Sensitivity to metabolic signals in late-gestation growth-restricted fetuses from rapidly growing adolescent sheep

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Wallace JM, Milne JS, Aitken RP, Hay WW Jr. Sensitivity to metabolic signals in late-gestation growth-restricted fetuses from rapidly growing adolescent sheep. Am J Physiol Endocrinol Metab 293: E1233–E1241, 2007. First published August 21, 2007; doi:10.1152/ajpendo.00294.2007.—Fetal sensitivity to insulin and glucose was investigated during fetal hyperinsulinemic-euglycemic (HI-euG, n = 18) and hyperglycemic-euinsulinemic (HG-euI, n = 12) clamps. Singleton bearing adolescent ewes were fed high (H) or control (C) nutrient intakes to induce compromised or normal placental/fetal size, respectively. Catheters were inserted in the umbilical vein (v), fetal artery, (a) and veins, and studies were conducted between day 126 and 133 of gestation. Umbilical blood flow (UmBF) was determined by the steady-state transplacental diffusion technique using 3H2O, and glucose fluxes were quantified by the Fick principle. For the HI-euG study, fetal glucose utilization was measured at spontaneously occurring fetal insulin concentrations and two additional higher levels, whereas fetal glucose was clamped at the initial baseline level. For the HG-euI study, fetal insulin was suppressed by somatostatin infusion, and fetal glucose utilization was determined at baseline (before somatostatin) glucose concentrations, and at 150 and 200% of this value. Placental weight (219 vs. 395 g), fetal weight (2,965 vs. 4,373 g), and UmBF (519 vs. 794 ml/min) were lower (P < 0.001) in H than in C groups. Relative to control fetuses, glucose extraction (Giv – al/Giv × 100) in the nonperturbed state was higher (21.7 vs. 15.9%) in growth-restricted fetuses despite lower glucose (0.78 vs. 1.05 mg/dl) and insulin (8.5 vs. 16.9 μU/ml) concentrations (all P < 0.001). During the HI-euG study, total fetal glucose utilization rate increased in response to higher insulin concentrations (65 and 64% in H and C groups). Similarly during the HG-euI study, a twofold increase in glucose supply increased fetal glucose utilization by 41 and 44% in H and C groups, respectively. Throughout both studies, absolute total fetal glucose utilization rates were reduced in H vs. C groups (P < 0.01) but were similar when expressed per kilogram fetus (HI-euG: 34.7, 49.5, and 57.5 in H vs. 34.7, 51.2, and 56.1 mol/min·kg−1·kg−1 in C, HG-euI: 28.7, 35.7, and 40.8 in H vs. 32.9, 34.5, and 43.8 mol/min·kg−1·kg−1 in C). These normal body weight-specific metabolic responses to short-term experimental increases in plasma insulin and glucose in response to chronic IUGR indicate maintained mechanisms of insulin action and glucose uptake/utilization capacity, which, if persistent, might predispose such IUGR offspring to excessive energy deposition in later life.

insulin action; glucose tolerance; intrauterine growth restriction; adolescent pregnancy; placenta

THE RISKS OF MISCARRIAGE, preterm birth, and low birth weight are particularly acute in young adolescent women (24, 28, 36, 37). Poor pregnancy outcome in this vulnerable cohort has variously been attributed to poor socioeconomic status, gynecological immaturity and growth, and nutritional status of the mother (reviewed in Ref. 49). With respect to the latter, continued maternal growth during pregnancy occurs in ~50% of adolescents and, in spite of larger pregnancy weight gains and increased fat stores, is associated with a reduction in birth weight compared with nonpregnant adolescent mothers (35). This effect is attributed to a competition for nutrients between the maternal body and her gravid uterus, unique to the adolescent growth period, and has been replicated in our ovine model. Thus, when singleton bearing adolescent ewes are overnourished to promote maternal growth throughout pregnancy, growth of both the placenta and fetus is impaired, and birth occurs prematurely relative to control adolescents of equivalent age (42, 43, 47). Although defects in proliferation of the fetal trophodermal and placental angiogenic gene expression are evident at midgestation (20, 32), placental insufficiency does not impact on fetal growth per se until the final third of gestation (49). By late pregnancy, these fetuses are asymmetrically growth restricted and, in spite of the ready availability of nutrients in the maternal circulation, are hypoglycemic, hyperlactacidemic, and have low anabolic hormone concentrations (44, 45). Such conditions imply a defect either in uteroplacental nutrient uptake, metabolism, or transport of essential nutrients, resulting in a reduction in umbilical supply. We have, however, demonstrated previously that uteroplacental metabolism per unit placenta in this model is normal in that uteroplacental glucose and oxygen consumption, together with lactate production, are decreased in proportion to the decrease in placental mass (44). In addition, although absolute placental glucose transport was reduced by ~47% in the growth-restricted vs. the control pregnancies, there was no difference between groups when expressed on a placental weight basis (46). Quantification of absolute umbilical nutrient uptakes of glucose, oxygen, and amino acids revealed that they are significantly attenuated in the growth-restricted fetus. However, all umbilical nutrient uptakes are equivalent to the normally growing control fetuses when expressed on a fetal weight-specific basis (41, 46) and yet, in spite of increased fetal glucose extraction (or clearance), fetal glucose and insulin concentrations remain low. This indicates that increased fetal glucose tolerance (utilization capacity) and/or increased insulin sensitivity may be operating to preserve normal fetal metabolism while growth is sacrificed. To test this hypothesis, the present study investigated fetal sensitivity to insulin and glu-
cose during fetal hyperinsulinemic-euglycemic and hypergly-

