1219; LKB Wallac, Turku, Finland). Results were corrected for procedural losses by determining the simultaneous recovery of 14CO2, after the addition of NaH14CO3 (ICN Pharmaceuticals Inc., USA) to samples of unlabeled blood and buffer included in each experiment. The mean recovery over all experiments was 89%.

Assays

IGF-I IGF-I concentrations in plasma were measured by double-antibody radioimmunoassay validated for fetal sheep plasma (Blum & Breier 1994). The minimal detectable dose of the assay was 0.07 ng/tube.

Antipyrine Antipyrine concentrations were measured by high performance liquid chromatography (HPLC) (Pimentel et al. 1986).

Specific activities Specific activities of serine and glycine in blood were determined after reversed phase separation of fluorescent o-phthalaldehyde derivatives by HPLC. Specifically, blood (0·1 ml) was deproteinized using sulfuric acid and sodium tungstate (Evans et al. 1993) and the supernatant (0·7 ml) evaporated to dryness. The residue was resuspended in 70 µl derivatizing reagent, held for 3 min at room temperature and 50 µl injected into the HPLC. The derivatizing reagent was prepared fresh every 5 days by dissolving 25 mg o-phthalaldehyde in 250 µl acetonitrile and 25 µl mercaptopropionic acid before dilution with 3 ml 1 M potassium borate buffer pH 9·6 containing 0·1 M triethylamine. The HPLC system (Waters, Milford, MA, USA) included a 300 × 3·9 mm C18 Nova-Pak column at 45 °C and a model 470 fluorescence detector (Excitation wavelength (Ex) 330 nm; Emission wavelength (Em) 450 nm). The mobile phase consisted of a buffer (10 mM sodium phosphate and 3 mM triethylamine pH 6·50), run in a stepped gradient with acetonitrile (from 0% to 25% acetonitrile) over 28 min, at 0·8 ml/min. Fractions of eluate containing serine and glycine were collected and counted using a dual-label scintillation counter (Rack-Beta model 1219; LKB Wallac) with external quench correction. The amounts of serine and glycine in each fraction were determined relative to derivatized external standards, prepared and run simultaneously with the samples.

Amino acid concentrations Amino acid concentrations were determined in separate blood samples by a different HPLC method. Specifically, blood samples (0·02 ml) and blood containing standard concentrations of amino acids were deproteinized as above, using sulfuric acid containing 50 µM DL-α-aminomeric acid as an internal standard, and sodium tungstate (total volume 0·2 ml). Fluorescent derivatives of amino acids were prepared as follows, using the Waters AccQ tag method. Borate buffer (0·06 ml) was placed in a tube and 20 µl sample or standard supernatant were added; 20 µl 10 mM AccQ-fluor reagent (6-aminquinindyl-N-hydroxysuccinimidyl carbamate) in acetonitrile were then added and mixed immediately. The contents were transferred to an HPLC injection vial and incubated at 55 °C for 10 min before 10 µl was injected. The HPLC system included a 300 × 3·9 mm C18 Nova-Pak column at 37 °C, and a model 474 fluorescence detector (Ex 250 nm; Em 395 nm). The mobile phase consisted of a buffer (80 mM sodium acetate, 3 mM triethylamine, 2·7 µM disodium calcium EDTA, adjusted to pH 6·43 with phosphoric acid) run in a stepped gradient with acetonitrile (0–3%) over 60 min. Retention times for serine, glycine and the internal standard were 35·3 ± 1·1, 41·3 ± 1·2 and 52·2 ± 1·5 min respectively.
Hindlimb serine uptake (µmol/min) estimated in the individual vessels as follows: Because glycine is made from two of the three available carbons of serine, the maximum percentage of glycine that could have been made from serine of fetal origin was estimated in the individual vessels as follows:

Calculations

Uterine and umbilical blood flows were calculated from antipyrine concentrations according to the Fick Principle (Meschia et al. 1966). Hindlimb blood flow was assumed to be a constant fraction of umbilical blood flow based on a previous study in late gestation pregnant sheep, in which both umbilical and hindlimb blood flows were determined using labeled microspheres (Harding et al. 1992). Uterine and umbilical blood flow and hindlimb blood flow from both hindlimbs (catheterized and uncatheterized) were measured and the ratio of hindlimb/umbilical blood flow calculated. An average of four measurements were taken for each animal. The mean ratio of hindlimb/umbilical blood flow was 0.12 ± 0.01 for 16 animals.

