Early treatment of the pregnant guinea pig with IGFs promotes placental transport and nutrient partitioning near term

Amanda N. Sferruzzi-Perri, Julie A. Owens, Prue Standen, Robyn L. Taylor, Gary K. Heinemann, Jeffrey S. Robinson, and Claire T. Roberts

Research Centre for Reproductive Health, Discipline of Obstetrics and Gynaecology, School of Paediatrics and Reproductive Health, University of Adelaide, Adelaide, South Australia, Australia

Submitted 2 July 2006; accepted in final form 8 October 2006

Sferruzzi-Perri AN, Owens JA, Standen P, Taylor RL, Heinemann GK, Robinson JS, Roberts CT. Early treatment of the pregnant guinea pig with IGFs promotes placental transport and nutrient partitioning near term. Am J Physiol Endocrinol Metab 292: E668–E676, 2007. First published October 24, 2006; doi:10.1152/ajpendo.00320.2006.—Appropriate partitioning of nutrients between the mother and conceptus is a major determinant of pregnancy success, with placental transfer playing a key role. Insulin-like growth factors (IGFs) increase in the maternal circulation during early pregnancy and are predictive of fetal and placental growth. We have previously shown in the guinea pig that increasing maternal IGF abundance in early to midpregnancy enhances fetal growth and viability near term. We now show that this treatment promotes placental transport to the fetus, fetal substrate utilization, and nutrient partitioning near term. Pregnant guinea pigs were infused with IGF-I, IGF-II (both 1 mg kg⁻¹ day⁻¹) or vehicle subcutaneously from days 20–38 of pregnancy (term = 69 days). Tissue uptake and placental transfer of the nonmetabolizable radio analogs [³H]methyl-D-glucose (MG) and [¹⁴C]aminoisobutyric acid (AIB) in vivo was measured on day 62. Early pregnancy exposure to elevated maternal IGF-I increased placental MG uptake by >70% (P = 0.004), whereas each IGF increased fetal plasma MG concentrations by 40–50% (P < 0.012). Both IGFs increased fetal tissue MG uptake (P < 0.048), whereas IGF-I also increased AIB uptake by visceral organs (P = 0.046). In the mother, earlier exposure to either IGF increased AIB uptake by visceral organs (P < 0.014), whereas IGF-I also enhanced uptake of AIB by muscle (P = 0.044) and MG uptake by visceral organs (P = 0.016) and muscle (P = 0.046). In conclusion, exogenous maternal IGFs in early pregnancy sufficiently increase maternal substrate utilization, placental transport of MG to the fetus, and fetal utilization of substrates near term. This was consistent with the previously observed increase in fetal growth and survival following IGF treatment.

insulin-like growth factor; fetal growth; glucose transport; system A amino acid transport

The relationship between maternal intake and utilization of substrates during pregnancy and their supply to the conceptus determines pregnancy success and life-long health of offspring (63). Adequate nutrients must be delivered to the growing placenta and fetus, as well as to the mother, to meet the energy requirements needed to maintain maternal health and her capacity to support the conceptus. To ensure the latter during normal pregnancy, the mother undergoes a number of physiological changes in her appetite, body composition, cardiovascular function, energy consumption, and metabolism (61). The placenta is central to these processes, because it is not only the organ responsible for the exchange of substrates between the mother and fetus but also synthesizes a number of steroid and peptide hormones, secreted into the maternal circulation, that modulate maternal physiology and adaptation to pregnancy.

Impaired supply of nutrients to the fetus causes intrauterine growth restriction (IUGR) (14, 15, 23), which currently affects 6% of pregnancies in developed countries and up to 40% in developing countries (1). IUGR is associated with perinatal morbidity and mortality (28, 54) and increases the risk of poor health in childhood and adult life (4). Understanding the factors essential for regulating nutrient partitioning during pregnancy may help to identify the causes of IUGR and possibly the development of novel therapeutics.

The insulin-like growth factors (IGFs) are implicated as major factors influencing nutrient partitioning between the mother and fetus. Substantial pregnancy-associated changes in maternal circulating IGF-I and IGF-II occur in several species (21, 30, 41, 59, 71, 74, 80), with IGF-II in particular also highly expressed in the placenta (36). Although maternal IGFs do not cross the placenta in physiologically significant quantities (10), they may act on the placenta and maternal tissues to regulate nutrient allocation between the mother, placenta, and fetus in various ways (78).

Maternal IGF-I may improve or maintain nutrient transfer to the fetus by enhancing placental transport and modification of nutrients or by increasing substrate availability in the mother for transfer to the fetus, as shown in the pregnant ewe (38, 53). This also may be the case in women, given that IGF-I stimulates lactate and amino acid uptake in cultured human placental trophoblasts (8, 49, 51, 82). Furthermore, IGF-I inhibits the release of vasoconstrictors in term human placental explants (69), which may increase placental blood flow and delivery of nutrients during pregnancy. However, there also is evidence to suggest that IGF-I may promote maternal anabolism over fetal growth. IGF-I administration in the second half of pregnancy increased maternal weight gain near term but did not alter fetal and placental growth in rats (31). In addition, elevated maternal circulating IGF-I induced by overnourishing singleton-bearing adolescent sheep correlated with increased maternal tissue accretion at the expense of the fetus and placenta (77, 78).