cemic-euinsulinemic clamps.

MATERIALS AND METHODS

Animals and experimental design. All procedures were licensed under the UK Animals (Scientific Procedures) Act of 1986 and by the Rowett Research Institute’s Ethical Review Committee.

Embryos from superfetated adult ewes (Border Leicester × Scottish Blackface), inseminated by a single sire, were recovered on day 4 after estrus and transferred synchronously in singleton into the uterus of recipient ewe lambs (Dorset Horn × Mule), exactly as described previously (48). This technique removes the potentially confounding influence of partial embryo loss and variation in fetal number and maximizes the homogeneity of the resulting fetuses. Donor ewes were multiparous, between 3 and 4 yr of age, and had a body condition score of 2.25 units at the time of embryo recovery. Body condition or adiposity score was subjectively assessed on a five-point scale (1 = emaciated, 5 = obese; see Ref. 34) and has previously been shown to provide an accurate assessment of both internal fat depot and carcass fat content (43). Embryo transfer was carried out during the breeding season, and the animals were housed in individual pens under natural lighting conditions at the Rowett Research Institute (57°N, 2°W). At the time of embryo transfer, the recipient ewe lambs were pubertal (~8.5 mo old), with a mean live weight of 45 ± 0.7 kg and a body condition score of 2.3 ± 0.03 units. Immediately following embryo transfer, recipients were allocated to one of two nutritional treatments on the basis of live weight, body condition score, and ovulation rate at the time of transfer. Where possible, care was also taken to randomize for the maternity of the embryo. Recipients were individually offered either a moderate (n = 8) or high (n = 11) quantity of the same complete diet. The dietary level in the moderate group was in fact a control intakes level calculated to maintain normal maternal adiposity throughout gestation and hence meet the estimated metabolizable energy requirements for optimal conceptus growth and pregnancy outcome in this genotype. In adolescent dams, this was achieved by allowing a moderate maternal weight gain (~50 g/day) during the first two-thirds of gestation followed by stepwise increases in maternal intake during the final third of gestation to meet the increasing demands of the developing fetus. In contrast, the high intakes were equivalent to approximately two times maintenance and were calculated to promote rapid maternal growth leading to obesity (43). The complete diet supplied 12 MJ metabolizable energy (ME) and 140 g crude protein per kilogram and was offered in two equal feeds at 0800 and 1600 daily. Animals offered moderate intakes received their entire ration immediately, whereas those offered high intakes had the level of feed gradually increased over a 2-wk period until the level of daily feed refusal was ~15% of the total offered (equivalent to ad libitum intakes). The level of feed offered was reviewed three times weekly and adjusted, on an individual basis and when appropriate, on the basis of body weight change data and the level of feed refused (recorded daily). Maternal body condition or adiposity score was assessed by one member of the team at approximately fortnightly intervals.

