Amniotic IGF-I supplements improve gut growth but reduce circulating IGF-I in growth-restricted fetal sheep

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Received 9 May 2001; accepted in final form 17 September 2001

Amniotic IGF-I supplements improve gut growth but reduce circulating IGF-I in growth-restricted fetal sheep. Am J Physiol Endocrinol Metab 282: E259–E269, 2002; 10.1152/ajpendo.00200.2001.—Insulin-like growth factor I (IGF-I) is an important regulator of fetal growth, and circulating concentrations are reduced in intrauterine growth-restricted (IUGR) fetuses. We investigated whether IGF-I administered into amniotic fluid could ameliorate IUGR in fetal sheep. Fetuses were assigned to control (n = 9), IUGR + saline (n = 12), or IUGR + IGF-I groups (daily intra-amniotic IGF-I injections of 20 μg, n = 13). IUGR was induced by placental embolization from 114 to 120 days. Treatment was from 120 to 130 days of gestation. Embolization produced asymmetrical IGF-I fetuses with decreased body weight and lighter, thinner-walled guts. Fetal plasma and amniotic IGF-I levels were reduced. During treatment, fetal plasma, but not amniotic, IGF-I levels recovered in the saline group but remained depressed in the IGF-I-treated group. IGF-I treatment restored gut weight and wall thickness to control levels and increased the number of crypt mitoses. Fetal weight was similar to that of controls, but spleen, liver, and thymic weights were reduced by 30–37%, and placentome growth was altered. Amniotic fluid IGF-I supplementation may provide the basis of future therapeutic approaches to IUGR, but the systemic effects require further investigation.

intrauterine growth restriction; hormone; metabolism; fetal therapy; placenta


INTRAUTERINE GROWTH RESTRICTION (IUGR) is a major cause of perinatal morbidity and mortality (49). Increased morbidity continues throughout childhood from sequelae such as growth failure (57), developmental delay (23), and cerebral palsy (18). The gut is one of the more severely affected organs in IUGR (3, 55). Epithelial permeability is increased, and mucosal immunity is reduced. Hence, IUGR infants are at increased risk of gut-acquired infections (50). Premature and compromised IUGR infants take longer to establish full enteral feeding and have an increased risk of gastrointestinal morbidity after birth (39), yet they are the very infants most in need of optimal nutrition. Furthermore, epidemiological evidence suggests that
intestinal growth in fetal (62) and neonatal animals (11). A 10-day infusion of IGF-I in large doses (26 μg·kg⁻¹·h⁻¹) to normally growing late-gestation ovine fetuses results in selective organ growth but no overall increase in body size (41). However, we have previously shown that a chronic enteral infusion of IGF-I in fetal sheep with an experimentally induced esophageal atresia, in doses calculated to replace the amount that would have been obtained from swallowed amniotic fluid (1–8 μg/day), can prevent the somatic and gastrointestinal growth restriction seen in the saline-treated controls (29). Furthermore, there were effects on organs other than the gut that suggest systemic absorption.

We wished to explore the enteral route of administration of IGF-I further and in a more clinically relevant paradigm. In this study, we have therefore chosen to administer IGF-I as a single daily injection into amniotic fluid, rather than directly into the gut, and we have utilized an ovine model of IUGR involving placental impairment. We have treated for 10 days in late gestation, as this duration of treatment has been shown to have effects on organ growth in normally growing fetuses of similar gestational age (41). We hypothesized that swallowed IGF-I would result in improved gastrointestinal and/or somatic growth of the IUGR fetuses. We chose a dose of 20 μg/day on the basis of our previous data from direct enteral administration (29), the estimated turnover of ovine amniotic fluid (58), and the potential for loss of IGF-I before swallowing.

MATERIALS AND METHODS

Animals. Approval for the experiment was obtained from the institutional animal ethics committee.

Thirty-four ewes carrying singleton fetuses underwent surgery at 110 days of gestation (term = 145 days). Under halothane anesthesia and aseptic technique, the maternal abdomen was opened and the fetus was accessed via a hysterotomy. Polyvinyl catheters were placed in the fetal femoral artery and vein via the tarsal vessels. The portal vein was catheterized via a mesenteric vein by use of a polyvinyl catheter with a silicone tip to reduce the risk of perforation of this thin-walled vessel. Growth catheters were inserted subcutaneously around each half of the fetal chest and attached to the sternum and spine (25). Four amniotic fluid catheters were inserted into the main uterine artery supplying each uterine horn via the tarsal vessels. The portal vein was catheterized via a mesenteric vein by use of a polyvinyl catheter with a silicone tip to reduce the risk of perforation of this thin-walled vessel. Growth catheters were inserted subcutaneously around each half of the fetal chest and attached to the sternum and spine (25). Four amniotic fluid catheters were attached to the fetal skin, one to either side of the fetal neck (the “upper” catheters) and one to each fetal hindlimb (the “lower” catheters). Vascular catheters were also placed into the main uterine artery supplying each uterine horn via an arterial branch near the tip of each horn. Catheters were also placed in the maternal carotid and femoral arteries and in the jugular vein. At surgery, the ewe received 5 ml of streptopen (250 mg of procaine penicillin and 250 mg of dihydrostreptomycin sulfate, Pittman Moore, Upper Hutt, New Zealand) intramuscularly, and the fetus received 80 mg of gentamicin into the amniotic fluid.