IGF-II also may act locally to modulate placental development and transport function, as occurs in mice. Indeed, placental amino acid transporter expression is altered by Igf2 deficiency in mice (58), and ablation of the trophoblast-specific peptide hormones, secreted into the maternal circulation, that modulate maternal physiology and adaptation to pregnancy.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The relationship between maternal intake and utilization of substrates during pregnancy and their supply to the conceptus determines pregnancy success and life-long health of offspring (63). Adequate nutrients must be delivered to the growing placenta and fetus, as well as to the mother, to meet the energy requirements needed to maintain maternal health and her capacity to support the conceptus. To ensure the latter during normal pregnancy, the mother undergoes a number of physiological changes in her appetite, body composition, cardiovascular function, energy consumption, and metabolism (61). The placenta is central to these processes, because it is not only the organ responsible for the exchange of substrates between the mother and fetus but also synthesizes a number of steroid and peptide hormones, secreted into the maternal circulation, that modulate maternal physiology and adaptation to pregnancy.

Impaired supply of nutrients to the fetus causes intrauterine growth restriction (IUGR) (14, 15, 23), which currently affects 6% of pregnancies in developed countries and up to 40% in developing countries (1). IUGR is associated with perinatal morbidity and mortality (28, 54) and increases the risk of poor health in childhood and adult life (4). Understanding the factors essential for regulating nutrient partitioning during pregnancy may help to identify the causes of IUGR and possibly the development of novel therapeutics.

The insulin-like growth factors (IGFs) are implicated as major factors influencing nutrient partitioning between the mother and fetus. Substantial pregnancy-associated changes in maternal circulating IGF-I and IGF-II occur in several species (21, 30, 41, 59, 71, 74, 80), with IGF-II in particular also highly expressed in the placenta (36). Although maternal IGFs do not cross the placenta in physiologically significant quantities (10), they may act on the placenta and maternal tissues to regulate nutrient allocation between the mother, placenta, and fetus in various ways (78).

Maternal IGF-I may improve or maintain nutrient transfer to the fetus by enhancing placental transport and modification of nutrients or by increasing substrate availability in the mother for transfer to the fetus, as shown in the pregnant ewe (38, 53). This also may be the case in women, given that IGF-I stimulates lactate and amino acid uptake in cultured human placental trophoblasts (8, 49, 51, 82). Furthermore, IGF-I inhibits the release of vasoconstrictors in term human placental explants (69), which may increase placental blood flow and delivery of nutrients during pregnancy. However, there also is evidence to suggest that IGF-I may promote maternal anabolism over fetal growth. IGF-I administration in the second half of pregnancy increased maternal weight gain near term but did not alter fetal and placental growth in rats (31). In addition, elevated maternal circulating IGF-I induced by overnourishing singleton-bearing adolescent sheep correlated with increased maternal tissue accretion at the expense of the fetus and placenta (77, 78).

IGF-II also may act locally to modulate placental development and transport function, as occurs in mice. Indeed, placental amino acid transporter expression is altered by Igf2 deficiency in mice (58), and ablation of the trophoblast-specific
Igf2 promoter (P0) reduces placental weight and adversely affects placental structural differentiation and transport capacity (17, 68). In the P0 mutant placenta, passive diffusion of inert hydrophilic solutes is reduced, whereas system A amino acid (17, 68) and glucose (16) transport is upregulated, apparently via direct impacts on placental transporter gene expression (16).

In guinea pigs and humans, maternal circulating concentrations of IGFs are substantial (30, 71) and may have major influences on nutrient partitioning during pregnancy. Indeed, we have shown in the guinea pig that increasing concentrations of IGF by infusion of IGF-I or IGF-II into the mother during early to midpregnancy enhanced fetal growth (largely due to increased muscle mass) and survival near term, but possibly in part via different mechanisms (67). In the IGF-I-treated mother, the nutritional requirements of the conceptus appeared to take priority over those of the mother, because maternal adipose stores were depleted in late pregnancy (without altering placental development), possibly increasing substrate availability for the conceptus. In contrast, IGF-II treatment in early to midpregnancy did not affect maternal body composition but enhanced development of the placental exchange region in late gestation, suggestive of improved placental functional capacity (67). Both IGFs increased fetal circulating amino acid concentrations near term, suggestive of a sustained improvement in nutrient transfer to the fetus (67). In addition, IGF supplementation increased concentrations of steroid hormones in the maternal circulation, which may have modified maternal physiological responses to pregnancy and, in turn, nutrient partitioning (67).

To dissect the mechanisms underlying maternal IGF influences in early to midpregnancy on substrate availability to, and hence, growth of, the fetus near term, we measured the consequences for placental uptake and transfer of nonmetabolizable radio analogs of glucose ([1-14C]methyl-D-glucose; MG) and amino acids ([1-13C]l-aminoisobutyric acid; AIB) in late gestation. In light of our group’s previous report (67) of differences in early to midpregnancy on substrate availability for the conceptus, we also have assessed MG and AIB uptake by several fetal and maternal tissues. In our group’s previous report (67) of differences in early to midpregnancy on substrate availability for the conceptus, we also have assessed MG and AIB uptake by several fetal and maternal tissues. In the present study we demonstrate that maternal IGF treatment during early to midpregnancy increases placental transfer from mother to fetus, as well as fetal utilization of MG, enhancing fetal growth near term, and in addition has sustained effects on maternal tissue glucose and amino acid uptake.

