Metabolic effects of IGF-I in the growth retarded fetal sheep

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Abstract

It has been shown that IGF-I has an anabolic effect in the normal fetus. However, there is evidence to suggest that there may be IGF-I resistance in the growth retarded fetus. Therefore, we investigated the effects of acute IGF-I infusion to chronically catheterised fetal sheep. At 128 days gestation, fetuses underwent a 4 h infusion of IGF-I (50 µg/kg/h). Three groups of animals were studied. Nine normally grown fetuses were studied as controls. Embolised animals (n=8) received microspheres into the uterine vasculature, and animals with spontaneous intra-uterine growth retardation (IUGR animals) (n=6) were fetuses found at post mortem to be spontaneously growth restricted.

The effects of IGF-I infusion on feto-placental carbohydrate and protein metabolism were similar in our control group to previous similar experiments. IGF-I infusion decreased fetal blood glucose, oxygen, urea and amino-nitrogen concentrations, and inhibited placental lactate production. The same fetal blood metabolite concentrations also fell during IGF-I infusion in the embolised fetuses, but the effect on placental lactate production was not seen. The only effect of IGF-I infusion in the spontaneous IUGR animals was a fall in fetal blood amino-nitrogen concentrations. We conclude that fetal IGF-I infusion does not have the same anabolic effects in the growth retarded fetus as the normal fetus. In addition, the effects of IGF-I were different in the two growth retarded groups. Our data support previous evidence that the growth retarded fetus has altered IGF-I sensitivity, and this may vary depending on the cause, severity and duration of growth retardation.

Introduction


IUGR may be due to genetic, toxic, infective or nutritional causes. Nutritional IUGR is characterised by reduced substrate delivery to the fetus, increased placental lactate production, and the fetus may become catabolic with loss of amino acids to the placenta (Owens et al. 1987, 1989). In the normal short-term, IGF-I infusions inhibit fetal amino acid oxidation and placental lactate production, and enhance fetal and/or placental amino acid and glucose uptake (Harding et al. 1994). These effects suggest that IGF-I might have the capacity to reverse some of the metabolic effects of IUGR.

Indirect experimental and clinical evidence suggests that altered hormonal sensitivity may be characteristic of IUGR. Tissues are IGF-I resistant in rats born with IUGR (Simmons et al. 1993), and short children born small for gestational age have relatively high circulating IGF-I concentrations (Hofman et al. 1997). It has therefore been proposed that the growth retarded fetus may be IGF-I resistant (Gluckman et al. 1997).

Despite the fact that IGF-I has profound metabolic effects in the normal fetus and there is evidence that IUGR may be associated with IGF-I resistance, the metabolic effects of IGF-I on the growth retarded fetus are unknown. Therefore, this study was designed to determine the acute effect of IGF-I infusion on metabolism in both experimentally induced and spontaneous IUGR fetuses.

Materials and Methods

Animal preparation

Pregnant ewes (Coopworth/Romney/Dorset cross) carrying single fetuses, underwent surgery under halothane...
anaesthesia at 112–118 days of gestation (term=145 days). Polyvinyl catheters were placed in both fetal femoral arteries and veins via the tarsal vessels. An amniotic catheter was sutured to one of the fetal hind limbs for administration of antibiotics, and the fetal common umbilical vein was catheterised (Young et al. 1974). Growth catheters were sutured around each side of the fetal chest from the sternum to the spine to measure change in fetal girth (Mellor & Murray 1982, Harding 1997). Catheters were placed in a uterine vein draining the pregnant horn and a uterine artery supplying each uterine horn, and advanced into the common uterine vein and arteries. Catheters were also placed in the maternal femoral artery and vein and carotid artery and jugular vein. Two sets of arterial and venous catheters were placed for back up, as failure of any one catheter would otherwise prevent completion of the study.

The ewes were housed in individual cages with a diet of chaffage and pelleted stock feed available ad libitum (metabolisable energy=9·7 MJ/kg dry matter, crude protein=19·4 g/100 g dry matter). Gentamicin (80 mg) was given intravenously to the fetus and streptopen (250 mg procaine penicillin/250 mg dihydrostreptomycin sulphate, Pittman Moore Ltd, Upper Hutt, New Zealand) was given by intramuscular injection to the ewe daily for the first 3 postoperative days. All catheters were flushed daily for the first 3 days with a solution of heparin (10 U/ml) in 0·9% saline. After this, catheters were flushed every other day until the end of the experiment. Growth catheters were measured twice daily and the mean of these two measurements was recorded. Immediately after IGF-I infusion, the ewe was killed with an overdose of phenobarbitone. The fetus and placenta were dissected and weighed and the positions of all the catheters were checked.