The ewes were housed in individual cages with free access to water and ad libitum quantities of a standard laboratory diet of chaff and pelleted stock feed.

At the end of the experiment (131 days of gestation), ewes were killed with an overdose of phenobarbitone. The uterus was removed intact from the ewe, and the fetal fluids were collected and weighed. The placement of catheters was checked, and the fetus and placenta were dissected and weighed. The bowel from the cardia of the stomach to the anus was rapidly removed and dissected free of its mesenteric attachments, and its length was measured. Enteric contents were removed, and the gut was weighed in sections. The first 2 cm of small intestine were assumed to represent duodenum. Because the jejunoeileal junction cannot be distinguished, the remaining small bowel was weighed in one section. The colon was weighed separately. Individual placentomes were dissected from the uterus, and membranes were categorized (63) and weighed. The uterus and membranes were weighed separately.

Experimental design. Animals were randomly assigned to one of three groups: control ewes (n = 9), growth-restricted ewes treated with saline (n = 12), and growth-restricted ewes treated with IGF-I (n = 13). Growth restriction was induced between 114 and 119 days of gestation by repetitive embolization of the uteroplacental bed with 20- to 50-μm polystyrene microspheres (Superose 12 diluted 1:100, Pharmacia Biotech, Uppsala, Sweden). The frequency and volume of injections (usually 1–2 ml into each uterine artery) were titrated against fetal arterial blood gas and metabolite concentrations (glucose and lactate; Yellow Springs Instruments, Dayton, OH) and against the daily increment in fetal girth, measured twice daily from the exteriorized growth catheters. Embolization was omitted if (1) fetal arterial oxygen pressure was <14.0 mmHg, (2) fetal blood lactate was >2.5 mmol/l, or (3) the growth catheters showed no growth for 2 days.

Treatment was given by daily intra-amniotic injection of 120 to 130 days of gestation. Saline-treated fetuses received 2 ml of saline. IGF-I-treated fetuses received 20 μg of recombinant human IGF-I (Pharmacia and Upjohn, Peapack, NJ) mixed with 2 ml of fresh amniotic fluid. Because we wished to investigate the effects of IUGR and IGF-I treatment of IUGR compared with normally grown fetuses, controls were neither embolized nor treated.

Plasma and amniotic fluid samples were collected simultaneously from maternal and fetal artery, fetal portal vein, and amniotic upper and lower catheters every 2–3 days, immediately before embolization or treatment. Blood was stored at –80°C until assay for glucose, urea, and α-aminonitrogen; after centrifugation at 4°C, plasma and amniotic fluid were frozen at –80°C until assayed for IGF-I and insulin.

Assays. Glucose and α-aminonitrogen were assayed by colorimetric assays (17, 34). Plasma and amniotic fluid IGF-I levels were measured by double-antibody RIA by use of a nonextraction method with excess IGF-II to abolish interference of the IGF-binding proteins. The minimal detectable dose was 0.02 ng/tube. The intra- and interassay coefficients of variation were 5.4 and 9.7%, respectively. IGF-I values are expressed in terms of international reference IGF-I (9). Insulin was also measured by RIA (10).

Mucosal scrapings for analysis of fetal gut enzymes were collected from duodenum (proximal 20 cm of small bowel), jejenum (20 cm distal to duodenal-jejunal junction), and ileum (20 cm proximal to the ileocecal valve). A 4-cm length from each section of gut was opened longitudinally, and mucosal scrapings were taken with a glass slide and then snap-frozen in liquid nitrogen. β-Galactosidase (lactase, EC 3.2.1.23) was assayed by the modified Dahlqvist method (59), total protein content by the Lowry method (42), and α-aminonacyl-peptide hydrolase (leucine aminopeptidase, EC 3.4.11.1) by colorimetry (22). The contribution of intracellular β-galactosidase to total β-galactosidase activity was cal-
culated by addition of PCMB (4-chloromercuric benzoic acid) to the buffer. This inhibits the intracellular enzyme but not the brush-border enzyme. Because the intracellular enzyme accounted for only 3.2% of the total enzyme activity, we report total β-galactosidase activity.