**METHODS**

**Animals.** This study was approved by the University of Adelaide Animal Ethics Committee. Pregnant female guinea pigs (IMVS colored strain, ~500 g, 3–4 mo old) were housed individually in the University of Adelaide Medical School Animal House (12:12-h light-dark cycle) and provided with food and water ad libitum. Females were assigned to form three groups of similar mean weight at mating. On day 20 of pregnancy (day 1 was the day a copulatory plug was observed or the day after females were paired with a male), females were anesthetized with atropine sulfate (0.05 mg/kg sc; Apex Laboratories, Somersby, New South Wales, Australia), xylazine hydrochloride (4 mg/kg im; Troy Laboratories, Smithfield, New South Wales, Australia), and ketamine hydrochloride (25 mg/kg ip; Troy Laboratories) and administered local analgesia with lidocaine hydrochloride (Troy Laboratories). A 200-μl mini osmotic pump (Alzet 2002; Alzet, Cupertino, CA) was surgically inserted beneath the skin on the back. Minipumps had previously been prepared to deliver vehicle (0.1 M acetic acid; n = 8) or 1 mg·kg−1·day−1 of IGF-I (n = 9) or IGF-II (n = 6; human recombinant protein; GroPep, Thebarton, South Australia, Australia) for 18 days at a flow rate of 0.51 μl/h. This treatment was shown to increase the concentration of IGF-I and IGF-II in the maternal circulation at midpregnancy 3.4- and 2.4-fold, respectively (67).

On day 60 of pregnancy, mothers were anesthetized according to their weight on day 20 of pregnancy, as described above, but with larger doses of xylazine hydrochloride (6 mg/kg im; Troy Laboratories) and ketamine hydrochloride (75 mg/kg ip; Troy Laboratories). Vascular catheters were inserted into the common carotid artery and external jugular vein and exteriorized through the skin on the back of the neck, and animals were allowed to recover.

On day 62 of pregnancy (term ~70 days) following an overnight fast, guinea pigs were administered a mixture of MG and AIB (both from Amersham, Amersham, UK) in physiological saline in a single bolus. To each dam, we aimed to treat with 100 μCi/kg of MG and 10 μCi/kg of AIB, but the precise dose actually administered was determined by weighing syringes containing each isotopic mixture before and after the completion of the isotopic mixture. The total administered volume of the isotopic mixture was 500 μl for each animal. Maternal blood was collected in heparinized tubes 20 min after radioactive analog administration, and animals were then killed by overdose of pentobarbitral sodium (Lethobarb; Virbac, Peakhurst, New South Wales, Australia). In animals with patent catheters, blood was sampled (1 ml) every 2 min following radioactive analog administration, with the catheter flushed with 1 ml of heparinized saline at each time point. Viable and resorbing implantation sites were counted, and the uterus and its contents were weighed. Fetal blood samples were collected by cardiac puncture into heparinized tubes immediately following dissection from the uterus. Fetal and maternal blood was centrifuged at 2,500 rpm for 15 min at 4°C to recover plasma, which was stored at −20°C. Maternal and fetal kidneys, liver, spleen, heart, brain, lungs, triceps muscle, gastrocnemius muscle, and retroperitoneal, perirenal, and interscapular adipose tissues were snap-frozen in liquid nitrogen for determination of MG and AIB content.

**MG and AIB content in plasma and tissues.** To determine the disintegrations per minute (dpm) of 3H and 14C by dual-isotope β-scintillation counting, we prepared quenched standards from tritiated water (H218O) and [14C]AIB, respectively. Ten quench standards were prepared using 1 ml of MQ water, 30 μl of glacial acetic acid, 14 ml of aqueous scintillation fluid (Beckman, Palo Alto, CA), 50 μl of 3H2O or [14C]AIB (55,000 dpm), and varying volumes (0, 10, 20, 40, 60, 80, 100, and 120 μl) of carbon tetrachloride in 20-ml plastic scintillation vials. These were shaken, covered with foil, and counted the following day after having been shaken once more by dual-isotope β-scintillation counting (LS 6500 Beckman) at 0–400 and 400–670 MeV for 3H and 14C, respectively.

For determination of plasma MG and AIB concentrations, 50 μl of plasma were deproteinized with 100 μl of 0.3 N Ba(OH)2 (Sigma Diagnostics, St. Louis, MO) and 100 μl of 0.3 N ZnSO4 (Sigma Diagnostics) at 4°C. After centrifugation at 4,000 rpm at 4°C for 15 min, 80 μl of deproteinized supernatant were mixed with 0.3 ml of water, 30 μl of glacial acetic acid, and 14 ml of scintillant (Ready Safe; Beckman Coulter, San Diego, CA) before counting to measure 3H and 14C by dual-isotope β-scintillation counting (LS 6500; Beckman Coulter) with quenched standards to indicate MG and AIB content, respectively.

For determination of fetal and maternal tissue MG and AIB concentrations, tissues (~100 mg) were solubilized with 0.7 ml of 1 M NaOH for 85 min at 65°C and then mixed with 2.1 ml of 6% perchloric acid to precipitate protein for 20 min at 4°C. After centrifugation at 4,000 rpm at 4°C for 15 min, 1 ml of deproteinized supernatant was mixed with 30 μl of glacial acetic acid and 14 ml of scintillant (Ready Safe; Beckman Coulter), and dual-isotope β-scint-
tillation counting (LS 6500; Beckman Coulter) was performed with quenched standards (as described above). The background count was then subtracted from the samples, before the sample count per gram or milliliter or total was adjusted to the same dose of isotope administered to the mother. Disintegrations per minute are represented per milliliter for plasma samples; for tissues, they are represented per gram or as total tissue uptake by multiplying the count per gram by the weight of the tissue. Perirenal, retroperitoneal, and interscapular fat counts were combined to provide a measure of muscle uptake; gastrocnemius and triceps counts were combined to provide a measure of muscle uptake; heart, spleen, kidney, liver, and lung counts were combined to provide a measure of visceral tissue uptake; and all tissues assessed for analog studies were combined to represent total tissue uptake. To estimate the transfer capacity of the placenta, we divided the total fetal tissue counts by placental weight.