Experimental groups
Twenty-three animals were studied. The primary intention was to compare normally grown fetuses to those with experimentally induced IUGR. However, it was anticipated that some fetuses would be spontaneously growth retarded and associated with metabolic compromise (low PO2, high PCO2, high lactate). These animals would not be embolised, but it was concluded that neither could they be considered as controls. Animals that were in this category or were found at post mortem to have IUGR were excluded from controls. Animals were defined as spontaneous IUGR if values fell outside ± 1 s.d. from the mean for 2 out of the following 4 criteria: fetal weight, placental weight, brain to body weight ratio or brain to liver weight ratio. Means and standard deviations were established from 40 animals which underwent the same surgery, nutrition and experimental protocol over a 7 year period. Data for the spontaneous IUGR animals were analysed separately from the embolised animals. All animals in the spontaneous IUGR group were metabolically compromised and met the growth criteria. Thus 3 groups of animals were studied: controls (n=9), embolised (n=8), and spontaneously growth retarded animals (n=6). All experiments were approved by the institutional Animal Ethics Committee.

Experimental protocol
The ewes were allowed 2–3 days to recover from surgery before beginning embolisation or saline injection. Ewes in the control group were given 2 ml saline to both uterine arteries twice a day for the next 7 days. Ewes in the embolised group were given 2 ml (3 × 105 spheres) 50 µ microspheres (New England Nuclear, Life Science Products, Boston, MA, USA) to each uterine artery twice daily. If the fetal arterial PO2 was below 15 mmHg or the lactate above 2·0 mM, 50 µ spheres were replaced with 2 ml (2 × 105) 15 µ spheres. Embolisation was stopped when the growth catheters indicated that fetal growth had slowed or stopped for several days.

Ten to fourteen days after surgery, at 126–129 days of gestation, a single IGF-I challenge was performed on each animal. A tracer infusion comprising 250 mg antipyrine, 1·5 mCi 3-O-[methyl-3H] d-glucose and 250 µCi [14C]urea in 32 ml saline, was infused into the fetal femoral vein at 3 ml/h for 7·5 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/kg estimated fetal weight) after 3·5 h infusion. The dose of IGF-I (50 µg/h/kg) was chosen based on previous work from this laboratory (Harding et al. 1994). A set of 5 blood samples was taken at 15 min intervals beginning after tracer steady state was reached (2·5 h). Blood was taken simultaneously from the fetal femoral artery (2·1 ml), umbilical vein (1·1 ml), uterine vein (2·6 ml), and maternal artery (4·2 ml). A second set of 5 samples at 15 min intervals was taken 3 h later over the last hour of the 4 h IGF-I infusion.

Sample handling
Blood was collected in sterile heparinised syringes and aliquoted into separate tubes in frozen gel blocks for later measurement of radioactivity, antipyrine, metabolites, and amino acids. Additional aliquots were centrifuged and the plasma collected for later IGF-I and insulin assay. All samples were then frozen at −80 °C. A separate aliquot of blood was set aside on ice for a maximum of 15 min before measurement of blood gases using a Chiron M845 blood gas analyser.

Assays
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. Insulin was also measured by radioimmunoassay (Breier et al. 1988). Anti-pyrine was measured by high performance liquid chromatography (Pimentel et al. 1986). Aliquots of blood were deproteinised with sulphuric acid and sodium tungstate (glucose, urea and amino-nitrogen assays) or perchloric acid (lactate assay). Glucose, urea and lactate were measured by standard colorimetric enzyme reactions (Hochella & Weinhouse 1965, Kunst et al. 1984, Kerscher & Ziegenhorn 1985) using a 96-well microplate reader. Amino-nitrogen was assayed by colorimetric reaction with β-naphthoquinone sulphonate (Evans et al. 1993). [14C]Urea and 3-O-[methyl-3H]glucose were counted in aliquots of deproteinised blood using a dual-channel scintillation counter (Rack-Beta model 1219; LKB Wallac, Turku, Finland) with external quench correction.

Data analysis

Uterine and umbilical blood flows were calculated according to the antipyrine steady-state diffusion method (Meschia et al. 1966). Uptakes of metabolites for mother and fetus were calculated by application of the Fick principle: uterine uptake = uterine blood flow × (maternal artery − uterine vein concentration); fetal uptake = umbilical blood flow × (umbilical vein − femoral artery concentration); placental uptake = uterine uptake − fetal uptake.

Clearance of the radioactive tracers was calculated by steady state diffusion methods (Kulhanek et al. 1974, Stacey et al. 1978). Fetal urea production rate (Gresham et al. 1972) was calculated as the [14C]urea clearance × (fetal artery − maternal artery urea concentration). Blood oxygen content was calculated from measured haemoglobin, oxygen saturation and PO2 values (Gull & Charlton 1993).

The results are reported as means ± s.e. For each animal the mean of the 5 measurements before and the 5 measurements during the IGF-I infusion was calculated, and the change within each group compared using a paired Student’s t-test. Relationships between variables were tested using simple linear regression. Post mortem data, and values obtained before IGF-I infusion in the embolised and spontaneous IUGR groups were each compared with controls using unpaired t-tests.

Results

Morphometry and growth

Mean fetal weight was reduced by approximately 15% and placental weight reduced by 30% in embolised fetuses compared with controls (Fig. 1 and Table 1). Fetal measurements and most organ weights were also reduced. However, fetal brain weight was unchanged, resulting in a marked increase in brain to body and brain to liver weight ratios in the embolised group (Table 2).