Surgery and animal care. Infusion and sampling catheters were surgically inserted at ~120 days of gestation. Water and food were withheld overnight before surgery. A temporary catheter was inserted in a maternal external jugular vein to facilitate accurate intravenous administration of the anesthesia induction agent, antibiotics, and analgesics. Anesthesia was induced by intravenous administration of thiopentone (25 mg/kg intraval sodium; Merial Animal Health, Essex, UK) and maintained by inhalation of halothane (Halothane M&B; Rhone-Murex, Essex, UK) in a mixture of oxygen and nitrous oxide. Just after anesthesia induction, ewes received antibiotic in the form of ampicillin (fixed dose of 500 mg Penbritin; Beechem Research, Hertfordshire, UK) and marbofloxacin (2 mg/kg Marboycil 10%; Vetoquinol, Buckingham, UK) and analgesics in the form of bu-}

premorphine (0.006 mg/kg Vetricus; Schering-Plough, Hertford-

shire, UK) and meloxicam (0.5 mg/kg Metacam; Boehringer In-

gelheim, Ingelheim/Rhein, Germany). Before closing the uterus, a further fixed dose of ampicillin (500 mg) was injected in the amniotic cavity. Following hysterotomy by electrocautery and using methods described previously (16), polyvinyl catheters for infusion (3 times) were placed in the fetal saphenous veins via pedal veins (1 and 2 catheters/leg, respectively), and catheters for sampling the umbilical circulation were placed in the lower fetal aorta via a pedal artery and in the common umbilical vein. A catheter for sampling the maternal circulation was placed in one maternal femoral artery via a groin incision. The hysterotomy was closed around the catheters, which were then were tunnelled subcutaneously, exteriorized through a flank incision, and kept in a pouch stitched to the ewe’s flank. Catheters were flushed daily with a heparin-saline solution (150 IU/ml, 0.9% wt/vol sodium chloride solution). Ewes were housed individually in polypropylene floor level crates and allowed to recover from surgery for a minimum of 3 days before study. The ewes had previously been acclimatized to these crates for short periods over several days before catheter insertion. Fetal arterial blood glucose, lactate, and oxygen concentrations were checked daily during the recovery period to monitor fetal wellbeing. Ewes were gradually realimented during the recovery period, and all were consuming control intakes to fully meet the ME requirement for stage of gestation by the time of study. Predicted fetal weight at study was based on an ultrasound examination of kidney size before surgery (3) and by palpating the fetus during surgery.

Study 1: Hyperinsulinemic-euglycemic clamp. This study was performed on 8 moderate (control)-intake and 10 high-intake animals. Ewes were fed as normal at 0700 on the day of study. To measure umbilical blood flow by the transplacental steady-state diffusion technique, a solution of tritiated water ($^3$H$_2$O, 16.7 μCi/ml; American Life Science, Buckinghamshire, UK) in saline was infused in a fetal vein catheter, starting with a bolus of 1 ml (50 μCi) administered over 1 min, after which the infusion rate was changed to 1 ml (50 μCi)/h for the remainder of the study. After infusion for at least 110 min to reach steady state, blood samples were withdrawn simultaneously from the maternal artery, fetal artery, and umbilical vein catheters. Four sets of blood samples were withdrawn at 10- to 15-min intervals in heparinized syringes and subsequently analyzed for baseline glucose, lactate, oxygen, insulin, and $^3$H$_2$O (fetal samples only) concentrations (period 1) and hematocrit. Directly following these baseline draws, a fetal hyperinsulinemic-euglycemic clamp was established at high (period 2) and higher still (period 3) insulin concentrations. Recombinant insulin (Humulin S; Eli Lilly, Basingstoke, England) was prepared in 0.9% sodium chloride containing 5% sterile sheep plasma. At the start of period 2, a 1.5-ml bolus was administered to fill the catheter line and provide 135 mU insulin to the fetus, and this was followed by a constant infusion of 1 mU insulin·min$^{-1}$·kg$^{-1}$ estimated fetal weight. Fetal euglycemia (at the mean arterial glucose concentration measured during the baseline period) was maintained with a fetal glucose infusion (25% dextrose in saline) and was adjusted according to frequent (5–10 min) fetal arterial glucose measurements as described previously (15). Once steady state had been achieved for at least 90 min, four sets of simultaneous blood samples were withdrawn and analyzed as detailed above. Study period 3 involved increasing the fetal insulin infusion threefold to 3 mU·kg$^{-1}$·min$^{-1}$ while maintaining fetal euglycemia for at least 90 min and then withdrawing a further four sets of blood samples. The fetus was transfused isovolumetrically with freshly collected heparinized maternal whole blood following all three periods to maintain blood volume and hemoglobin concentration on this and the subsequent study day if applicable.