Sections of duodenum, jejunum, ileum, and colon (20 cm distal to ileocecal valve) were fixed in buffered formalin and then embedded in paraffin wax for light microscopy. Sections 4 μm thick were cut at right angles to the long axis and stained with hematoxylin and eosin. For each fetus, 20 measurements from each of 4 sections of each region of bowel were made of the muscularis externa, submucosa, crypt depth, and villus height. From these, total bowel wall thickness (excluding villi) was calculated. Measurements were made using a Leica light microscope with a JVC TK-1281 video attachment connected to a computer with the Mocha Image Analyzer program (Jandel Scientific, Corte Madera, CA).

To assess the effect of IGF-I treatment on mitosis in the cells of the crypts of the small intestine, slides of gut section were stained for proliferating cell nuclear antigen (PCNA) by use of an anti-PCNA antibody (mouse anti-human antibody no. M087901, Dako, Carpenteria, CA). The secondary antibody was a horse anti-mouse antibody with biotin attached (Vectastain ABC kit, Vector Laboratories, Burlingame, CA). An avidin-biotin complex (Vectastain ABC kit) was then added, and the stain was developed with diaminobenzidine.

Data analysis. All values are presented as the means ± SE or median (range) as appropriate. Blood gas, metabolite, and hormone concentrations in arterial and amniotic samples in the three groups were compared separately during the embolization (114–119 days) and treatment periods (120–130 days) with repeated-measures ANOVA. Comparable data from portal venous samples were analyzed by factorial ANOVA, because occasional missing points due to catheter difficulties did not allow repeated-measures ANOVA to be used. Fetal arterial and portal venous data from the same time point were compared by paired t-test. Parametric data from fixed time points were compared using factorial ANOVA. The Games-Howell post hoc test was employed for multiple comparisons, with significance set at the 0.05 level. Nonparametric continuous data were compared by the Kruskall-Wallis test; nominal data were compared by logistic regression analysis. Data were analyzed using Statview (SAS Institute, Cary, NC).

**RESULTS**

There were no significant differences among groups in ewe weight, condition, fetal size at surgery, or fetal sex (Table 1).

**Effects of embolization.** Five fetuses died during embolization, two that were randomized to later receive IGF-I and three that would have received saline. These animals were excluded from further analysis. Embolization resulted in acute falls in oxygen content (P < 0.05, Fig. 1A) and fetal arterial glucose concentration (control 1.12 ± 0.03, IGF-I 0.86 ± 0.07, saline 0.98 ± 0.05 mM, P < 0.01, for both embolized groups vs. control, Fig. 1C). At the end of the embolization period, fetal arterial hemoglobin concentration was higher in embolized animals (P < 0.05 for IGF-I vs. control, P = 0.06 for saline vs. control; factorial ANOVA; Fig. 1B). There was an acute increase in fetal arterial lactate concentration in embolized fetuses (Fig. 1D) that did not reach statistical significance. There was a corre-
During the treatment period, growth rate in controls remained unchanged, and saline- and IGF-I-treated fetuses continued to grow at a slower rate than controls (control 4.7±0.3 mm/day; saline 4.0±0.3 mm/day, \( P < 0.05 \), vs. control; IGF-I 3.6±0.3 mm/day, \( P < 0.001 \), vs. control). At postmortem, body weight and liver weight of saline-treated fetuses were reduced by 21% (\( P < 0.05 \)) and 29% (\( P < 0.01 \)), respectively, compared with controls (Table 2). The body weight of IGF-I-treated fetuses was not significantly different from that of controls, but liver weight was even further reduced [by 37% (\( P < 0.001 \)) and 11% (\( P = 0.08 \))]. Splenic and thymic weights were each reduced by ~37% compared with controls (Table 2). Total placental weight and median placentome weight were reduced to a similar degree in IGF-I- and saline-treated animals (~30%, \( P < 0.001 \), vs. controls, Table 3). However, there was a trend for IGF-I-treated animals to have fewer placentomes <2 g in weight (Table 3). Furthermore, IGF-I treatment significantly altered the distribution of placentome types from types A and B to types C and D (\( P < 0.001 \), Table 3).

Effects of embolization and IGF-I treatment on gut size and enzyme function. Embolization reduced the weight of all regions of the bowel and thus total gut weight, although the reduction in large bowel weight did not reach statistical significance (\( P = 0.06 \), Table 4). Gut weight as a proportion of fetal body weight was not significantly reduced. Embolization significantly reduced large bowel length and weight of contents present in the bowel lumen (Table 4).

IGF-I treatment reversed the reduction in large bowel weight and length and the reduction in weight of total and large bowel contents (\( P < 0.05 \), vs. saline). The increase in duodenal weight with IGF-I treatment was not statistically significant (\( P = 0.08 \), Table 4).

Lactase and leucine aminopeptidase levels in mucusal scrapings were not significantly different between groups (Table 5). Enzyme levels in all treatment groups decreased significantly from proximal to distal bowel (\( P < 0.0001 \), Table 5).