In the spontaneous IUGR group, mean fetal weight was reduced by approximately 30% and placental weight by 50%. There was a reduction in organ weights, with relative preservation of brain weight similar to that of the embolised group (Fig. 1, Tables 1 and 2).

Over the last 5 days of the experiment, fetal growth, as measured by girth increment, was faster in controls (2·9 ± 0·8 mm/day) than in embolised (0·9 ± 0·7 mm/day, P<0·001) or spontaneous IUGR fetuses (1·6 ± 1·1 mm/day, P=0·05) (Fig. 2).

Before IGF-I infusion

Fetal plasma IGF-I concentrations were lower in embolised and spontaneous IUGR, fetuses than in controls, with no differences in maternal IGF-I concentrations (Table 3). Fetal and maternal plasma insulin concentrations did not differ between groups.

Maternal oxygen contents did not differ between groups before fetal IGF-I infusion (Table 4). Fetal oxygen content was lower in spontaneous IUGR fetuses than in controls. Fetal and uterine oxygen uptakes were lower in the
embolised fetuses than in controls (Table 5). There were no differences between groups in placental oxygen uptakes.

Fetal but not maternal blood glucose concentrations were lower in the embolised and spontaneous IUGR fetuses than in controls (Table 4). Fetal glucose uptake was lower in the embolised and spontaneous IUGR fetuses than in controls (Table 5). There were no differences between groups in placental oxygen uptakes.

There were no differences between groups in fetal or maternal blood concentrations of lactate, urea or amino-nitrogen. There were also no differences in fetal, maternal or placental uptakes of lactate, urea or amino-nitrogen.

All substrate uptakes calculated per kilogram were not different between the groups, except for fetal oxygen uptake/kg, which was lower in the embolised animals than in controls (232 ± 24 vs 310 ± 22 µmol/min/kg, \( P=0.03 \)).

Uterine blood flow was lower in embolised and spontaneous IUGR animals than in controls (Table 6). Uterine blood flow calculated per kilogram was also lower in embolised animals than in controls (633 ± 83 vs 918 ± 66 ml/min/kg). Umbilical blood flow was lower in embolised animals than in controls, but was not different per kilogram fetal weight.

There were no differences between groups in placental urea clearances (a measure of placental capacity to transport by simple diffusion) or fetal urea production (a measure of amino acid oxidation). However, placental 3-O-[methyl-\(^3\)H]glucose clearance (a measure of placental

### Table 1
Morphometric data at postmortem. Values are expressed as means ± s.e. for 9 control, 8 embolised, and 6 spontaneous IUGR animals

<table>
<thead>
<tr>
<th>Measure</th>
<th>Control</th>
<th>Embolised</th>
<th>Spontaneous IUGR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (days)</td>
<td>128 ± 0.4</td>
<td>127 ± 0.5</td>
<td>127 ± 1.4</td>
</tr>
<tr>
<td>Fetal weight (g)</td>
<td>3886 ± 115</td>
<td>3299 ± 240*</td>
<td>2579 ± 350***</td>
</tr>
<tr>
<td>Fetal crown rump (cm)</td>
<td>43.1 ± 0.6</td>
<td>41.1 ± 0.6*</td>
<td>40.4 ± 1.7*</td>
</tr>
<tr>
<td>Fetal girth (cm)</td>
<td>33.5 ± 0.6</td>
<td>31.4 ± 0.7*</td>
<td>30.5 ± 0.5**</td>
</tr>
<tr>
<td>Fetal hind limb (cm)</td>
<td>32.4 ± 0.5</td>
<td>31.6 ± 0.9</td>
<td>30.2 ± 2.1*</td>
</tr>
<tr>
<td>Adrenal (g)</td>
<td>0.50 ± 0.04</td>
<td>0.46 ± 0.02</td>
<td>0.30 ± 0.04**</td>
</tr>
<tr>
<td>Brain (g)</td>
<td>43.3 ± 0.9</td>
<td>41.9 ± 1.4</td>
<td>43.0 ± 1.8</td>
</tr>
<tr>
<td>Heart (g)</td>
<td>29.2 ± 1.1</td>
<td>26.3 ± 1.0</td>
<td>21.9 ± 1.3***</td>
</tr>
<tr>
<td>Kidney (g)</td>
<td>34.1 ± 1.9</td>
<td>28.0 ± 2.1*</td>
<td>23.0 ± 0.8***</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>147.3 ± 7.1</td>
<td>116.7 ± 8.2*</td>
<td>81.4 ± 6.8***</td>
</tr>
<tr>
<td>Lung (g)</td>
<td>100.6 ± 5.6</td>
<td>93.0 ± 5.3</td>
<td>76.4 ± 5.4*</td>
</tr>
<tr>
<td>Spleen (g)</td>
<td>11.3 ± 0.8</td>
<td>6.0 ± 0.7***</td>
<td>4.5 ± 0.1***</td>
</tr>
<tr>
<td>Thyroid (g)</td>
<td>1.0 ± 0.1</td>
<td>0.98 ± 0.1</td>
<td>0.57 ± 0.05***</td>
</tr>
<tr>
<td>Thymus (g)</td>
<td>15.3 ± 1.3</td>
<td>8.1 ± 1.7**</td>
<td>5.6 ± 0.9***</td>
</tr>
<tr>
<td>Placenta (g)</td>
<td>483 ± 38</td>
<td>326 ± 28**</td>
<td>227 ± 28**</td>
</tr>
<tr>
<td>Uterus (g)</td>
<td>723 ± 28</td>
<td>756 ± 39</td>
<td>496 ± 50***</td>
</tr>
</tbody>
</table>