Study 2: Hyperglycemic-euinsulinemic clamp. This study was performed on six moderate (control)-intake and six high-intake animals. With the exception of one high-intake animal, all animals had previ-
ously undergone a hyperinsulinemic-euglycemic clamp with a minimum rest phase of 36 h between studies. Period 1 was repeated exactly as detailed above for study 1. Directly following the baseline draws, a fetal hyperglycemic-euinsulinemic clamp was established. The aim was to achieve a fetal arterial glucose concentration 150 and 200% above baseline during periods 2 and 3, respectively. A fetal somatostatin infusion (S-9129; Sigma-aldrich.com) was used to suppress fetal insulin. Somatostatin was prepared in 0.9% sodium chloride containing 5% sterile sheep plasma. At the start of period 2, a bolus was administered to fill the catheter line and provide 100 μg somatostatin/kg fetus. This was followed by a constant infusion of 4 μg·min⁻¹·kg⁻¹ estimated fetal weight throughout periods 2 and 3. Fetal hyperglycemia was achieved using 25% dextrose and commenced 30–45 min after the start of the somatostatin infusion to suppress fetal insulin. Once steady state at the desired glucose level had been achieved for 90–120 min, and four sets of simultaneous samples were withdrawn as above. This procedure was then repeated during period 3 but at the higher glucose concentration. For fetal glucose concentrations, steady state was defined as being around the sampling period mean value, without a consistent trend to increase or decrease.

At least 16 h after the final study, the ewe and fetus were humanely killed by intravenous administration of an overdose of pentobarbitone sodium (200 mg/ml Euthate; Willows Francis Veterinary, Crawley, UK) on 131 ± 0.4 days of gestation. The fetus and placentomes were dissected and weighed, and catheter location was verified. Major fetal organs were dissected and weighed.

Biochemical analyses. The plasma 3H2O concentrations were measured in triplicate using 150 μl of plasma, 500 μl distilled water, and 15 ml Ultima Gold scintillation fluid (Packard Bioscience) and counted on a Packard scintillation counter (Tri-carb 1900TR with internal quench correction). Plasma 3H2O concentrations were converted to whole blood concentrations according to Veen et al. (40). Plasma glucose and lactate concentrations were measured in duplicate with a Yellow Springs Instruments (YSI, Yellow Springs, OH) dual biochemistry analyzer (model 2700). The YSI instrument was calibrated with known standards after every fourth determination. Whole blood oxygen content and hematocrit were measured in duplicate using a Radiometer OSM-3 hemoximeter (Radiometer, Copenhagen, Denmark) and hematocrit centrifuge (Hawksley, Sussex, UK), respectively. Plasma insulin concentrations were measured in duplicate by radioimmunoassay as described previously (4). The limit of detection was 4 μU insulin/ml, and the intra-assay coefficient of variation was 5.1%.

Calculations and data analyses. Umbilical blood flow and nutrient uptakes were calculated as described previously (26) and according to the following equations.

Umbilical blood flow

\[
= \text{net transplacental diffusion rate of } ^3\text{H}_2\text{O/umbilical arteriovenous blood concentration difference of } ^3\text{H}_2\text{O}
\]

where net transplacental diffusion rate of 3H2O is calculated according to the Fick principle as fetal 3H2O infusion rate minus rates of accumulation and metabolism in the fetus.

Net umbilical (fetal) uptake of substrate = umbilical blood flow × umbilical venoarterial blood substrate concentration difference

Total fetal glucose uptake rate (fetal glucose utilization rate)

\[
= \text{net umbilical uptake of glucose} + \text{fetal glucose infusion rate during steady state}
\]

Fetal glucose extraction = (umbilical venous glucose concentration – umbilical arterial glucose concentration)/umbilical venous glucose concentration

Fetal glucose clearance = total fetal glucose uptake (utilization) rate/umbilical venous glucose concentration.

The significance of differences between the overnourished and control nutritional treatment groups was determined by Student’s unpaired t-test, whereas differences within animals were assessed by Student’s paired t-test. Correlation analysis was by Pearson’s Product Moment Test, where appropriate.