Effect of embolization and IGF-I treatment on histology of the gut. Embolization reduced villus height in the duodenum and ileum but increased villus height in...
Placental size, placentome weight, and type

Table 2. Fetal body proportions and organ weights

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 9)</th>
<th>Saline (n = 9)</th>
<th>IGF-I (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal weight, kg</td>
<td>4.38 ± 0.28</td>
<td>3.46 ± 0.14*</td>
<td>3.73 ± 0.16</td>
</tr>
<tr>
<td>Crown-rump length, cm</td>
<td>44.1 ± 0.7</td>
<td>41.9 ± 0.6</td>
<td>43.0 ± 0.4</td>
</tr>
<tr>
<td>Chest girth, cm</td>
<td>34.9 ± 0.6</td>
<td>31.4 ± 0.4*</td>
<td>32.5 ± 0.5*</td>
</tr>
<tr>
<td>Hindlimb, cm</td>
<td>34.6 ± 0.9</td>
<td>32.9 ± 0.7</td>
<td>32.5 ± 0.4</td>
</tr>
<tr>
<td>Brain, g</td>
<td>45.6 ± 1.1</td>
<td>45.3 ± 1.7</td>
<td>45.4 ± 1.3</td>
</tr>
<tr>
<td>Brain/kg body wt</td>
<td>10.7 ± 0.5</td>
<td>13.2 ± 0.5*</td>
<td>12.4 ± 0.7*</td>
</tr>
<tr>
<td>Liver, g</td>
<td>146.4 ± 5.1</td>
<td>104.3 ± 6.2*</td>
<td>92.9 ± 12.0*</td>
</tr>
<tr>
<td>Liver/kg body wt</td>
<td>34.2 ± 1.9</td>
<td>30.6 ± 2.5</td>
<td>24.2 ± 2.8*</td>
</tr>
<tr>
<td>Spleen, g</td>
<td>9.5 ± 0.7</td>
<td>8.0 ± 0.7</td>
<td>6.1 ± 0.5</td>
</tr>
<tr>
<td>Spleen/kg body wt</td>
<td>2.3 ± 0.2</td>
<td>2.3 ± 0.1</td>
<td>1.6 ± 0.1*§</td>
</tr>
<tr>
<td>Thyroid, g</td>
<td>17.4 ± 2.8</td>
<td>12.2 ± 1.3</td>
<td>11.0 ± 1.1‡</td>
</tr>
<tr>
<td>Thyroid/kg body wt</td>
<td>3.8 ± 0.4</td>
<td>3.5 ± 0.4</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>Lung, g</td>
<td>102.6 ± 10.0</td>
<td>94.1 ± 9.8</td>
<td>103.5 ± 6.7</td>
</tr>
<tr>
<td>Lung/kg body wt</td>
<td>24.0 ± 2.3</td>
<td>27.1 ± 2.3</td>
<td>28.4 ± 2.5</td>
</tr>
<tr>
<td>Heart/kg body wt</td>
<td>7.4 ± 0.3</td>
<td>7.9 ± 0.4</td>
<td>7.5 ± 0.3</td>
</tr>
<tr>
<td>Kidneys/kg body wt</td>
<td>6.7 ± 0.5</td>
<td>7.2 ± 0.6</td>
<td>7.0 ± 0.3</td>
</tr>
<tr>
<td>Adrenals/kg body wt</td>
<td>0.13 ± 0.01</td>
<td>0.14 ± 0.01</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td>Thyroid/kg body wt</td>
<td>0.29 ± 0.02</td>
<td>0.26 ± 0.02</td>
<td>0.29 ± 0.03</td>
</tr>
<tr>
<td>Uterus, g</td>
<td>906.9 ± 114.6</td>
<td>654.6 ± 29.7*</td>
<td>669.6 ± 39.4*</td>
</tr>
<tr>
<td>Uterus/kg body wt</td>
<td>213.8 ± 29.5</td>
<td>183.4 ± 5.3</td>
<td>180.4 ± 10.2</td>
</tr>
<tr>
<td>Membranes, g</td>
<td>338.6 ± 26.4</td>
<td>225.8 ± 11.8*</td>
<td>269.2 ± 38.2</td>
</tr>
<tr>
<td>Fetal fluids, ml</td>
<td>1349.9 ± 264.3</td>
<td>971.2 ± 66.2</td>
<td>979.4 ± 168.9</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05, †P < 0.01, ‡P < 0.001 vs. controls; §P < 0.01 vs. saline-treated animals.

IGF-I treatment resulted in shorter villi but deeper crypts in all regions of the small bowel (Table 5). Thus villus-to-crypt ratio was reduced in all regions (Table 5). IGF-I treatment resulted in a substantial thickening of all components of the bowel wall in small bowel and in the colon (Table 5). The vacuolation of the villus enterocytes in the ileum was enhanced further by IGF-I treatment, so that the whole of the villus was affected (Fig. 2).