Umbilical and fetal hindlimb glycine uptakes were calculated from blood glycine concentrations by application of the Fick Principle:

Fetal glycine uptake
\[
= \text{umbilical blood flow (ml/min)} \times ([\text{UV}] - [\text{FA}] \text{ mM})(\mu\text{mol/min})
\]

Hindlimb glycine uptake
\[
= \text{hindlimb blood flow (ml/min)} \times ([\text{FA}] - [\text{FV}] \text{ mM})(\mu\text{mol/min})
\]

Blood serine concentration differences were too small to be measured accurately across the umbilical circulation. Therefore, [14C]serine was used to calculate placental and hindlimb uptakes of serine as follows:

Placental serine uptake (µmol/min)
\[
= \frac{\text{SA glycine} \times 3}{\text{SA serine} \times 2}
\]

Serine conversion to glycine

The rate of 14CO2 production from tracer serine was calculated as follows (Cetin et al. 1992, Moores et al. 1994):

Fetal 14CO2 production rate (d.p.m./min)
\[
= \text{umbilical blood flow (ml/min)} \times (14CO*2FA - 14CO*2UV \text{ d.p.m./ml})
\]

Hindlimb 14CO2 production rate (d.p.m./min)
\[
= \text{hindlimb blood flow (ml/min)} \times (14CO*2FV - 14CO*2FA \text{ d.p.m./ml})
\]

Uteroplacental 14CO2 production rate (d.p.m./min)
\[
= \text{uterine blood flow (ml/min)} \times (14CO*2UOV - 14CO*2MA \text{ d.p.m./ml})
\]

Placental 14CO2 production rate (d.p.m./min)
\[
= \text{uteroplacental 14CO2 production rate} - \text{fetal 14CO2 production rate}
\]

Where MA is maternal artery and UOV is utero-ovarian vein.

The percentage of infused [14C]serine excreted as 14CO2 via the umbilical and uterine circulations, and across the hindlimb were calculated as:

Net serine oxidation (%)
\[
= \frac{14CO*2 \text{ production rate} (\text{d.p.m./min})}{\text{serine infusion rate} (\text{d.p.m./min})} \times 100
\]

As 1 mole of serine, if completely oxidized, can result in production of 3 moles of 14CO2, the net oxidation of serine in the fetus, hindlimb, placenta and uteroplacenta, either directly or via intermediate metabolites, can be estimated:

Serine oxidation rate (µmol/min)
\[
= \frac{14CO*2 \text{ production rate} (\text{d.p.m./min})}{\text{SA} \text{ FA} (\text{d.p.m./µmol})} \times 3
\]

Serine oxidation in the carcass as a proportion of total fetal serine oxidation was estimated on the basis of the assumption that the arteriovenous difference of the tracer across the hindlimb was representative of that across the entire fetal carcass, and that the ratio of carcass blood flow to umbilical blood flow was ∼0.9 (Rudolph & Heymann 1970):

Carcass serine oxidation as portion of fetal oxidation
\[
= \frac{(14CO*2FV - 14CO*2FA \text{ d.p.m./ml})}{(14CO*2FA - 14CO*2UV \text{ d.p.m./ml})} \times 0.9
\]

Statistics

The results are reported as mean ± s.e. For each animal the mean of the five measurements before and the five during
the IGF-I infusion was calculated, and the change with IGF-I infusion within each group was compared using a paired Student’s t-test.

Results

Mean fetal weight was 3·9 ± 0·4 kg and placental weight was 508 ± 30 g. The mean rate of infusion of IGF-I was 49 ± 3 µg/h per kg. Fetal plasma IGF-I concentrations increased approximately 3·5-fold during infusion (from 94 ± 8 to 313 ± 28 ng/ml). Maternal plasma IGF-I concentrations did not change during IGF-I infusion (182 ± 24 and 171 ± 20 ng/ml). During fetal infusion of IGF-I, fetal blood glucose concentrations decreased slightly (from 0·83 ± 0·08 to 0·76 ± 0·08 mM, P=0·03), as did fetal plasma insulin concentrations (from 0·56 ± 0·11 to 0·18 ± 0·04 ng/ml, P=0·02), but maternal glucose and insulin concentrations did not change (glucose 2·37 ± 0·10 and 2·33 ± 0·10 mM, insulin 0·73 ± 0·17 and 0·70 ± 0·11 ng/ml). During fetal infusion of IGF-I umbilical blood flow decreased slightly from 689 ± 37 to 616 ± 41 ml/min, P=0·02) and uterine blood flow was unchanged (1460 ± 128 and 1380 ± 117 ml/min).