RESULTS

Maternal weight and adiposity score changes in relation to pregnancy outcome. The dietary-induced changes in maternal weight and adiposity score and morphometric data relating to pregnancy outcome are detailed in Table 1. The external assessment of body condition revealed that the moderate dietary intakes maintained maternal adiposity at the desired initial control level throughout gestation. In contrast, the high dietary intakes promoted rapid maternal weight gain and obesity (P < 0.001). At necropsy on day 131 of gestation, the relative perirenal fat mass of the dams was two times higher (P < 0.001) in the high compared with the control intake group. The weight of the gravid uterus and total and average placental weights were reduced in high- compared with moderate-intake groups (P < 0.001). The average relative reduction in placental weight was 45% and was associated with a 32% decrease in fetal weight (P < 0.001). At this stage of gestation, placental mass was more perturbed than fetal weight, resulting in a significantly higher fetal-to-placental weight ratio (P < 0.01) in the overnourished group. Placental weight was strongly correlated with fetal weight within the high group (r = 0.874, n = 11, P < 0.001) and for the high- and moderate-
intake groups combined ($r = 0.865, n = 19, P < 0.001$). Fetal liver weights but not brain weights were significantly smaller in high- vs. moderate-intake group fetuses ($93 \pm 9.2$ vs. $144 \pm 8.4$ g, $P < 0.001$, and $41.1 \pm 0.5$ vs. $41.3 \pm 0.8$ g, not significant, respectively). Consequently, the brain-to-liver weight ratio was elevated in high- compared with moderate-intake group fetuses ($0.48 \pm 0.047$ vs. $0.29 \pm 0.015$, $P < 0.002$). The mass of both the fetal pancreas and perirenal fat depot was reduced in high- compared with moderate-intake group fetuses ($2.3 \pm 0.25$ vs. $4.2 \pm 0.47$ g, $P < 0.01$, and $17.7 \pm 1.57$ vs. $24.1 \pm 2.13$ g, $P < 0.05$, respectively) but were equivalent when expressed on a fetal weight-specific basis ($0.78 \pm 0.081$ vs. $0.94 \pm 0.084$ g/kg fetus and $6.0 \pm 0.42$ vs. $5.5 \pm 0.47$ kg fetus, respectively).

Spontaneous umbilical blood flow, nutrient concentrations, and flux rates. On study day 1 (~day 128 of gestation), spontaneous absolute umbilical blood flow was reduced by $34\%$ ($P < 0.001$) in the growth-restricted pregnancies of the high-intake group. These baseline fetal glucose concentrations were positively correlated with placental and fetal weight within the high ($r = 0.882$ and $0.841$, respectively, $n = 10, P < 0.01$) but not the moderate-intake group. Absolute umbilical glucose uptake was reduced by $29\%$ ($P < 0.01$) in high- compared with moderate-intake pregnancies but was identical between groups when normalized for fetal weight; thus, the high-intake group had a higher fetal glucose extraction (or clearance rate; $P < 0.001$).

Fetal arterial plasma lactate concentrations were equivalent between groups and, although absolute umbilical lactate uptake was reduced by $28\%$ in the growth-restricted pregnancies, this did not quite achieve formal significance ($P > 0.09$). Fetal weight-specific lactate uptake was equivalent between groups (Table 2).

Comparison of high- and moderate-intake pregnancies revealed that spontaneous arterial plasma insulin concentrations were elevated in the dams ($P < 0.05$) and attenuated in the fetus ($P < 0.001$). Irrespective of treatment group, a negative association was detected between maternal and fetal insulin concentrations ($r = -0.532, n = 18, P < 0.05$). Spontaneous fetal arterial insulin concentrations were positively correlated with fetal weight within the high ($r = 0.782, n = 10, P < 0.01$)- and moderate ($r = 0.867, n = 8, P < 0.01$)-intake groups. Within the growth-restricted pregnancies of the high-intake group, fetal insulin concentrations were more perturbed than glucose concentrations, and thus the fetal insulin-to-glucose ratio was significantly lower ($P < 0.01$) than in the normally growing fetuses of the moderate-intake control group (Table 2).

Nutrient concentrations and fluxes during hyperinsulinemic-euglycemic clamp. The mean fetal arterial insulin and glucose concentrations achieved and maintained during the hyperinsulinemic-euglycemic clamp are detailed in Table 3, together with the fetal glucose flux rates. As per the experimental design, fetal arterial glucose during periods 2 and 3 were clamped in both groups of fetuses at concentrations equivalent to the baseline period (period 1). Overall, mean variance in fetal glucose concentrations during the clamp was <2%. Fetal plasma insulin concentrations increased approximately four- and sixfold from period 1 to period 2 in the moderate- and high-intake groups, respectively (high insulin, $P < 0.001$) and a further fourfold in both groups between periods 2 and 3 (higher still insulin, $P < 0.001$). The rise in fetal insulin between periods 1 and 2 in response to fetal insulin infusion was associated with a significant increase in total fetal glucose utilization rate in both moderate ($P < 0.02$)- and high ($P <

Table 2. Spontaneous umbilical blood flow, fetal blood oxygen, glucose and lactate content, umbilical uptakes and extractions, and maternal blood glucose and insulin content at ~128 days of gestation (study day 1) in adolescent dams offered a moderate (control)- or high-nutrient intake from day 4 of gestation.