IGF-I treatment increased the proportion of crypt cells in the duodenum and jejunum that stained positive for PCNA above that in saline and control animals. However, in the ileum, the proportion of PCNA-positive cells was reduced by IGF-I treatment (P < 0.01 vs. saline-treated fetuses; Table 5).

The proportion of PCNA-positive crypt cells was increased in all fetal regions throughout the amniotic fluid (saline 28%, IGF-I 34% less than control, P < 0.01; Fig. 3A) and in amniotic fluid (saline 12%, P = 0.1, and IGF-I 13% less than control, P < 0.01; Fig. 3A). During the treatment period, IGF-I levels in maternal plasma rose in controls but not in ewes from either embolized group (P < 0.05 compared with controls). During the treatment period, IGF-I levels in saline-treated animals recovered in fetal plasma (not significantly different from controls) but remained low in amniotic fluid (P = 0.07 vs. controls). In contrast, in IGF-I-treated animals, IGF-I levels failed to recover in fetal plasma (P < 0.05 compared with saline and control animals) but increased fivefold in amniotic fluid (P < 0.01, Fig. 3A). There were no significant differences in IGF-I levels between the fetal arterial and portal venous blood. There were also no significant differences in samples taken from the upper and lower amniotic catheters, demonstrating good mixing throughout the amniotic fluid (data not shown).

Insulin levels in amniotic fluid and plasma. Maternal plasma insulin levels were unaffected by embolization or by treatment (Fig. 3B). During embolization, fetal plasma insulin levels fell by 50% (P < 0.01 vs. control, Fig. 3B). During the treatment period, fetal plasma insulin levels remained low in both saline- and IGF-I-treated animals.

Table 3. Placental size, placentome weight, and type

<table>
<thead>
<tr>
<th>Placental Weight</th>
<th>Placentomes in Each Weight Category, n</th>
<th>Placenta % of Placentomes of Each Type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total, g</td>
<td>g/kg fetal wt</td>
</tr>
<tr>
<td>Control (n = 9)</td>
<td>486 ± 35</td>
<td>112 ± 5.6</td>
</tr>
<tr>
<td>Saline (n = 9)</td>
<td>339 ± 18†</td>
<td>95.0 ± 3.6*</td>
</tr>
<tr>
<td>IGF-I (n = 11)</td>
<td>338 ± 25§</td>
<td>90.6 ± 5.2†</td>
</tr>
</tbody>
</table>

Values are means ± SE or median (range) as appropriate. Placenta types (Ref. 63) differed in IGF animals from saline and control animals (P < 0.001; logistic regression analysis). *P < 0.05, †P < 0.01, ‡P < 0.001 vs. control; §P = 0.09 vs. saline-treated animals.

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and reduce the growth of the organs most sensitive to the effects of circulating IGF-I concentrations, the spleen and thymus. Third, IGF-I administration into the amniotic cavity altered placental morphology. Thus intra-amniotic, and hence enteral, administration of IGF-I to the UGR fetus has both local and systemic effects on fetal and placental growth and endocrine status.

Clinical attempts to improve fetal growth by maternal supplementation with protein (33) or oxygen (6) have not been successful. This may reflect the fact that most cases of IUGR in developed countries are due to placental insufficiency, and therefore improving maternal nutrition and/or oxygenation does not necessarily have any effect on fetal nutrition or oxygenation. In experimentally induced IUGR in sheep, the onset of growth restriction can be prevented by chronic intra-amniotic infusion of glucose and amino acids (13) or by chronic enteral infusion of IGF-I (29), commenced at the same time as the onset of the growth-restricting insult. However, there is no previous evidence that aspects of established IUGR may be successfully treated. In our study, treatment was for 10 days. The size of IGF-I-treated fetuses at the end of the treatment period was intermediate between saline-treated and control fetuses and was not significantly different from that of controls. This may suggest that a longer duration of treatment could reverse the somatic growth restriction, as well as the effects on the gut.

The most marked effects of IUGR and of IGF-I treatment were on the gut, although microscopic changes in other organs may not have been detected because other organs were not examined histologically. Embolization reduced gut weight and wall thickness and resulted in persistence of the apical endocytic complex (AEC), an extensive endocytic transport system capable of transporting large-molecular-weight molecules (16). In fetal sheep, the AEC has usually completely disappeared, apart from a few remaining vacuoles in the villus tip.