Statistics. All data were analyzed using SPSS (version 13; SPSS, Chicago, IL). To determine the effect of IGF treatment in early pregnancy on maternal parameters, we performed general linear model univariate ANOVA with Bonferroni post hoc tests. To assess the impact of IGF treatment on placental and fetal parameters, we performed linear mixed model repeated-measures ANOVA with Sidak post hoc tests, using the mother as the subject and the fetus or placenta as the repeated measure. The number of viable pups per litter was used as a covariate when required. Using Pearson’s two-tailed bivariate correlation analyses, we analyzed associations between MG and AIB uptake and placental structure and maternal circulating hormone concentrations (as previously reported in Ref. 67). Data are expressed as means ± SE or estimated marginal means ± SE, as required. Data were considered statistically significant when \( P < 0.05 \).

RESULTS

**Effect of exogenous maternal IGF treatment on litter composition and fetal and placental weights.** Litters from mothers that had been treated with IGF-I during early to midpregnancy had a greater number of viable fetuses (means ± SE: vehicle, 2.5 ± 0.2; IGF-I, 3.3 ± 0.1; IGF-II, 3.0 ± 0.2; \( P = 0.001 \)) and fewer resorptions (means ± SE: vehicle, 0.61 ± 0.1; IGF-I, 0.10 ± 0.01; IGF-II, 0.25 ± 0.1; \( P = 0.007 \)) compared with vehicle-treated mothers near term (number of dams and total viable fetuses per group are as follows: vehicle, 8 and 20; IGF-I, 9 and 30; and IGF-II, 6 and 18, respectively). However, total litter size was unaffected by earlier maternal IGF treatment (means ± SE: vehicle, 3.1 ± 0.1; IGF-I, 3.4 ± 0.1; IGF-II, 3.3 ± 0.1). Maternal IGF-I treatment increased fetal weight (g) near term (means ± SE: vehicle, 69.7 ± 2.4; IGF-I, 76.8 ± 1.9; IGF-II, 71.1 ± 2.6; \( P = 0.05 \)); however, there was no effect of treatment on placental weight (data not shown).

**Exogenous maternal IGF-I treatment increases uptake of MG by the placenta.** Treating the mother with IGF-I during early to midpregnancy increased placental MG uptake compared with vehicle-treated mothers near term in per gram and total placenta terms (\(+78\%\), \( P = 0.001 \), and \(+70\%\), \( P = 0.004 \), respectively) (Fig. 1A). Placentas from mothers treated with IGF-I had higher MG uptake per gram of placenta compared with those treated with IGF-II (\( P = 0.019 \)). Earlier maternal IGF treatment did not alter placental uptake of AIB on a per gram of tissue or whole tissue basis (Fig. 1B).

**Exogenous maternal IGF treatment increases fetal plasma MG concentrations but does not alter plasma AIB.** Maternal IGF-I and IGF-II treatment during early to midpregnancy increased the concentration of fetal plasma MG near term (+50%, \( P = 0.002 \), and 41%, \( P = 0.012 \), respectively) but not that of AIB (Fig. 2A). Across all treatments, fetal plasma MG was positively correlated with fetal weight (\( r = 0.29 \), \( P = 0.031 \)) and crown-rump length (\( r = 0.28 \), \( P = 0.036 \)). Across all treatments, placental MG uptake per gram of tissue and total uptake were positively correlated with fetal plasma MG concentrations (\( r = 0.92 \), \( P < 0.001 \), and \( r = 0.91 \), \( P < 0.001 \), respectively).

**Exogenous maternal IGF treatment increases fetal tissue MG uptake.** Early-to-midpregnancy maternal IGF-I increased MG uptake (dpm/g) by fetal spleen (+42%, \( P = 0.05 \), perirenal fat (+49%, \( P = 0.043 \)), lung (+63%, \( P = 0.037 \)), liver (+36%, \( P = 0.037 \)), and kidney (+51%, \( P = 0.001 \)) and increased total MG uptake (dpm) by retroperitoneal fat (+74%, \( P = 0.002 \)), perirenal fat (+85%, \( P = 0.003 \)), liver (+84%, \( P = 0.013 \)), kidney (+81%, \( P < 0.001 \), heart (+88%, \( P = 0.043 \), gastrocnemius (+99%, \( P = 0.015 \)), and triceps (+77%, \( P = 0.036 \)) compared with vehicle near term (Table 1). Early-to-midpregnancy maternal IGF-II increased MG uptake (dpm/g) by fetal lung (+50%, \( P = 0.043 \)) and liver (+34%, \( P = 0.037 \)) and increased total MG uptake (dpm) by lung (+52%, \( P = 0.016 \), liver (+43%, \( P = 0.05 \)), gastrocnemius (+49%, \( P = 0.049 \)), and triceps (+44%, \( P = 0.05 \)) compared with vehicle near term (Table 1). MG uptake by the fetal kidney in total (dpm) and relative terms (dpm/g) was 23 and 39% greater with maternal IGF-I treatment compared with IGF-II, respectively (\( P = 0.049 \) and \( P = 0.018 \), respectively). Total MG uptake by the fetal retroperitoneal fat was 42% greater following maternal IGF-I treat-
with fetal plasma and tissue MG counts (data not shown). Placental MG transfer capacity (total fetal tissue counts divided by placental weight) was increased by earlier maternal IGF-I (+50%, P = 0.029) or IGF-II (+28%, P = 0.033) treatments (means ± SE: vehicle, 349 ± 43; IGF-I, 506 ± 39; IGF-II, 446 ± 36).