<table>
<thead>
<tr>
<th>Maternal Nutrient Intake</th>
<th>Moderate ($n = 8$)</th>
<th>High ($n = 10$)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Umbilical blood flow, ml/min</td>
<td>794±51</td>
<td>519±38</td>
<td>$P &lt; 0.001$</td>
</tr>
<tr>
<td>Umbilical blood flow/kg fetus, ml·min⁻¹·kg⁻¹</td>
<td>193±12</td>
<td>180±7</td>
<td>NS</td>
</tr>
<tr>
<td>Fetal arterial blood oxygen content, mM</td>
<td>3.0±0.21</td>
<td>2.8±0.25</td>
<td>NS</td>
</tr>
<tr>
<td>Umbilical oxygen uptake, μmol/min</td>
<td>1,303±94</td>
<td>891±77</td>
<td>$P &lt; 0.01$</td>
</tr>
<tr>
<td>Umbilical oxygen uptake/kg fetus, μmol·min⁻¹·kg⁻¹</td>
<td>315±18</td>
<td>307±13</td>
<td>NS</td>
</tr>
<tr>
<td>Umbilical oxygen delivery, μmol/min</td>
<td>3,701±347</td>
<td>2,379±266</td>
<td>$P &lt; 0.01$</td>
</tr>
<tr>
<td>Umbilical oxygen extraction, %</td>
<td>36±2.7</td>
<td>39±2.0</td>
<td>NS</td>
</tr>
<tr>
<td>Maternal arterial plasma glucose, μmol/ml</td>
<td>3.9±0.16</td>
<td>4.1±0.12</td>
<td>NS</td>
</tr>
<tr>
<td>Fetal arterial plasma glucose, μmol/ml</td>
<td>1.05±0.047</td>
<td>0.78±0.048</td>
<td>$P &lt; 0.002$</td>
</tr>
<tr>
<td>Umbilical glucose uptake, μmol/min</td>
<td>143±9.3</td>
<td>101±8.9</td>
<td>$P &lt; 0.01$</td>
</tr>
<tr>
<td>Umbilical glucose uptake/kg fetus, μmol·min⁻¹·kg⁻¹</td>
<td>34.7±2.29</td>
<td>34.7±1.29</td>
<td>NS</td>
</tr>
<tr>
<td>Fetal glucose extraction, %</td>
<td>15.9±0.95</td>
<td>21.7±1.0</td>
<td>$P &lt; 0.001$</td>
</tr>
<tr>
<td>Fetal glucose/oxygen quotient</td>
<td>0.66±0.02</td>
<td>0.68±0.03</td>
<td>NS</td>
</tr>
<tr>
<td>Fetal arterial plasma lactate, μmol/ml</td>
<td>1.96±0.11</td>
<td>2.14±0.46</td>
<td>NS</td>
</tr>
<tr>
<td>Umbilical lactate uptake, μmol/min</td>
<td>105±9.9</td>
<td>76±12.9</td>
<td>NS</td>
</tr>
<tr>
<td>Umbilical lactate/kg fetus, μmol·min⁻¹·kg⁻¹</td>
<td>25.7±2.75</td>
<td>25.1±3.61</td>
<td>NS</td>
</tr>
<tr>
<td>Maternal arterial insulin, μU/ml</td>
<td>37.9±6.5</td>
<td>63.3±9.8</td>
<td>$P &lt; 0.05$</td>
</tr>
<tr>
<td>Fetal arterial plasma insulin, μU/ml</td>
<td>16.9±1.14</td>
<td>8.5±1.08</td>
<td>$P &lt; 0.001$</td>
</tr>
<tr>
<td>Fetal arterial glucose-to-insulin ratio</td>
<td>0.06±0.005</td>
<td>0.11±0.012</td>
<td>$P &lt; 0.01$</td>
</tr>
</tbody>
</table>

Values are means ± SE.
0.05)-intake groups (Table 3). Total fetal glucose utilization rate was higher still in both groups during period 3 when compared with period 1 (P < 0.01) but was not statistically different from that observed in period 2. Similarly, when expressed on a fetal weight-specific basis, total glucose uptake/utilization rate per kilogram fetus increased between periods 1 and 2 in moderate (P < 0.05)- and high (P < 0.01)-intake groups and remained elevated during period 3 (P < 0.01 and P < 0.001 relative to period 1, respectively). The relationship between fetal insulin and total fetal glucose uptake during the hyperinsulinemic-euglycemic clamp is depicted in Fig. 1. In absolute terms, the growth-restricted fetuses of high-intake dams display reduced total fetal glucose uptake rates during all three periods when compared with the normally growing fetuses of moderate-intake dams (P < 0.01, Fig. 1A). However, when expressed per kilogram fetus, total fetal glucose uptake rates are virtually identical between nutritional treatment groups (Fig. 1B). Similarly, the mean individual percentage increase in total fetal glucose utilization rates between periods 1 and 3 was equivalent between moderate and high groups (63.7 ± 10.15 and 64.3 ± 6.94%, respectively).