Blood serine and glycine concentrations and uptakes

Blood serine concentrations decreased in all three fetal vessels (fetal artery, fetal vein and umbilical vein) with fetal infusion of IGF-I (Table 1). Placental uptake of serine from the fetus did not change during fetal IGF-I infusion. However, fetal hindlimb uptake of serine decreased during fetal IGF-I infusion. Blood concentrations of glycine in the fetal artery decreased during fetal infusion of IGF-I, and there was a trend toward a decrease in glycine concentration in the umbilical vein (Table 1). Fetal uptake of glycine remained unchanged during fetal IGF-I infusion. However, fetal hindlimb uptake of glycine decreased during fetal IGF-I infusion.

Conversion of serine to other metabolites

The effect of fetal IGF-I infusion on the conversion of serine to glycine could not be determined directly in the placenta or fetus because the arteriovenous differences in radioactivity in the glycine fraction across the umbilical circulation and fetal hindlimb were below the limit of detection. However, there was evidence that glycine was being made from infused serine, as there were measurable carbon-14 counts in the glycine fraction that must have originated from infused serine. As measured by specific activity ratios in the individual vessels, the percentage of circulating [14C] glycine made from infused serine increased during fetal infusion of IGF-I in the fetal artery (from 31·6 ± 3·4 to 35·3 ± 4·5%, P=0·02), fetal vein (from 38·0 ± 4·5 to 40·9 ± 5·2%, P=0·05), and umbilical vein (from 34·4 ± 3·8 to 36·8 ± 4·4%, P=0·08).

Serine oxidation

During IGF-I infusion there was no change in 14CO2 production rates in the fetus (45·4 ± 6·8 to 36·8 ± 8·1 µmol/min), uteroplacenta (49·5 ± 7·8 to 44·9 ± 6·8 µmol/min), or placenta (1·7 ± 5·6 to 7·3 ± 9·2 µmol/min). Hindlimb production of 14CO2 did not significantly change during fetal IGF-I infusion (2·0 ± 1·1 and 2·5 ± 1·3 µmol/min, n=7). However, inspection of the data showed that two animals had negative arteriovenous differences in 14CO2 across the hindlimb before fetal IGF-I infusion (Fig. 1). If these two animals were excluded, then hindlimb 14CO2 production rates decreased in the remaining five animals (from 3·3 ± 1·0 to 0·8 ± 0·7 µmol/min, P=0·04).

[14C]serine oxidation in the carcass as a proportion of total fetal [14C]serine oxidation decreased with fetal infusion of IGF-I (from 0·90 ± 0·32 to 0·82 ± 0·30 µmol/min, P=0·05, n=7, Fig. 2), indicating that serine oxidation was reduced in the fetal carcass and was occurring elsewhere. Exclusion of the same two animals that had negative arteriovenous differences in 14CO2 across the hindlimb before IGF-I infusion did not alter this finding (decrease from 1·20 ± 0·39 to 0·13 ± 0·09, P=0·05, n=5).

Net serine oxidation as a percentage of infused serine did not change with fetal IGF-I infusion in the fetus (11·4 ± 1·5 and 11·3 ± 2·2%, uteroplacenta (12·0 ± 1·2 and 12·4 ± 0·8%), or hindlimb (0·59 ± 0·28 and

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Serine and glycine blood concentrations (concn) and uptakes before and during IGF-I infusion. Values are mean ± s.e. for 8 animals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serine</strong></td>
<td><strong>Glycine</strong></td>
</tr>
<tr>
<td>Before</td>
<td>During</td>
</tr>
<tr>
<td>Fetal artery concn (µmol/l)</td>
<td>423 ± 46</td>
</tr>
<tr>
<td>Fetal vein concn (µmol/l)</td>
<td>411 ± 54</td>
</tr>
<tr>
<td>Umbilical vein concn (µmol/l)</td>
<td>422 ± 47</td>
</tr>
<tr>
<td>Fetal uptake (µmol/min)</td>
<td>−1·3 ± 3·0</td>
</tr>
<tr>
<td>Hindlimb uptake (µmol/min)</td>
<td>1·2 ± 0·2</td>
</tr>
</tbody>
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*P<0·05, **P<0·01, ***P<0·001, †P=0·06 compared with value before IGF-I infusion.