*\( P<0.05 \), **\( P<0.01 \), ***\( P<0.001 \) compared with control values.

### Table 2
Organ weights as a percentage of body weight at postmortem. Values are means ± s.e. expressed as a percentage of body weight (multiplied by a factor of 100, if specified) for 9 controls, 8 embolised, and 6 spontaneous IUGR animals

<table>
<thead>
<tr>
<th>Organ</th>
<th>Control (× 100)</th>
<th>Embolised</th>
<th>Spontaneous IUGR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal</td>
<td>1.3 ± 0.1</td>
<td>1.5 ± 0.2</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>Heart</td>
<td>0.75 ± 0.03</td>
<td>0.82 ± 0.05</td>
<td>0.85 ± 0.04*</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.88 ± 0.05</td>
<td>0.86 ± 0.05</td>
<td>0.90 ± 0.05</td>
</tr>
<tr>
<td>Liver</td>
<td>3.80 ± 0.17</td>
<td>3.60 ± 0.24</td>
<td>3.14 ± 0.17*</td>
</tr>
<tr>
<td>Lung</td>
<td>2.58 ± 0.10</td>
<td>2.87 ± 0.19</td>
<td>2.97 ± 0.18</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.29 ± 0.02</td>
<td>0.18 ± 0.02**</td>
<td>0.17 ± 0.01**</td>
</tr>
<tr>
<td>Thyroid (× 100)</td>
<td>1.4 ± 0.1</td>
<td>2.4 ± 0.4</td>
<td>2.2 ± 0.1**</td>
</tr>
<tr>
<td>Thymus</td>
<td>0.39 ± 0.03</td>
<td>0.25 ± 0.04**</td>
<td>0.22 ± 0.02**</td>
</tr>
<tr>
<td>Brain</td>
<td>1.1 ± 0.04</td>
<td>1.3 ± 0.1*</td>
<td>1.7 ± 0.3***</td>
</tr>
<tr>
<td>Brain/liver</td>
<td>0.30 ± 0.03</td>
<td>0.37 ± 0.03**</td>
<td>0.55 ± 0.05***</td>
</tr>
<tr>
<td>Placenta</td>
<td>12.4 ± 0.8</td>
<td>10.1 ± 1.0</td>
<td>8.8 ± 0.3***</td>
</tr>
</tbody>
</table>

*\( P<0.05 \), **\( P<0.01 \), ***\( P<0.001 \) compared with controls.
capacity to transport by facilitated diffusion) was lower in the embolised and spontaneous IUGR animals than in controls (Table 6). Placental 3-O-[methyl-3H]glucose clearance per kilogram of placental weight was also lower in the embolised than in the control animals (100 ± 12 ml/min/kg vs 202 ± 32 ml/min/kg, P=0·01).

During IGF-I infusion

Because spontaneous IUGR fetuses were smaller than those in the other groups, the IGF-I infusion rate per kilogram fetal weight was highest in this group, although the differences did not reach statistical significance (spontaneous IUGR 62·7 ± 5, embolised 42·4 ± 4, controls 51·4 ± 3 μg/h/kg). This resulted in a greater increase in fetal plasma IGF-I concentrations in both IUGR groups than in controls (4·4-, 4·9- and 2·5-fold respectively), and achieved similar fetal plasma IGF-I concentrations in all groups during the infusion (Table 3). Maternal plasma IGF-I concentrations did not change during IGF-I infusion. Fetal plasma insulin concentrations fell during fetal IGF-I infusion in controls. There was no change in maternal plasma insulin concentrations in any of the groups.

During fetal IGF-I infusion, fetal blood glucose concentrations fell in control and embolised fetuses, with no change in the spontaneous IUGR group (Table 4). There was no change in maternal blood glucose concentrations in any group and no change in glucose uptakes.

During fetal IGF-I infusion blood lactate concentrations increased by approximately 25% in control but not in embolised or spontaneous IUGR fetuses (Table 4). Maternal blood lactate concentrations fell by 13% in the control group but not in the embolised or spontaneous IUGR groups. Fetal and uterine lactate uptakes did not change in any group. Placental lactate production fell by approximately 45% in the control group (Table 5). The percentage change in placental lactate production in the control and spontaneous IUGR groups was significantly correlated with fetal (P=0·003, r=0·78) and placental (P=0·001, r=0·83) weight (Fig. 3). This correlation was absent in the embolised group.