The major rise in fetal insulin concentrations during periods 2 and 3 did not significantly influence either absolute or fetal weight-specific umbilical oxygen (Fig. 2) or lactate (data not shown) uptake rates in either high- or moderate-intake groups, and, within nutritional treatment groups, oxygen and lactate uptake rates were similar to those reported above under spontaneous insulin concentrations (Table 2).

Nutrient concentrations and fluxes during hyperglycemic-euglycemic clamp. The mean fetal arterial glucose and insulin concentrations achieved and maintained during the
hyperglycemic-euinsulinemic clamp (study day 2) are detailed in Table 4, together with the fetal glucose flux rates. As detailed above for study day 1, baseline fetal insulin and glucose concentrations were lower in growth-restricted fetuses of high-intake dams compared with control fetuses during period 1. As per the experimental design, the fetal somatostatin infusion during periods 2 and 3 prevented any increase in fetal insulin concentrations during the hyperglycemic clamp. Indeed, fetal insulin concentrations were generally significantly lower in periods 2 and 3 compared with period 1 for both nutritional treatment groups. During periods 2 and 3, fetal plasma glucose concentrations were clamped at 154 and 210% of baseline period 1 values in the moderate group and at 159 and 217% of period 1 values in the high group. Total fetal glucose utilization rates were not altered between periods 1 and 2 but were higher during period 3 (P < 0.05) in both moderate and high groups. When expressed on a fetal weight-specific basis, the total glucose uptake rate per kilogram fetus increased between periods 1 and 2 in growth-restricted fetuses (P < 0.05) and between periods 1 and 3 in both growth-restricted (P < 0.02) and control fetuses (P < 0.05). The relationship between fetal glucose and total glucose uptake rate during the hyperglycemic-euinsulinemic clamp is depicted in Fig. 3. In absolute terms, the growth-restricted fetuses of high-intake dams display reduced total fetal glucose uptake rates during all three periods. This was equivalent to an average decrease of 44% (P < 0.01), 33% (P < 0.01) and 41% (P < 0.001) relative to normally growing fetuses of moderate-intake dams during periods 1, 2, and 3, respectively. However, when expressed per kilogram fetal weight, total fetal glucose uptake rates were similar between nutritional treatment groups (Fig. 3B). The mean percentage increase in total fetal glucose utilization rate between periods 1 and 3 was similar between groups (44 ± 20.1, moderate and 40 ± 7.2%, high), despite considerably more variance among individual values in the moderate-intake control group.

The increase in fetal glucose concentrations during periods 2 and 3 did not influence either absolute or fetal weight-specific umbilical oxygen (Fig. 4) or lactate (data not shown) uptake rates in either high- or moderate-intake groups. Furthermore, within nutritional treatment groups, umbilical oxygen and lactate uptake rates were similar to those reported during spontaneous conditions on study day 1 and 2.

DISCUSSION

In keeping with previous studies using this nutritionally mediated model of placental and fetal growth restriction (44, 46), the growth-restricted fetuses exhibited low circulating glucose and insulin concentrations during baseline assessments on two independent study days in late gestation. This occurred in spite of normal fetal weight-specific glucose uptakes and increased glucose extraction (to partially compensate for reduced glucose supply) in the growth-restricted group. The design of the present study allowed us to investigate fetality to insulin and glucose during fetal hyperinsulinemic-euglycemic and hyperglycemic-euinsulinemic clamps in this particular study.
or high (emerging from observations in a different ovine model of localization (14). Evidence to support these possibilities is plasma membrane glucose transporter abundance, activity, or signal transduction activity and/or tissue-specific increases in have not yet been examined but may include increased insulin to prevent a reduction in glucose transport and insulin action.

Putative mechanisms that may underlie this adaptive response and hence total nutrient supply become limiting (44 – 46).