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However, as was the case with hindlimb $^{14}$CO$_2$ production rates, net serine oxidation in the fetal hindlimb decreased with fetal IGF-I infusion if the same two animals were excluded from the data (from $0.90 \pm 0.23$ to $0.27 \pm 0.19\%$, $P=0.04$, $n=5$, Fig. 3).

**Discussion**

The aim of this study was to determine the effects of acute infusion of IGF-I on fetoplacental amino acid metabolism, and specifically on the metabolism of the closely related amino acids serine and glycine. Serine was chosen as the tracer amino acid because of its unique role in fetal amino acid metabolism. In the late gestation fetal lamb there is no measurable umbilical uptake of serine. All the serine required by the fetus appears to be synthesized within the fetus, largely in the fetal liver from glycine (Cetin *et al.* 1991, Thureen *et al.* 1995). This fetal serine is then taken up in large amounts by the placenta where some is oxidized and some is converted back to glycine and recycled back to the fetus (Marconi *et al.* 1989, Cetin *et al.* 1992). There is virtually no loss of fetal serine to the maternal circulation. Thus the use of serine as a tracer is likely to provide information on the effects of IGF-I on fetoplacental amino acid exchange and the metabolism and oxidation of serine and glycine in both fetus and placenta.

In postnatal life, IGF-I has important effects on protein metabolism, including both increased protein synthesis and inhibition of protein breakdown (Tomas *et al.* 1990, Douglas *et al.* 1991). Previous studies in chronically catheterized fetal sheep have suggested that IGF-I has similar effects in the fetus. There is limited indirect experimental evidence of increased fetoplacental amino nitrogen uptake, reduced protein breakdown and reduced net amino acid oxidation in response to acute IGF-I infusion in the fetus (Harding *et al.* 1994, Liechty *et al.* 1996, Boyle *et al.* 1998).

In our previous study, IGF-I infusion reduced both maternal and fetal circulating amino nitrogen concentrations and tended to increase amino nitrogen uptake by fetus and placenta, although this did not reach statistical significance (Harding *et al.* 1994). These effects were taken as indirect evidence that IGF-I increased fetoplacental amino acid uptake. However, in the current study we found no evidence that IGF-I infusion altered...
net fetoplacental amino acid uptake when measured directly. There was no change in net umbilical or placental uptake of serine or glycine. These findings are also consistent with those of Liechty and colleagues (1996), who found that fetal infusion of IGF-I did not increase umbilical uptake of leucine or phenylalanine in fetal sheep.

In the current study, however, IGF-I infusion did reduce the uptake of both serine and glycine across the fetal hind limb. As umbilical uptakes were unchanged, this suggests that IGF-I infusion led to redistribution of serine and glycine uptakes from the carcass to elsewhere in the fetus. Such redistribution of amino acid uptake away from the carcass to other fetal tissues may afford part of the explanation for the selective effects of chronic fetal infusion of IGF-I on promoting the growth of some fetal organs but not others. For example, Lok and colleagues (1996) showed that a 10-day infusion of IGF-I to fetal sheep led to an increase in size of most major fetal organs but had little effect on hind limb muscle and carcass weight. Thus one of the roles of IGF-I in the regulation of fetal growth may involve the regulation of substrate distribution, particularly of amino acids, between different fetal tissues.