During fetal IGF-I infusion there was a small fall in both fetal and maternal blood urea concentrations in the control and embolised groups, with no change in the spontaneous IUGR group (Table 4). There was no change in fetal urea production in any group. Fetal IGF-I infusion caused a fall in fetal blood amino-nitrogen concentrations in all groups (Table 4). There was no change in maternal amino-nitrogen concentrations, and no change in fetal, placental or uterine uptakes.

During fetal IGF-I infusion there was no change in uterine blood flow in any group (Table 6). Umbilical blood flow fell in control but not in embolised or spontaneous IUGR animals. There was no change in placental clearance of [14C]urea in any group (Table 6). Placental clearance of 3-O-[methyl-3H]glucose fell approximately 40% in the control group, with no change in the embolised or spontaneous IUGR groups.

Calculation of all uptakes and clearances per kilogram of utero-placental or fetal weight did not alter any of the effects of IGF-I infusion reported above.

Discussion

This study has shown that acute IGF-I infusion to the spontaneously or induced growth retarded fetal sheep does not have the same anticatabolic effects as seen in the normal fetus. It provides the first in vivo demonstration that IUGR is associated with apparent IGF-I resistance in utero. The effects of IGF-I infusion on fetoplacental carbohydrate and protein metabolism were similar in our control group to those reported previously (Harding et al. 1994). Fetal blood glucose, oxygen, urea and amino-nitrogen concentrations fell, and placental lactate production was inhibited. The same fetal metabolite concentrations also fell in the embolised fetuses, but the effect of IGF-I on placental lactate production was not seen. A fall in fetal amino-nitrogen concentrations was the only effect of IGF-I infusion in the spontaneous IUGR animals.
Our findings add to limited earlier data suggesting that altered IGF-I sensitivity may be a feature of the growth retarded fetus. Tissues from rats born with IUGR are resistant to the effects of IGF-I on glucose and protein metabolism (Frampton et al. 1990, Simmons et al. 1993), and small for gestational age children showed increased levels of IGF-I when matched for height, age and ponderal index with idiopathic short children, suggesting possible IGF-I insensitivity (Hofman et al. 1997). Relative IGF-I resistance may explain a number of features of IUGR, including slow postnatal growth in some cases.

This apparent resistance to the metabolic effects of IGF-I appears to involve placental as well as fetal tissues. Inhibition of placental lactate production, which may be the most important metabolic effect of IGF-I in utero (Liu et al. 1994), was not seen in either IUGR group. Furthermore, within the non-embolised animals the correlation between fetal and placental size, and change in placental lactate production in response to IGF-I, suggests that IGF-I may be an important physiological regulator of placental metabolism across the continuum of fetal and placental sizes. The absence of this correlation in the embolised group may reflect placental tissue damage caused by embolisation, such that placental weight does not reflect functional placental tissue able to respond to IGF-I. Alternatively, placental sensitivity to IGF-I, like that of the fetus, may depend upon the cause, duration and severity of the IUGR.

Previous investigators have shown that sensitivity to other hormones is also altered in IUGR. Small for gestational age short children are insulin resistant (Hofman et al. 1997). There is also evidence for insulin resistance in slow growing fetal sheep (Harding & Johnston 1995). Growth retarded neonatal rats have delayed development of growth hormone (GH) sensitivity (Woodall et al. 1996), and there is evidence of altered GH sensitivity in IUGR children (Deibler et al. 1989). Thus, our data support the growing body of evidence that there may be a multihormone resistance in IUGR.

Mechanisms for IGF-I resistance in IUGR may include receptors and binding proteins. IGF-I influences cellular metabolism through a receptor mediated mechanism (LeRoith et al. 1991), and receptor numbers or their binding capacities may be decreased in the IUGR fetus.

### Table 3: Maternal and Fetal Plasma IGF-I and Insulin Concentrations Before and During IGF-I Infusion

<table>
<thead>
<tr>
<th></th>
<th>Control Before</th>
<th>Control During</th>
<th>Embolised Before</th>
<th>Embolised During</th>
<th>Spontaneous IUGR Before</th>
<th>Spontaneous IUGR During</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maternal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF-I</td>
<td>172 ± 23</td>
<td>159 ± 22</td>
<td>212 ± 25</td>
<td>211 ± 26</td>
<td>150 ± 25</td>
<td>156 ± 25</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.68 ± 0.15</td>
<td>0.68 ± 0.12</td>
<td>1.10 ± 0.14</td>
<td>1.25 ± 0.19</td>
<td>2.49 ± 1.3</td>
<td>2.00 ± 0.92</td>
</tr>
<tr>
<td><strong>Fetal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF-I</td>
<td>92 ± 6</td>
<td>312 ± 24a</td>
<td>57 ± 10a</td>
<td>260 ± 18c</td>
<td>47 ± 7c***</td>
<td>223 ± 67a**</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.54 ± 0.12</td>
<td>0.18 ± 0.04a</td>
<td>0.81 ± 0.35</td>
<td>0.43 ± 0.10</td>
<td>1.56 ± 1.27</td>
<td>1.07 ± 0.86</td>
</tr>
</tbody>
</table>

*P<0.05, †P<0.001 compared with value before IGF-I infusion in the same group; ‡P<0.05, ***P<0.001 compared with value before IGF-I infusion in controls.