### Table 5. Enzyme activity, histology, and immunohistochemistry of the bowel

<table>
<thead>
<tr>
<th></th>
<th>Duodenum</th>
<th></th>
<th>Jejunum</th>
<th></th>
<th>Ileum</th>
<th></th>
<th>Colon</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Saline</td>
<td>IGF-I</td>
<td>Control</td>
<td>Saline</td>
<td>IGF-I</td>
<td>Control</td>
<td>Saline</td>
</tr>
<tr>
<td></td>
<td>(n = 8)</td>
<td>(n = 7)</td>
<td>(n = 9)</td>
<td>(n = 9)</td>
<td>(n = 6)</td>
<td>(n = 7)</td>
<td>(n = 7)</td>
<td>(n = 10)</td>
</tr>
<tr>
<td>Lactase, U/g</td>
<td>42.2 ± 3.9</td>
<td>43.7 ± 1.6</td>
<td>43.4 ± 3.1</td>
<td>35.5 ± 4.0</td>
<td>31.9 ± 3.2</td>
<td>31.9 ± 2.5</td>
<td>5.9 ± 1.5</td>
<td>5.9 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>protein§</td>
<td>8.3 ± 0.7</td>
<td>8.9 ± 1.1</td>
<td>10.2 ± 0.7</td>
<td>7.9 ± 0.8</td>
<td>9.1 ± 0.7</td>
<td>10.2 ± 2.0</td>
<td>9.8 ± 2.5</td>
</tr>
<tr>
<td>Villus ht, μm</td>
<td>593 ± 10</td>
<td>550 ± 13 8</td>
<td>449 ± 8 8</td>
<td>608 ± 10 3</td>
<td>637 ± 10 0</td>
<td>504 ± 7 7</td>
<td>604 ± 12 0</td>
<td>561 ± 8 8</td>
</tr>
<tr>
<td>Crypt depth, μm</td>
<td>137 ± 2</td>
<td>138 ± 2</td>
<td>193 ± 4 8</td>
<td>145 ± 2</td>
<td>127 ± 1 3</td>
<td>159 ± 4 9</td>
<td>113 ± 2</td>
<td>113 ± 2</td>
</tr>
<tr>
<td>v/c Ratio</td>
<td>4.5 ± 0.1</td>
<td>4.4 ± 0.1</td>
<td>2.6 ± 0.1 6</td>
<td>4.5 ± 0.1</td>
<td>5.4 ± 0.1 6</td>
<td>4.1 ± 0.1 4</td>
<td>5.1 ± 0.1</td>
<td>5.6 ± 0.1 4</td>
</tr>
<tr>
<td>Mucosa, μm</td>
<td>143 ± 3</td>
<td>140 ± 3</td>
<td>242 ± 6 6</td>
<td>72 ± 1</td>
<td>58 ± 1 5</td>
<td>159 ± 5 7</td>
<td>75 ± 1</td>
<td>82 ± 3 6</td>
</tr>
<tr>
<td></td>
<td>342 ± 0</td>
<td>323 ± 0</td>
<td>56 ± 1 10</td>
<td>56 ± 1 10</td>
<td>56 ± 1 10</td>
<td>56 ± 1 10</td>
<td>56 ± 1 10</td>
<td>56 ± 1 10</td>
</tr>
<tr>
<td>Wall – villi, μm</td>
<td>434 ± 6</td>
<td>426 ± 6</td>
<td>728 ± 17 6</td>
<td>337 ± 5</td>
<td>260 ± 3 6</td>
<td>529 ± 12 0</td>
<td>294 ± 5</td>
<td>327 ± 5 6</td>
</tr>
<tr>
<td>% Crypt cells</td>
<td>163 ± 3</td>
<td>175 ± 1 6</td>
<td>261 ± 1 3 6</td>
<td>9.7 ± 0.6</td>
<td>7.2 ± 0.6 6</td>
<td>261 ± 1 3 6</td>
<td>7.6 ± 0.9</td>
<td>6.8 ± 0.7</td>
</tr>
</tbody>
</table>

Values are means ± SE. LAP, leucine aminopeptidase; v/c, villus/crypt; m externa = muscularis externa; wall-villi, total wall thickness minus villus height; PCNA, proliferating cell nuclear antigen. †P < 0.001 vs. saline-treated animals; *P < 0.001 for enzyme levels in duodenum vs. jejunum, duodenum vs. ileum, and for jejunum vs. ileum within each treatment group.
enterocytes of the distal ileum, by 131 days of gestation (60). This is precisely the time that our ewes were killed, and the histology of our control fetuses is entirely consistent with this. However, in saline-treated animals, extensive vacuolation remained in the villi of the ileum at 131 days. Persistence of the AEC has also been reported in fetal sheep with experimental esophageal atresia and was proposed to represent delayed development due to the absence of luminal trophic factors (61). In the experiment we report here, there was no obstacle in any of the treatment groups to the swallowing of amniotic fluid and all of the trophic factors it contains. Furthermore, one of the major trophic factors for the gut is IGF-I, and the AEC was even more pronounced in IGF-I-treated fetuses. Thus we speculate that persistence of the AEC may reflect a functional change, whereby the nutrient-deprived fetus attempts to increase nutrient uptake from amniotic fluid by maintaining the transcellular transport capability of the gut mucosa. The reduction in crypt mitosis in the ileum may be consistent with an adaptive response, with the emphasis on function rather than on growth.