S disposition. Although such adaptive mechanisms may have a beneficial effect for ensuring fetal survival, albeit at the expense of growth, they may also have implications beyond the fetal period. Thus, if these maintained mechanisms of insulin action persist, the prenatally growth-restricted fetuses may be predisposed to increased fat deposition when exposed to high glycemic diets during postnatal life. In fact, placental growth restriction of fetal growth in a different model of IUGR in sheep, produced by prepregnancy carunclectomy, has shown increased insulin action to dispose of both glucose and free fatty acids (FFA’s), and increased visceral adiposity in the IUGR offspring when studied as young lambs at 1 mo of age (8).

Similarly, increases in body fatness have been reported in low-relative to high-birth-weight offspring of prolific ewes, particularly when artificially reared to grow rapidly to a target weight of 20 kg (11). Other reports provide evidence of increased insulin sensitivity with respect to glucose disposal in human IUGR infants as early as 48 h after birth (2, 25). These latter observations in the human neonate are consistent with our results herein in the late-gestation IUGR sheep fetus, where a similar increase in the glucose-to-insulin ratio was observed under baseline conditions. Together these observations indicate a continuum of increased insulin sensitivity from the fetal period after chronic IUGR through at least childhood that might predispose to enhanced glucose and potentially FFA disposal, thereby contributing to the potential for producing increased adiposity over time.

If the putative prenatal effects on amino acid metabolism and net protein accretion are similarly programmed, this increase in fat may occur at a relatively reduced muscle mass and stature. Indeed, this scenario has been documented to occur in the low-birth-weight offspring of prolific ewes described above whereby increased body fat deposition occurred at reduced
muscle mass (12). Furthermore, such a scenario has been documented to occur during childhood in a number of studies in growth-restricted human infants born in developed countries (17, 18, 27). The differences in body composition appear to persist into adulthood when individuals born small for gestational age have less lean tissue mass and greater fat mass, which uniquely has a more central localization (9, 19, 31). In the human IUGR infant, catch-up growth is associated with hyperinsulinemia, reflecting insulin resistance, and impaired glucose tolerance (5, 6, 29). Similarly, in a preliminary study of female offspring from the overnourished adolescent model, rapid catch-up growth following prenatal growth restriction was associated with increased insulin secretion (perhaps representing a compensatory response to insulin resistance, although increased insulin secretion capacity cannot be excluded) following an intravenous glucose challenge at 9 mo of age and elevated fasting glucose at 15 and 24 mo of age (Milne JS, Aitken RP, and Wallace JM, unpublished observations). It remains to be established whether similar alterations in metabolism persist in male offspring, but sex-dependent effects are beginning to emerge which indicate that the mechanisms and strategies that operate during the pre- and early postnatal life to ensure survival differ between the sexes (23, 30).

The results of the present study do not indicate any programmed defect in fetal pancreatic development per se. Absolute pancreatic mass was reduced by 45% in growth-restricted fetuses and was associated with a 50% reduction in baseline circulating fetal insulin concentrations on the first study day. However, fetal weight-specific pancreas mass was equivalent between groups, and, similarly, pancreatic insulin content in comparable cohorts of noninstrumented late-gestation fetuses was equivalent in growth-restricted and normally growing fetuses (4,348 ± 330 vs. 4,008 ± 457 μIU insulin/mg pancreas, respectively, n = 18 fetuses/group; Wallace JM and Milne JS, unpublished observation). All of the above are commensurate with the relatively late onset of IUGR in this paradigm. In contrast, extensive studies of the fetal pancreas in the maternal hyperthermia model reveal a selective reduction in β-cell mass, reduced insulin and insulin mRNA contents, and a lower mitotic index (21). This in turn is commensurate with the relatively early onset of a more severe form of growth restriction in this model at a time when the endocrine pancreas is undergoing major development.

Comparable studies in human fetuses cannot be conducted for obvious technical and ethical reasons. Therefore, the ultimate goal in studying fetal metabolism in these ovine models of fetal growth restriction is to develop and evaluate strategies targeted at reversing the physiological changes in glucose utilization that evolve when glucose supply is limited, a universal condition in all animal models, as well as human fetal growth restriction. IUGR occurs in 8 and 17% of human pregnancies in the developed and developing world, respectively (39), and carries serious risks to the fetus, neonate, child, and even adult (10, 13). To date, however, there have been no successful means to correct or prevent worsening of fetal IUGR in human pregnancies once they are detected by ultrasound. The results of the current study suggest that the growth-restricted fetus can respond appropriately to acute changes in both insulin and glucose supply. It remains to be determined, however, whether the reintroduction of specific nutrients and hormones over the longer term (by infusion in the fetus or indirectly in the mother) can benefit fetal protein accretion and growth.

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