### Table 4: Blood Substrate Concentrations Before and During IGF-I Infusion

<table>
<thead>
<tr>
<th></th>
<th>Control Before</th>
<th>Control During</th>
<th>Embolised Before</th>
<th>Embolised During</th>
<th>Spontaneous IUGR Before</th>
<th>Spontaneous IUGR During</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maternal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxygen</td>
<td>5.51 ± 0.16</td>
<td>5.50 ± 0.16</td>
<td>5.67 ± 0.27</td>
<td>5.59 ± 0.25</td>
<td>5.90 ± 0.56</td>
<td>5.88 ± 0.48</td>
</tr>
<tr>
<td>Glucose</td>
<td>2.38 ± 0.09</td>
<td>2.31 ± 0.10</td>
<td>2.55 ± 0.10</td>
<td>2.51 ± 0.09</td>
<td>2.52 ± 0.43</td>
<td>2.59 ± 0.33</td>
</tr>
<tr>
<td>Lactate</td>
<td>0.56 ± 0.05</td>
<td>0.49 ± 0.04a</td>
<td>0.59 ± 0.05</td>
<td>0.64 ± 0.07</td>
<td>0.87 ± 0.24</td>
<td>0.79 ± 0.24</td>
</tr>
<tr>
<td>Urea</td>
<td>5.22 ± 0.49</td>
<td>4.83 ± 0.40a</td>
<td>5.16 ± 0.36</td>
<td>4.59 ± 0.42a</td>
<td>3.71 ± 0.51</td>
<td>3.88 ± 0.48</td>
</tr>
<tr>
<td>Amino-nitrogen</td>
<td>4.26 ± 0.26</td>
<td>4.32 ± 0.25</td>
<td>4.30 ± 0.11</td>
<td>4.24 ± 0.14</td>
<td>4.20 ± 0.49</td>
<td>4.25 ± 0.39</td>
</tr>
<tr>
<td><strong>Fetal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxygen</td>
<td>3.44 ± 0.06</td>
<td>2.97 ± 0.13b</td>
<td>3.22 ± 0.33</td>
<td>2.80 ± 0.23a</td>
<td>2.70 ± 0.27**</td>
<td>2.21 ± 0.27a</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.82 ± 0.07</td>
<td>0.74 ± 0.08a</td>
<td>0.47 ± 0.06**</td>
<td>0.36 ± 0.07b</td>
<td>0.49 ± 0.06**</td>
<td>0.43 ± 0.05</td>
</tr>
<tr>
<td>Lactate</td>
<td>1.48 ± 0.19</td>
<td>1.97 ± 0.18a</td>
<td>1.78 ± 0.35</td>
<td>2.22 ± 0.61</td>
<td>1.52 ± 0.27</td>
<td>1.77 ± 0.39</td>
</tr>
<tr>
<td>Urea</td>
<td>5.31 ± 0.41</td>
<td>5.03 ± 0.37a</td>
<td>5.76 ± 0.37</td>
<td>5.34 ± 0.42a</td>
<td>4.80 ± 0.57</td>
<td>4.93 ± 0.48</td>
</tr>
<tr>
<td>Amino-nitrogen</td>
<td>7.34 ± 0.21</td>
<td>6.81 ± 0.18b</td>
<td>7.72 ± 0.52</td>
<td>7.17 ± 0.43b</td>
<td>7.79 ± 0.45</td>
<td>7.26 ± 0.35a</td>
</tr>
</tbody>
</table>

*P<0.05, †P<0.01 compared with value before IGF-I infusion in the same group; ‡P<0.001 compared with value before IGF-I infusion in controls.
the circulation and in tissues IGF-I is bound to binding proteins which can modulate the action of IGF-I (Humbel 1990). In particular, IGF binding protein-1 (IGFBP-1) inhibits the action of IGF-I in vivo in rats (Mortensen et al. 1997), and experimental IUGR is associated with increased IGFBP-1 plasma concentrations and liver message in several species (Unterman et al. 1990, McLellan et al. 1992). Thus, IGFBP-1 levels may have been increased and/or binding capacities altered in the IUGR groups in our study.

This study is also the first in vivo demonstration of a different response to IGF-I in different animal models of IUGR. The spontaneous IUGR group was composed of animals that became growth retarded for unknown reasons. These fetuses were more severely growth retarded than the embolised fetuses, had smaller placentas, and the duration of the growth retardation was likely to have been longer. The finding that different models of IUGR behave differently in response to IGF-I is further supported by data from Owens (1996), who found that IGF-I has anabolic effects on protein metabolism in the fetal sheep made growth retarded by restriction of placental implantation (carunclectomy). This apparent difference from our study in the effects of IGF-I could be due to the fact that carunclectomy results in a long period of growth retardation, or because of the method of inducing IUGR. Thus, it appears that the degree of IGF-I sensitivity may vary with the cause, severity and duration of IUGR.