The only growth-promoting effects of IGF-I treatment were seen in the gut, consistent with the mode of action being via swallowed IGF-I. The increased gut growth and increased mitosis in intestinal crypts are consistent with numerous other reports of IGF-I action on the gut (11, 29, 56, 62). Swallowed IGF-I may exert its local effects by acting directly on its receptors expressed on the enterocyte luminal membrane (24) or via paracrine or autocrine effects, with IGF-I acting at IGF type I receptors in the gut to stimulate local release of IGF-I, IGF-II, and IGF-binding proteins (56).

Previous studies of enteral IGF-I administration found changes mainly in the proximal gut (62). Here, we report changes along the whole length of the gut. Indeed, the colon showed the biggest change in weight and also showed substantial thickening of the wall in response to IGF-I treatment. These findings suggest that the less marked effects of IGF-I treatment on growth in the ileum are not due to a decreasing effect of treatment more distally in the bowel (62) or to effects in the distal bowel being secondary to systemic rather than local effects (52). Furthermore, we have data confirming that radiolabeled IGF-I administered into amniotic fluid can be recovered from the contents of the ileum and colon in an intact form (7). Ingested IGF-I may thus be transported along the length of the gut in an intact form and have effects along the whole length of the bowel. Alternatively, there may have been enterohepatic recirculation of swallowed IGF-I, as has been suggested from tracer studies in rats (32).

Embolization decreased fetal plasma levels of IGF-I and insulin in both treatment groups. This was the expected response, because insulin levels in the fetus are regulated by glucose and amino acid concentrations (19), and IGF-I levels are similarly regulated by glucose and insulin (46). However, fetal plasma IGF-I levels recovered during saline treatment and remained low during amniotic IGF-I treatment. This difference was not expected. It cannot be explained by the reduced fetal insulin levels, because they were similar in the two groups. One possible explanation is that gastrointestinal uptake of low doses of IGF-I resulted in reduced systemic levels by suppressing endogenous hepatic or skeletal muscle IGF-I production. Downregulation of hepatic IGF-I expression has been shown to occur after chronic intravenous administration of a high dose of IGF-I (30). Such reduced systemic IGF-I production would be consistent with the observed re-

Fig. 2. Light micrographs of ileum (×200 magnification). A: control; B: saline; C: IGF-I. Solid bar, 100 μm. Arrows point to vacuolated villus enterocytes. There are a few remaining vacuoles at the villus tips in A, as is normal for a fetus of this gestational age. Note the increased vacuolation in B, extending further toward the villus base, and even more marked changes in C. PP, Peyer’s Patch.
duction in spleen and thymus weight in these fetuses (29, 41) and may also account for the reduced liver weight. Alternatively, it is possible that amniotic IGF-I treatment reduced liver size directly, and that the lower systemic IGF-I concentrations were a result rather than a cause of the smaller liver in these fetuses.

Another possible explanation for the reduced circulating IGF-I levels is the suppression (or release) of a gut-mediated regulator of IGF-I production. Ghrelin, an endogenous ligand for the growth hormone secretagogue receptor recently isolated from rat stomach (31), is a potential candidate that we are investigating. This would provide a possible mechanism for the differential regulation of organ growth in the face of a limited nutrient supply. It is thus possible that IGF-I administered into the amniotic fluid in our study has promoted gastrointestinal growth at the expense of growth of other organs. The effects of a higher dose of IGF-I, and of a combination of IGF-I and additional nutrients administered into amniotic fluid, warrant further investigation.

Embolization also reduced IGF-I levels in amniotic fluid. The source and regulation of IGF-I in amniotic fluid are not known. Although most amniotic fluid proteins are of maternal origin (1), fetal urine and fetal tracheal and oronasopharyngeal secretions all contain IGF-I and together account for the majority of daily amniotic fluid production (26). Amniotic fluid IGF-I levels may thus reflect fetal levels, either via routes we have mentioned or via transmembrane fluxes, which provide an unknown contribution to amniotic fluid.