Exogenous maternal IGF-I treatment increases fetal tissue AIB uptake. Maternal IGF-I treatment during early to midpregnancy increased total AIB uptake (dpm) by the fetal heart by 82% (means ± SE: vehicle, 7.8 ± 2.4; IGF-I, 14.3 ± 1.7; IGF-II, 9.3 ± 1.6; P = 0.038). There was no effect of either maternal IGF treatment on AIB uptake by other fetal tissues (on a per gram or total tissue basis); however, uptake of AIB per gram of tissue was greatest in the fetal liver and lowest in the retroperitoneal fat of vehicle and IGF-II-treated mothers and lowest in the interscapular fat of IGF-I-treated mothers (data not shown). Maternal IGF-I treatment increased AIB uptake (dpm/g) by combined fetal visceral tissues by 41% (means ± SE: vehicle, 61 ± 13; IGF-I, 135 ± 12; IGF-II, 101 ± 11; P = 0.05) and fetal muscle AIB by 59% compared to baseline.

Table 1. Effect of maternal IGF treatment in early to midpregnancy on fetal tissue methyl glucose uptake near term

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>IGF-I</th>
<th>IGF-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of mothers</td>
<td>6</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Number of fetuses</td>
<td>17</td>
<td>18</td>
<td>16</td>
</tr>
<tr>
<td>Methyl glucose uptake</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain (dpm/g)</td>
<td>149.6 ± 20.2</td>
<td>190.7 ± 18.0</td>
<td>179.2 ± 17.1</td>
</tr>
<tr>
<td>(total dpm)</td>
<td>368.9 ± 49.1</td>
<td>495.5 ± 45.4</td>
<td>462.0 ± 41.6</td>
</tr>
<tr>
<td>Heart (dpm/g)</td>
<td>146.9 ± 29.5</td>
<td>219.7 ± 25</td>
<td>176.0 ± 23.6</td>
</tr>
<tr>
<td>(total dpm)</td>
<td>616.6 ± 16.3*</td>
<td>116.1 ± 13.†</td>
<td>83.9 ± 13.1†</td>
</tr>
<tr>
<td>Liver (dpm/g)</td>
<td>153.6 ± 16.9*</td>
<td>209.3 ± 16.9†</td>
<td>205.8 ± 14.4†</td>
</tr>
<tr>
<td>(total dpm)</td>
<td>558.8 ± 81.8*</td>
<td>832.9 ± 81.4†</td>
<td>798.5 ± 69.5†</td>
</tr>
<tr>
<td>Lungs (dpm/g)</td>
<td>117.7 ± 21.5*</td>
<td>192.0 ± 19.8†</td>
<td>177.0 ± 18.5†</td>
</tr>
<tr>
<td>(total dpm)</td>
<td>158.9 ± 32.7*</td>
<td>293.0 ± 30.3†</td>
<td>241.3 ± 28.2†</td>
</tr>
<tr>
<td>Kidneys (dpm/g)</td>
<td>187.1 ± 17.0*</td>
<td>282.2 ± 15.7†</td>
<td>229.2 ± 14.4†</td>
</tr>
<tr>
<td>(total dpm)</td>
<td>101.8 ± 14.3*</td>
<td>183.8 ± 13.2†</td>
<td>132.1 ± 12.1†</td>
</tr>
<tr>
<td>Spleen (dpm/g)</td>
<td>115.3 ± 15.0*</td>
<td>163.6 ± 13.4†</td>
<td>147.2 ± 13.1†</td>
</tr>
<tr>
<td>(total dpm)</td>
<td>18.3 ± 3.4</td>
<td>18.5 ± 3.1</td>
<td>15.3 ± 3.0</td>
</tr>
<tr>
<td>Triceps muscle (dpm/g)</td>
<td>71.5 ± 11.2</td>
<td>101.6 ± 11.6</td>
<td>97.6 ± 9.7</td>
</tr>
<tr>
<td>(total dpm)</td>
<td>12.8 ± 2.7*</td>
<td>22.7 ± 2.8†</td>
<td>18.4 ± 2.4†</td>
</tr>
<tr>
<td>Gastrocnemius muscle (dpm/g)</td>
<td>100.9 ± 17.0</td>
<td>154.2 ± 14.9</td>
<td>127.8 ± 13.9</td>
</tr>
<tr>
<td>(total dpm)</td>
<td>12.3 ± 3.1*</td>
<td>24.5 ± 2.7†</td>
<td>18.8 ± 2.5†</td>
</tr>
<tr>
<td>Interscapular fat (dpm/g)</td>
<td>94.0 ± 22.7</td>
<td>77.6 ± 22.5</td>
<td>66.4 ± 19.2</td>
</tr>
<tr>
<td>(total dpm)</td>
<td>84.4 ± 18.6</td>
<td>79.8 ± 18.5</td>
<td>68.9 ± 15.8</td>
</tr>
<tr>
<td>Perirenal fat (dpm/g)</td>
<td>65.2 ± 10.1†</td>
<td>97.2 ± 9.0†</td>
<td>71.2 ± 8.5†</td>
</tr>
<tr>
<td>(total dpm)</td>
<td>44.6 ± 8.9*</td>
<td>86.1 ± 7.9†</td>
<td>57.5 ± 7.5†</td>
</tr>
<tr>
<td>Retroperitoneal fat (dpm/g)</td>
<td>54.8 ± 7.8</td>
<td>75.6 ± 7.2</td>
<td>62.0 ± 6.7</td>
</tr>
<tr>
<td>(total dpm)</td>
<td>35.1 ± 5.2*</td>
<td>61.4 ± 4.8†</td>
<td>42.9 ± 4.5†</td>
</tr>
</tbody>
</table>