We calculated uptakes of serine and glycine across the fetal hind limb based upon assumptions that the arteriovenous difference across the hindlimb was representative of that of the whole carcass, and that the ratio of carcass to umbilical blood flow did not change during the infusion. The possibility that IGF-I may have altered local blood flow either within the carcass or in its distribution between umbilical and carcass flow in these experiments cannot be excluded. However, we did observe a small (approximately 10%) decrease in umbilical blood flow during the infusion. Such a decrease in umbilical blood flow would be expected to be associated with a redistribution in fetal cardiac output away from the carcass towards essential organs such as the heart and brain. If there was no change in amino acid uptake across the hindlimb, this decrease in hindlimb blood flow would result in an increase in the arteriovenous difference across the hindlimb – that is, there would be an apparent increase in hindlimb amino

*Figure 2* [$^{14}$C]serine oxidation in the carcass as a proportion of total fetal [$^{14}$C]serine oxidation before and during IGF-I infusion. Symbols indicate individual animals. Lines join the values before and during IGF-I infusion for each animal.
acid uptake. However, we found an apparent decrease in uptake for both glycine and serine across the hindlimb during infusion of IGF-I. Thus important changes in carcass blood flow are unlikely to explain our observations with regard to either amino acid uptake or circulating concentrations.

Previous studies have suggested that acute fetal infusion of IGF-I inhibits fetal protein breakdown (Liechty et al. 1996, Boyle et al. 1998). Our findings are consistent with such an effect. We found that IGF-I infusion reduced the circulating fetal concentrations of serine and glycine. In the absence of a change in amino acid uptake, the most likely explanation for this finding is a reduction in protein breakdown leading to a reduced release of these amino acids into the fetal circulation. However, the possibility of reduced synthesis of both amino acids by the fetus cannot be excluded in our studies.

The reduction in circulating serine and glycine concentrations in the fetus is unlikely to be due to the observed small decreases in fetal insulin and glucose concentrations. Insulin regulates glucose and amino acid uptake into fetal tissues (Fowden 1989), so that a decrease in insulin concentrations would be expected to increase rather than decrease circulating amino acid concentrations.

Previous studies have suggested that acute fetal infusion of IGF-I leads to a decrease in net fetal amino acid oxidation (Harding et al. 1994, Liechty et al. 1996). However, in the current study we did not find any effect of IGF-I infusion on fetal, placental or uteroplacental 14CO2 production rates. One possible explanation for this discrepancy may be the method of measurement of amino acid oxidation. In our previous study (Harding et al. 1994) we used fetal urea production as a measure of amino acid oxidation, and Liechty and colleagues (1996) measured the rate of production of 14CO2 from labeled leucine. However, it is also notable that Liechty et al. (1996) found that IGF-I infusion caused a decrease in [14C]leucine oxidation only when fed and fasting states were compared, but not in the fed state alone. Similarly, the basal rate of fetal production of urea before IGF-I infusion was four to five times lower in the animals in the current study than in those in our previous study. Thus it is possible that the effect of IGF-I infusion on fetal amino acid oxidation depends on the basal oxidation rate, with IGF-I acutely suppressing oxidation if the basal rate is high, but having no detectable effect if the basal amino acid oxidation rate is already low.

Figure 3 Net hindlimb serine oxidation before and during IGF-I infusion. Symbols indicate individual animals. Lines join the values before and during IGF-I infusion for each animal.
Although in this study IGF-I infusion did not alter net fetal amino acid oxidation, it did appear to decrease serine oxidation in the fetal carcass. We used two measures of serine oxidation by fetal carcass tissues in this study. One was calculated as the oxidation of serine in the carcass as a proportion of total fetal serine oxidation, and the other was the rate of $^{14}$CO$_2$ production across the hindlimb. Using data from the carcass oxidation measurements, IGF-I infusion reduced $^{14}$CO$_2$ production as a proportion of total fetal $^{14}$CO$_2$ production. Using the hindlimb measurements, $^{14}$CO$_2$ production was reduced during IGF-I infusion in five of seven animals. However the net change was statistically significant only if we excluded the results from two animals that had negative arteriovenous differences in $^{14}$CO$_2$ across the hindlimb before infusion. These negative arteriovenous differences across the hindlimb are difficult to explain. It is unlikely that $^{13}$CO$_2$ became fixed in tissues, and it is also unlikely that $^{14}$CO$_2$ is converted to bicarbonate ions in amounts sufficient to cause negative excretion of $^{14}$CO$_2$ in these two animals. Thus it seems most likely that there was a methodological problem with the measurements in these animals. This likelihood, and the consistency of the data across the two measurements, suggest that IGF-I infusion did inhibit serine oxidation in tissues of the fetal carcass in our study. This reduced oxidation would also be consistent with the reduced carcass uptakes of serine and glycine that we observed. Thus once again the data are consistent with an effect of IGF-I on distribution of amino acids as possible oxidative substrates between the different tissues in the fetus.