Fetal plasma IGF-I levels in both our IUGR groups before IGF-I infusion were almost half those of controls. This is consistent with many previous reports of the association between low circulating IGF-I levels and IUGR (Jones et al. 1988, Bernstein et al. 1991, Lassarre et al. 1991, Owens et al. 1994). Although IGF-I levels in the growth retarded animals were lower than controls before IGF-I infusion, IGF-I levels increased to approximately the same value as controls. Therefore, the differences seen between groups are unlikely to be due to different IGF-I levels achieved during the infusion.

Table 5 Substrate uptakes before and during IGF-I infusion. Values are expressed as means ± s.e. in µmol/l for 9 control, 8 embolised, and 6 spontaneous IUGR animals

<table>
<thead>
<tr>
<th></th>
<th>Control Before</th>
<th>Control During</th>
<th>Embolised Before</th>
<th>Embolised During</th>
<th>Spontaneous IUGR Before</th>
<th>Spontaneous IUGR During</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Uterine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxygen</td>
<td>1872 ± 149</td>
<td>1780 ± 106</td>
<td>1320 ± 180*</td>
<td>1367 ± 247</td>
<td>1480 ± 343</td>
<td>1531 ± 317</td>
</tr>
<tr>
<td>Glucose</td>
<td>278 ± 41</td>
<td>270 ± 24</td>
<td>219 ± 32</td>
<td>263 ± 57</td>
<td>212 ± 29</td>
<td>320 ± 90</td>
</tr>
<tr>
<td>Lactate</td>
<td>143 ± 42</td>
<td>120 ± 33</td>
<td>84 ± 30</td>
<td>152 ± 67</td>
<td>67 ± 19</td>
<td>116 ± 48</td>
</tr>
<tr>
<td>Amino-nitrogen</td>
<td>99 ± 55</td>
<td>137 ± 58</td>
<td>4 ± 43</td>
<td>−6 ± 51</td>
<td>103 ± 107</td>
<td>178 ± 106</td>
</tr>
<tr>
<td><strong>Placental</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxygen</td>
<td>646 ± 151</td>
<td>629 ± 104</td>
<td>634 ± 111</td>
<td>601 ± 165</td>
<td>776 ± 315</td>
<td>869 ± 305</td>
</tr>
<tr>
<td>Glucose</td>
<td>204 ± 39</td>
<td>198 ± 30</td>
<td>180 ± 37</td>
<td>203 ± 54</td>
<td>166 ± 28</td>
<td>296 ± 128</td>
</tr>
<tr>
<td>Lactate</td>
<td>−210 ± 50</td>
<td>−117 ± 29*</td>
<td>−92 ± 24</td>
<td>−129 ± 81</td>
<td>−129 ± 40</td>
<td>−189 ± 61</td>
</tr>
<tr>
<td>Amino-nitrogen</td>
<td>−50 ± 47</td>
<td>−61 ± 64</td>
<td>−23 ± 106</td>
<td>−128 ± 78</td>
<td>9 ± 86</td>
<td>−139 ± 194</td>
</tr>
<tr>
<td><strong>Fetal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxygen</td>
<td>1202 ± 83</td>
<td>1120 ± 72</td>
<td>750 ± 78***</td>
<td>755 ± 91</td>
<td>719 ± 51***</td>
<td>706 ± 58*</td>
</tr>
<tr>
<td>Glucose</td>
<td>79 ± 7</td>
<td>72 ± 10</td>
<td>53 ± 7*</td>
<td>61 ± 12</td>
<td>54 ± 11</td>
<td>57 ± 11</td>
</tr>
<tr>
<td>Lactate</td>
<td>66 ± 50</td>
<td>20 ± 33</td>
<td>6 ± 8</td>
<td>−12 ± 33</td>
<td>60 ± 29</td>
<td>73 ± 20</td>
</tr>
<tr>
<td>Amino-nitrogen</td>
<td>141 ± 42</td>
<td>187 ± 61</td>
<td>110 ± 47</td>
<td>75 ± 44</td>
<td>113 ± 94</td>
<td>171 ± 149</td>
</tr>
</tbody>
</table>

*P<0.05 compared with value before IGF-I infusion in the same group; **P<0.01 compared with value before IGF-I infusion in controls.