Disintegrations per minute (dpm) were adjusted for background and the treatment administered to the mother. Data are expressed as estimated marginal means ± SE adjusted for the number of viable fetuses per litter. *†Different symbols denote significant difference between groups (P < 0.05).
Exogenous maternal IGF treatment increases uptake of AIB in the mother. Maternal IGF-II increased spleen AIB uptake (dpm/g) by 102% (means ± SE: vehicle, 94.9 ± 23; IGF-I, 109 ± 21; IGF-II, 188 ± 23; P = 0.039). Maternal IGF-I and IGF-II increased AIB uptake (dpm/g) by maternal visceral tissue by 54 and 83%, respectively (means ± SE: vehicle, 670 ± 91; IGF-I, 1,031 ± 81; IGF-II, 1,225 ± 84; P = 0.014 and P = 0.002, respectively), and combined internal organs and tissues by 52 and 80%, respectively (means ± SE: vehicle, 736 ± 96; IGF-I, 1,118 ± 92; IGF-II, 1,319 ± 91; P = 0.007 and P = 0.002, respectively). Maternal IGF-I increased total AIB uptake by maternal muscle (+82%, P = 0.044), and both IGF-I and IGF-II treatment of the mother increased total AIB uptake by visceral tissues (both +71% and P = 0.048) and combined internal organs and tissues (both +70% and P = 0.044) (Fig. 4B). Across all treatments, the uptake of AIB per gram of tissue was greatest in the maternal liver and lowest in the brain and interscapular fat of vehicle-treated mothers and lowest in the brain in IGF-treated animals (data not shown).

 Associations of MG uptake with placental morphology. To determine the association of placental and fetal MG content with previously determined structural correlates of placental function near term (67), we determined correlations between these parameters. Overall, MG uptake by placenta (dpm/g) was correlated positively with the placental total surface area for exchange (r = 0.39, P = 0.039). Total placental MG uptake correlated positively with volume for placental exchange (r = 0.37, P = 0.019), the placental total surface area for exchange (r = 0.47, P = 0.001), and volume of maternal blood spaces within the labyrinth (r = 0.32, P = 0.035). Overall, fetal

with IGF-II (means ± SE: vehicle, 38 ± 5.9; IGF-I, 51 ± 5.6; IGF-II, 32 ± 5.3; P = 0.05). Maternal IGF-I treatment compared with vehicle and IGF-II increased total AIB uptake into fetal visceral tissues (+43%, P = 0.046, and +40%, P = 0.047, respectively) (Fig. 2C). Total AIB uptake by fetal muscle was 73% greater following maternal IGF-I treatment compared with IGF-II (P = 0.029) (Fig. 2C). Overall, there was no effect of maternal IGF treatment on AIB placental transfer capacity (data not shown).

Exogenous maternal IGF-I treatment delays the clearance of MG from the maternal circulation in late pregnancy. To determine whether early-to-midpregnancy exogenous IGF treatment of the mother altered the clearance of MG or AIB from the maternal circulation in late pregnancy, following a bolus injection, we sampled maternal blood every 2 min for 20 min. Maternal IGF-I treatment increased maternal plasma MG at 4 (+87%, P = 0.07), 6 (+61%, P = 0.07), 8 (+67%, P = 0.068), 10 (+55%, P = 0.048), 12 (+81%, P = 0.02), 14 (+83%, P = 0.03), and 18 min (+63%, P = 0.07) after analog administration (Fig. 3). Maternal IGF-II did not alter the clearance of MG. There was no effect of treatment with either IGF on AIB clearance from maternal plasma (data not shown).

Exogenous maternal IGF-I treatment increases uptake of MG in the mother. Maternal IGF-I increased MG uptake (dpm/g) by maternal muscle by 78% (means ± SE: vehicle, 221 ± 68; IGF-I, 394 ± 61; IGF-II, 289 ± 63; P = 0.058), visceral tissue by 97% (means ± SE: vehicle, 975 ± 222; IGF-I, 1,921 ± 200; IGF-II, 1,609 ± 207; P = 0.014), and combined internal organs and tissues by 101% (means ± SE: vehicle, 1,454 ± 351; IGF-I, 2,935 ± 339; IGF-II, 2,371 ± 332; P = 0.007). Exogenous maternal IGF-I increased the total MG uptake into maternal muscle (+82%, P = 0.046), visceral tissue (+89%, P = 0.016), and all tissues combined (+102%, P = 0.019) (Fig. 4A). There was no effect of maternal IGF treatment on MG uptake by individual maternal organs or tissues, and overall, the uptake of MG per gram of tissue was greatest in the maternal kidney and lowest in the interscapular fat (data not shown).