Fig. 3. A: IGF-I levels in amniotic fluid, fetal arterial and maternal arterial plasma; B: insulin levels in fetal and maternal arterial plasma in control (●, n = 9) and saline (▲, n = 9)- and IGF-I (○, n = 11)-treated animals. Solid bar, period of embolization. Values are means ± SE. Levels in portal venous plasma were not significantly different from arterial plasma and are not shown. Amniotic fluid IGF-I levels from lower catheters were not significantly different from those from upper catheters and are not shown. Amniotic fluid IGF-I levels fell with embolization (P < 0.05) and increased with IGF-I treatment (P < 0.01). Fetal plasma IGF-I and insulin levels fell with embolization (P < 0.01). IGF-I, but not insulin, levels recovered in the saline group. Fetal IGF-I levels remained depressed in the IGF-I-treated group (P < 0.05). Maternal insulin levels were unchanged throughout the study and were not different between groups. Maternal plasma IGF-I levels did not change during the embolization period, but by the end of the treatment period, the rise seen in control ewes did not occur in the embolized groups, and levels were significantly lower in both embolized groups at the end of the study period.
However, amniotic fluid IGF-I levels remained low in both embolized groups after embolization was completed, despite recovery of fetal plasma IGF-I levels in the saline-treated animals. This dissociation of fetal plasma and amniotic fluid IGF-I levels raises the possibility that amniotic fluid IGF-I arises from an extrafetal source, either the placenta or the mother. There are data suggesting that the placenta can regulate fetal plasma IGF-I levels (28), and such regulation may be disturbed by placental embolization. It is not known whether the placenta also contributes to circulating maternal IGF-I levels in pregnancy, or whether it plays a role in regulating those levels. However, placental damage caused by embolization may explain the fall in maternal IGF-I plasma levels seen toward the end of the experiment by reducing placental production either of IGF-I or of the placental variant growth hormone that may regulate maternal IGF-I levels in pregnancy (12). Reduced maternal IGF-I levels have been reported in human studies of IUGR because of placental dysfunction (27), and animal data also suggest that maternal IGF-I levels influence fetal and placental growth (21), even though IGF-I does not cross the placenta.

Embolization reduced both total placental weight and the weight of individual placentomes. IGF-I treatment did not alter total placental weight but tended to decrease the number of very small placentomes, although this was not statistically significant. However, IGF-I treatment did alter placentome morphology, with fewer types A and B and more types C and D placentomes in IGF-I-treated animals. Types C and D placentomes have been shown to have a greater proportion of fetal to maternal tissue (63), and a shift from type A to types C and D has been reported to occur in undernutrition, high-altitude hypoxia, and maternal dexamethasone administration in early gestation (37, 47). Because there were no differences in metabolite or oxygen levels between the two embolized groups, the difference between groups is not due to more severe hypoxia or nutrient deprivation in the IGF-I group. However, it is possible that IGF-I treatment augmented the response of placental tissues to the reduced nutrient supply caused by embolization, resulting in relative overgrowth of fetal tissues. The mechanism whereby ovine fetal placental tissues grow and differentiate in late gestation is not known, but in vitro studies of human placental tissue have demonstrated IGF-I-stimulated trophoblast proliferation and differentiation (43). IGF type I receptors (IGFIR) are present in the sheep placenta from early in gestation (51) and increase in number with increasing gestational age (35). The numbers of placental IGFIR are reported to increase in human pregnancies complicated by IUGR, possibly as an adaptive mechanism (2). Previous studies of the effects of 10-day high-dose intravenous IGF-I administration to fetal sheep also demonstrated an effect on placental growth, with reduction in the number of placentomes of small size (41).

Onset of growth restriction has previously been shown to be prevented by administration of low-dose enteral IGF-I (29) or of intravenous nutrients to the fetus (13), beginning at the same time as the growth-restricting insult. Our finding that some of the effects of established IUGR may be reversed with only 10 days of intra-amniotic IGF-I therapy suggests that a longer duration of IGF-I therapy may result in overall fetal growth.

Furthermore, because the greatest effects of amniotic IGF-I supplementation were on the fetal gut, coadministration of both IGF-I and nutrients into amniotic fluid may result in improved fetal nutrient supply by the enteral route and further improve fetal growth. However, addition of nutrients to a hypoxic fetus may simply result in downregulation of umbilical uptake by the fetus (14) or in an obligatory increase in oxidative metabolism with adverse effects on fetal oxygenation. These possibilities will require further investigation, perhaps utilizing different paradigms of IUGR as well as further exploration of the optimal dose and duration of treatment.

The experimental paradigm in this study resulted in asymmetrical IUGR with fetal metabolic changes similar to those seen in IUGR human fetuses. We have demonstrated for the first time that some of the established effects of IUGR may be reversed in utero. The effects of IGF-I treatment on the gut may be of clinical importance in view of the risk of gut-associated morbidity, some of which may be permanent (39), in IUGR infants. However, the suppression of circulating fetal IGF-I levels and the reduction in growth of some organs are of concern and require further investigation. We found that injected IGF-I was rapidly mixed throughout amniotic fluid and that IGF-I levels fivefold baseline were maintained for \( \approx 24 \) h postinjection (7). Our studies of radiolabeled IGF-I injected into amniotic fluid also suggest that the interval between doses could be extended to several days (7), making this a more clinically feasible approach. Together, these findings suggest that intra-amniotic supplementation of IGF-I may form the basis of possible future therapeutic strategies in human IUGR.

We acknowledge the technical expertise of Toni Smith-Wong, Christine Keven, and Janine Street.

This work was supported by the Health Research Council of New Zealand, the New Zealand Lottery Grants Board, and the Fisher and Paykel Trust.

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