Table 6 Tracer clearances, urea production and blood flow before and during IGF-I infusion. Values are expressed as means ± s.e. for 9 control, 8 embolised, and 6 spontaneous IUGR animals

<table>
<thead>
<tr>
<th></th>
<th>Control Before</th>
<th>Control During</th>
<th>Embolised Before</th>
<th>Embolised During</th>
<th>Spontaneous IUGR Before</th>
<th>Spontaneous IUGR During</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Uterine blood flow (ml/min)</strong></td>
<td>1460 ± 128</td>
<td>1380 ± 117</td>
<td>841 ± 96**</td>
<td>862 ± 374</td>
<td>939 ± 113*</td>
<td>1269 ± 355</td>
</tr>
<tr>
<td><strong>Umbilical blood flow (ml/min)</strong></td>
<td>703 ± 36</td>
<td>618 ± 36b</td>
<td>495 ± 58**</td>
<td>463 ± 127</td>
<td>553 ± 156</td>
<td>587 ± 198</td>
</tr>
<tr>
<td>[14C]Urea clearance (ml/min)</td>
<td>60 ± 9</td>
<td>50 ± 6</td>
<td>41 ± 6</td>
<td>37 ± 7</td>
<td>49 ± 13</td>
<td>181 ± 156</td>
</tr>
<tr>
<td>3-O[methyl-3H]glucose clearance (ml/min)</td>
<td>93 ± 12</td>
<td>57 ± 7*</td>
<td>32 ± 5***</td>
<td>28 ± 4</td>
<td>49 ± 10*</td>
<td>29 ± 6</td>
</tr>
<tr>
<td><strong>Fetal urea production (µmol/min)</strong></td>
<td>11 ± 10</td>
<td>9 ± 8</td>
<td>17 ± 5</td>
<td>21 ± 4</td>
<td>27 ± 11</td>
<td>11 ± 4</td>
</tr>
</tbody>
</table>

*P<0.05; **P<0.01 compared with value before IGF-I infusion in the same group; ***P<0.001 compared with value before IGF-I infusion in controls.
Fetal IGF-I infusion reduced placental 3-O-[methyl-\(^3\)H]glucose transfer in the controls but not in the embolised and spontaneous IUGR animals. The reason why IGF-I reduced placental clearance in the controls is not clear. It is unlikely to be due to the reduced umbilical blood flow seen during IGF-I infusion, since reduced umbilical blood flow did not affect other metabolite uptakes or \(^{14}\)C]urea clearance, and placental transfer of glucose is primarily membrane rather than flow limited (Simmons et al. 1979). Glucose is transported across the placenta from mother to fetus by facilitative diffusion through specific glucose transporter proteins (Simmons et al. 1979, Currie et al. 1997, Hauguel-De Mouzon et al. 1997). However, it is unlikely that IGF-I reduced glucose transporter activity, since IGF-I is reported to increase both glucose transporter-4 and glucose transporter-1 activity in rats (Simmons et al. 1993, Lund et al. 1994). Placental 3-O-[methyl-\(^3\)H]glucose clearance was less in both IUGR groups than in controls before IGF-I infusion. This is consistent with previous reports of reduced exchange area and glucose transporter density in human placenta with IUGR (Jansson et al. 1993). It has also been suggested that there may be impaired glucose transporter function in IUGR fetal rat lung and muscle (Simmons et al. 1992, 1993).

There was a fall in fetal blood urea concentrations with IGF-I infusion in the control and embolised groups. The fall in fetal urea concentration could reflect an inhibition of protein oxidation, but this is not supported by the lack of change in fetal urea production, a measure of fetal amino acid oxidation. Urea is excreted by the kidneys in fetal sheep at approximately half the transplacental excretion rate (Gresham et al. 1972). Since IGF-I increases glomerular filtration rate and plasma renal flow in rats, fetal sheep and humans (Hirschberg & Kopple 1989, Hammerman & Miller 1997, Marsh et al. 1998), the fall in fetal urea concentrations in our studies may, in part, be the result of increased excretion by the fetal kidney. There was also a fall in maternal blood urea concentrations in the control and embolised groups. This is unlikely to be a direct effect of IGF-I, since it has been shown that IGF-I does not cross the placenta (Bassett et al. 1990), and may simply reflect the fall in fetal urea concentrations.

We have previously found a 30% decrease in fetal urea production in normal fetuses given IGF-I using a similar experimental protocol (Harding et al. 1994). However, fetal urea production before IGF-I in our current study was approximately 4–5 times lower than the values reported previously. This could be due to a healthier group of animals with higher glucose and lower protein oxidation rates than those seen previously. Liechty et al. (1996) have demonstrated that, when excess glucose is made available for oxidation, amino acid oxidation decreases. Therefore, it is possible that in our current study IGF-I failed to decrease amino acid oxidation because this was already low, and there was no increase in glucose utilization. In addition, placental lactate production, maternal blood lactate and urea concentrations were all higher in our

![Figure 3](image-url)
current study than those found previously. Thus the differences between the two studies may, in part, be explained by the fact that the two groups of animals were different metabolically before IGF-I was given.

In summary, we have shown that short term IGF-I infusion to the growth retarded fetus does not have the same effects on feto-placental metabolism as in the normal fetus. Our data suggest that the degree of IGF-I sensitivity in utero may vary depending on the cause, duration and severity of growth retardation. The mechanisms by which IGF-I sensitivity is altered in response to different growth retarding insults remain to be explored.

Acknowledgements

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References