Fig. 3. Effect of exogenous maternal IGFs in early to midpregnancy on the clearance of MG from the maternal circulation near term. Maternal blood was sampled every 2 min for 20 min following bolus injection of MG. Data are from 4 to 6 mothers per treatment, and dpm were adjusted for background and the treatment administered to the mother. Values are expressed as mean dpm/ml (±SE). *P < 0.048, statistically significant difference compared with the vehicle group.

Fig. 4. Effect of exogenous maternal IGFs in early to midpregnancy on total maternal tissue uptake of MG (A) and AIB (B) near term. Data are from 6 to 7 mothers per treatment, and dpm were adjusted for background and the treatment administered to the mother. Values are expressed as mean total dpm (±SE). *P < 0.048, statistically significant difference compared with the vehicle group.
plasma and tissue MG values correlated positively with volume for placental exchange ($r = 0.35$, $P = 0.027$ and $r = 0.38$, $P = 0.025$, respectively) and volume of maternal blood spaces ($r = 0.47$, $P = 0.003$ and $r = 0.40$, $P = 0.023$, respectively).

**Associations of MG uptake with maternal hormones.** Treating the mother with IGF during early to midpregnancy altered the associations of placental and fetal MG content near term with previously determined maternal circulating estradiol concentrations (67). However, maternal progesterone concentrations did not correlate with any parameter analyzed (data not shown). Maternal plasma estradiol concentration of vehicle-treated mothers negatively correlated with transfer of MG by maternal tissues ($r = -0.96$, $P = 0.003$), but in IGF-I-treated mothers there was no correlation, and following IGF-II treatment, these parameters were positively correlated ($r = 0.84$, $P = 0.036$).

**DISCUSSION**

In the present study we have demonstrated for the first time that treating the mother with either IGF during early to midpregnancy has a sustained effect on placental transport of nutrients to the fetus and influences nutrient partitioning, and likely metabolism, in the mother and conceptus near term. This finding extends, and provides new insight into, our previous observations that increasing the abundance of IGF-I and IGF-II in the maternal circulation during early to midpregnancy enhanced fetal growth and survival near term (67).

In the current study, early-to-midpregnancy maternal IGF treatment increased placental uptake and transfer of MG to the fetus in late pregnancy. Because glucose is the primary source of energy for the fetus and the placenta (60), enhanced placental transfer of glucose would directly increase fetal energy supplies to promote growth and survival. This was certainly the case for maternal IGF-I treatment in the current study and for both maternal IGF treatments in the previous, larger study (67), where fetal growth and survival were increased near term.

In mothers treated with IGF-II in particular, in early to midpregnancy, enhanced placental transfer and uptake of MG by the fetus near term was demonstrated but may be secondary to sustained effects on placental structural development. IGF-II treatment in early to midpregnancy increased the cross-sectional area, proportion, and volume of the placental exchange region, as well as the total surface area of placenta functioning in exchange in late gestation (67). These structural alterations were predicted to enhance the capacity of the placenta to deliver substrate (67) and are consistent with the increase in MG transfer in late pregnancy observed in the current study. Indeed, we also report positive correlations between a variety of placental structural parameters and placental MG transport to the fetus.

In contrast to IGF-II, maternal IGF-I treatment in early to midpregnancy did not alter the structure of the placental exchange region (67). Circulating IGF-I in the mother may influence other determinants of placental transport capacity, including the abundance, localization, and affinity of transporter proteins at the exchange interface, placental blood flow, and the concentration gradients of substrate between the mother and fetus, and placental consumption and modification of substrates. Uptake and transport of glucose across the placenta occurs via facilitated carrier-mediated diffusion (66), which in the human, mouse, rat, and sheep placenta primarily involves glucose transporter (GLUT1) and GLUT3, respectively (6). Furthermore, GLUT1 expression on the basal membrane of the placental barrier is positively regulated by IGF-I (7). The latter observation is especially noteworthy, because GLUT1 protein density on the basal membrane in the human placenta is thought to be the rate-limiting step in transplacental glucose transfer (76). This is consistent with reports of accelerated fetal growth in insulin-dependent diabetes mellitus being associated with increased GLUT1 activity on the basal membrane (29) and enhanced placental glucose transport capacity (43).

IGF-I also may have enhanced MG uptake and transfer to the fetus indirectly by increasing maternal blood flow to the placenta. During pregnancy, the maternal spiral arterioles that supply the uterus are transformed by invading placental trophoblasts into dilated vessels, unresponsive to maternal vasoconstrictors (2, 55, 62). This normal physiological process permits uninterrupted high-conductance flow of blood from the mother that constitutes 30–40% of the total maternal cardiac output (57). In culture, IGF-I stimulates trophoblast migration and invasion (52) through elevated matrix metalloproteinase-9 activity (40), activation of integrins (46, 47), and altered cytoskeletal organization (45). It also inhibits vasoconstrictors, such as thromboxane B2 and prostaglandin F2α, in human term placental explants (69). Placental synthesis of IGF-I expression is normally low (particularly compared with IGF-II) in most species analyzed, including the guinea pig and human (35–37). However, in the IGF-I-treated mother, elevated maternal circulating IGF-I concentrations may act in an endocrine fashion and influence the function of trophoblast surrounding or migrating within uterine spiral arteries. Therefore, elevated maternal circulating IGF-I may promote uterine vascular remodeling, which would permit increased perfusion of the placenta and indirectly increased substrate flux to the fetus.