Before fetal IGF-I infusion, serine oxidation in the carcass accounted for most of the oxidation occurring in the entire fetus. However, during IGF-I infusion none of the serine was oxidized in the carcass. As there was no evidence that serine oxidation was occurring in the placenta, it again seems likely that serine oxidation had shifted to elsewhere in the fetus. The fetal liver is a possible location. In the adult mammalian liver it has been shown that serine is primarily cleaved to methylenetetrahydrofolate and glycine, and glycine can then be oxidized to CO$_2$ (Yoshida & Kikuchi 1972). In addition, it has been shown that there is significant glycine oxidation in the liver of the late gestation fetal sheep, and $^{14}$CO$_2$ produced by the liver from $[^{14}$C]glycine can account for at least 70% of the total $^{14}$CO$_2$ produced by the fetus (Thureen et al. 1995). Thus, the infused serine in the current study could have been converted to $[^{14}$C]glycine, which is a possible candidate for oxidation in the fetal liver during IGF-I infusion.

Fetal IGF-I infusion increased the amount of glycine being made from serine. The conversion of serine to glycine yields methylenetetrahydrofolate, which is a major source of one-carbon units that can be used in a variety of biosyntheses. Therefore, increased conversion of serine to glycine could have important effects on metabolism by increasing the availability of one-carbon units for use in the fetus and/or placenta. Methylenetetrahydrofolate can also be a donor for remethylation of the amino acid homocysteine to form methionine. Thus increased glycine production from serine could have important implications for sulfur amino acid metabolism.

Some baseline data before fetal IGF-I infusion in this study differed from those reported previously. Mean fetal rates of production of $^{14}$CO$_2$ in the current study were only slightly lower than but not significantly different from uterine $^{14}$CO$_2$ excretion rates, suggesting that oxidation of serine in the placenta was so small that it was within the error of measurement. This is in contrast to a previous report that uterine excretion of $^{14}$CO$_2$ from tracer serine was significantly greater than fetal production, indicating that oxidation of serine in the placenta (Cetin et al. 1992). In addition, previous investigators have estimated that 60% of fetal glycine is derived from fetal serine as measured by tracer enrichment ratios in fetal arterial plasma in the late gestation fetal sheep (Cetin et al. 1992). This estimate of glycine produced from serine is twice that calculated in the present study (approximately 30%). The difference between studies might be due to the difference in circulating serine concentrations. We found that fetal blood serine concentrations in our animals were approximately half of those reported previously ($423 \pm 46 \mu$M compared with $767 \pm 45 \mu$M). Although the other study (Cetin et al. 1992) reported serine concentrations in plasma rather than in blood, serum plasma and erythrocyte concentrations are tightly correlated (Cetin et al. 1991), so this does not explain the difference between studies. However, a significant positive correlation has been reported between plasma glycine-serine enrichment ratio and fetal arterial plasma serine concentration, and our data were consistent with this (Cetin et al. 1992). Therefore, the lower amount of glycine made from serine in our study may be attributable to the lower fetal serine concentrations. Another possible explanation for the differences between the two studies may be differences in the metabolic or nutritional state of the animals. Mean fetal weight in the study by Cetin’s group was approximately 1 kg less than mean fetal weight in this study, even though animals in both experiments were studied at the same gestational age.

In summary, we have shown that acute infusion of IGF-I to the fetal sheep does not increase fetoplacental uptake of serine or glycine or inhibit their oxidation. However, it does appear to reduce fetal protein breakdown. More interestingly, IGF-I appears to influence the distribution of amino acids as oxidative substrates between different fetal tissues, with acute fetal IGF-I infusion leading to reduced serine and glycine uptake and oxidation by the fetal carcass in favor of other fetal tissues. In addition, IGF-I increases the conversion of serine to glycine in the fetus. Thus IGF-I appears to have an important role in the regulation of amino acid metabolism in different fetal tissues, with potentially important implications for fetal growth and metabolism.
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