Despite effects of exogenous maternal IGFs on placental transport of MG, there was no effect on placental uptake and transfer of AIB in late gestation. This was unexpected, because fetuses from IGF-treated mothers had elevated plasma α-amino nitrogen concentrations (67). These outcomes suggest that maternal IGF treatment may not alter the sodium-dependent system A transporters, which are responsible for AIB and neutral amino acid (alanine, serine, proline, and glutamine) transfer, but rather may target other amino acid transporter systems. There are at least nine different amino acid transporter systems identified in the
human and rat placenta (64), which display overlapping substrate specificity (5). Determining the fetal circulating amino acid profile in response to earlier maternal IGF treatment would provide more insight into which amino acid transporters may be affected in the placenta, providing a direct link with the observed increased fetal growth (67).

Both maternal IGF treatments increased MG fetal tissue utilization, whereas IGF-I also increased that of AIB in the fetal visceral organs near term. Furthermore, there was increased utilization of MG and AIB per gram of several fetal tissues of IGF-treated mothers, without an alteration in wet weight. We therefore speculate that the larger fetuses of the IGF-treated mothers (67) may have an accelerated growth rate and that particular fetal tissues may be functionally more active and therefore metabolizing substrate more efficiently, contributing to the rapid growth of the fetus in late gestation. This altered fetal growth trajectory may have been established during treatment and sustained until term, because fetuses of mothers treated with either IGF in early to midpregnancy were also heavier in midgestation (70).

Interestingly, in the current study, early to midpregnancy treatment of the guinea pig with either IGF also increased uptake of MG and AIB by maternal tissues in late pregnancy. Despite increased maternal substrate utilization, maternal organ weights were not increased and fetal and placental growth was not compromised near term in response to earlier maternal IGF treatment (67). These findings suggest that the IGF-treated mothers may be metabolically more active than vehicle-treated controls. Total energy expenditure increases by 19% by the third trimester of pregnancy compared with nonpregnant women (11), and during normal pregnancy, endogenous hepatic glucose production (3, 13, 48), the 24-h respiratory quotient, and basal and sleeping metabolic rates all increase (9, 12, 20, 75). These maternal physiological changes presumably contribute to maternal adaptation to pregnancy to meet the increasing demands of the placenta and fetus.

Increased maternal AIB and MG utilization in late pregnancy in response to IGF treatment may reflect a maternal metabolic strategy that enables maternal organs to increase their functional capacity and energy expenditure. Indeed, we have found in our guinea pig cohorts that total conceptus mass may be equal to as much as 50% of maternal weight near term, which is likely to place huge metabolic demands on the mother. Certainly, maternal plasma glucose concentrations tended to be increased in response to earlier maternal IGF treatment (67), suggestive of increased maternal glucose production available for conceptus growth. Interestingly, in the current study, mothers treated with IGF-I during early to midpregnancy displayed reduced clearance of MG from the maternal plasma, although fetal, placental, and maternal substrate uptakes were improved near term. This may have reflected substantial differences in the response of other maternal and fetal tissues, not analyzed in the current study, to maternal IGF treatment. Such tissues include other fat depots and skeletal muscles, the gastrointestinal tract, uterus, and skin, which are known to vary in their endocrine characteristics, including insulin and related molecule sensitivities.

In conclusion, we have shown that increased maternal circulating IGF concentrations in early pregnancy sustainedly increase maternal substrate utilization, placental MG transfer to the fetus, and fetal utilization of MG and AIB, enhancing fetal growth and survival near term. Whether the latter are indirect consequences of enhanced maternal adaptation to pregnancy or whether the IGF-treated mother increased her own substrate utilization in organs that must increase their functional activity to meet the challenge of pregnancy remain to be elucidated. Nevertheless, we speculate that effects of maternal IGF-II treatment on placental MG uptake and transfer are secondary to impacts on placental differentiation (67), whereas the precise mechanism of IGF-I actions is yet to be identified. However, clearly, enhanced placental transfer function results from both. Overall, this study has provided further evidence for a major role of maternal IGFs in the regulation of nutrient partitioning during pregnancy. Because insufficient supply of nutrients to the fetus is associated with IUGR (14, 15, 23), therapeutic approaches that increase maternal IGFs in early to midpregnancy may be effective in ameliorating or preventing its development in pregnancies at risk. The challenge is to identify those women at risk of IUGR early in pregnancy before the fetus is compromised.

ACKNOWLEDGMENTS

We thank GroPep Pty Ltd for supplying recombinant human IGFs. We thank Kirsty Pringle, Jasper Button, and Cherise Fletcher for assistance in the guinea pig postmortems.

GRANTS

This work was supported by a National Health and Medical Research Council of Australia Project Grant (to C. T. Roberts) and a Channel 7 Children’s Research Foundation Grant (to J. A. Owens and C. T. Roberts).

REFERENCES

13. Catalano PM, Tzybri ED, Wolfe RR, Roman NM, Amini SB, Sims EA. Longitudinal changes in basal hepatic glucose production and suppression

E674 MATERNAL IGF TREATMENT PROMOTES PLACENTAL TRANSPORT


19. During a brief fast in human pregnancy.


MATERNAL IGF TREATMENT PROMOTES PLACENTAL TRANSPORT


