Insulin Deficiency Alters the Metabolic and Endocrine Responses to Undernutrition in Fetal Sheep Near Term

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Insulin deficiency affects the adult metabolic response to undernutrition, but its effects on the fetal response to maternal undernutrition remain unknown. This study examined the effects of maternal fasting for 48 h in late gestation on the metabolism of fetal sheep made insulin deficient by pancreatectomy (PX). The endocrine and metabolic responses to maternal fasting differed between intact, sham-operated and PX fetuses, despite a similar degree of hypoglycemia. Compared with intact fetuses, there was no increase in the plasma concentrations of cortisol or norepinephrine in PX fetuses during maternal fasting. In contrast, there was a significant fasting-induced rise in plasma epinephrine concentrations in PX but not intact fetuses. Umbilical glucose uptake decreased to a similar extent in both groups of fasted animals but was associated with a significant fall in glucose carbon oxidation only in intact fetuses. Pancreatectomized but not intact fetuses lowered their oxygen consumption rate by 15–20% during maternal fasting in association with increased uteroplacental oxygen consumption. Distribution of uterine oxygen uptake between the uteroplacental and fetal tissues therefore differed with fasting only in PX fetuses. Both groups of fetuses produced glucose endogenously after maternal fasting for 48 h, which prevented any significant fall in the rate of fetal glucose utilization. In intact but not PX fetuses, fasting-induced glucogenesis was accompanied by a lower hepatic glycogen content. Chronic insulin deficiency in fetal sheep therefore leads to changes in the counterregulatory endocrine response to hypoglycemia and an altered metabolic strategy in dealing with nutrient restriction in utero. (Endocrinology 153: 4008–4018, 2012)
there are reductions in the rates of umbilical uptake and fetal use of glucose in fetal sheep together with a slowing of growth as seen in insulin-deficient, PX fetuses of well-nourished ewes (11, 13, 15–17). Thus, the role of the fetal endocrine pancreas and of the β-cells, in particular, is not primarily to regulate glycaemia but rather to match fetal glucose use to the placental glucose supply (4, 14). When undernutrition occurs near term, there is also activation of glucose production by fetal sheep, which appears to be related to increases in the fetal cortisol and catecholamines concentrations (15, 18). However, little is known about the metabolic and endocrine consequences of undernutrition in hyperglycemic fetuses deficient in insulin. This study therefore quantified the rates of glucose and oxygen metabolism in relation to the endocrine changes induced by short-term maternal fasting in PX fetuses close to term.

Materials and Methods

Animals

A total of 10 Welsh Mountain ewes carrying single fetuses were used. During the experimental period, the ewes were housed individually and maintained on concentrate (200 g/d; Beart Ltd., Stowbridge, Suffolk, UK) and hay and water ad libitum. Half the daily ration of concentrates was fed at 0800 h with the remainder given at 1700 h. Food but not water was withheld for 18–24 h before surgery. All procedures were carried out under the Animals (Scientific Procedures) Act 1986.

Surgical procedures

Under general anesthesia (1.5% halothane in a 2:1 mixture of O₂ and N₂O₂), fetuses were either pancreatectomized (PX, n = 5) or sham operated (intact controls, n = 5) at 115–119 d of gestation (normal term 145 ± 2 d) and then catheterized at a second operation 10–11 d later using the same anesthetic regime. Catheters were inserted into the uterine vein, umbilical vein, fetal dorsal aorta, and caudal vena cava and into the maternal aorta via a femoral artery of all animals as described previously (15). At the end of the experimental period, ewes and fetuses were given a lethal dose of anesthetic (200 mg/kg Na pentobarbital, iv) before collection of fetal tissues. To minimize animal use, some of the sham-operated controls (n = 4) were also used in a contemporaneous, independent study of the metabolic effects of fetal adrenalectomy because the experimental protocol and the procedures and timing of sham operation and catheterization were identical to this study (18).

Experimental procedures

Blood samples (0.5–1.0 ml) were taken from the fetus and mother daily between 0900 and 1000 h to monitor well-being. At least 6 d after vascular catheterization (136–138 d), measurements of fetal glucose and oxygen metabolism were made in the fed state in all 10 animals. Thereafter food but not water was withdrawn from the ewes and a second set of measurements were made in the fasted state at 139–141 d of gestation when the ewes had been without food for 48 h. As ruminants, sheep do not derive glucose from the gut but produce it endogenously from precursors derived primarily from the rumen in fed conditions (19). Consequently, even though digesta are still present in the gut, glucose levels fall rapidly in response to food withdrawal in pregnant ewes to reach a plateau by 48 h with little, if any, further decline in value when fasting continues for 5–7 d (17, 20).

In both fed and fasted animals, tritiated water (0.30 MBq/ml; Amersham International, Bucks, UK) and universally labeled [¹⁴C]glucose [0.37 MBq/ml in 0.09% NaCl (wt/vol); ICN Biochemicals, High Wycombe, Bucks, UK] were infused together into the fetal caudal vena cava for 2–4 h at known rates between 0.08 and 0.09 ml/min after an initial priming dose (3–4 ml). Simultaneous blood samples (3.5 ml) were taken anaerobically from the umbilical vein, fetal dorsal aorta, uterine vein, and maternal dorsal aorta before (0 min) and, when steady state had been established, at known times approximately 120, 140, 160, and 180 min after beginning the infusion. The simultaneous blood samples were analyzed immediately for blood pH, gas tensions, packed cell volume and O₂ content (0.5 ml), and labeled carbon dioxide (¹⁴CO₂) where appropriate (1.0 ml). The remainder of the sample (2 ml) was added to a chilled tube containing EDTA for subsequent analyses. An aliquot (0.5 ml) of the EDTA-treated blood was deproteinized with zinc sulfate (0.3 M) and labeled carbon dioxide (¹⁴CO₂) were measured in all five sets of simultaneous samples in the fed state. No pancreatic remnants were found after euthanasia from these additional fetuses in the conscious state immediately before euthanasia to compare cortisol concentrations with fetuses in the fasted state. No pancreatic remnants were found after pancreatectomy.

Biochemical analyses

The blood gas tensions, packed cell volume, O₂ content, and whole-blood concentrations of glucose, [¹⁴C]-glucose, and ¹⁴CO₂ were measured in all five sets of simultaneous samples in the fed and fasted states. Blood ¹⁴CO₂ content was measured immediately by acidification of the blood and trapping of the liberated ¹⁴CO₂ in a quaternaryammonium compound con-
been corrected for recovery. Blood O₂ content was calculated from the percentage O₂ saturation and the hemoglobin concentrations measured using an OSM2 hemoximeter (Radiometer, Copenhagen, Denmark) that had been calibrated for ovine blood. Blood pH and partial pressures of O₂ and CO₂ were measured using an ABL5 radiometer (Radiometer, Copenhagen, Denmark) and corrected for a fetal body temperature of 39 C.

Glucose concentrations were determined enzymatically in whole blood and plasma using a colorimetry assay (15) and an automated analyzer, respectively (2300 StatPlus; Yellow Springs Instruments, Yellow Springs, OH). Plasma ³H₂O concentrations were measured using scintillation counting and converted to blood concentrations, allowing for the differences in water content between blood and plasma in both fetus and mother as described previously (22, 23). Labeled glucose was determined using chemical methods published previously (24). It was separated from all other ¹⁴C labeled products by anion exchange chromatography after preincubation with and without glucose oxidase (15, 22). The mean recovery of [¹⁴C]glucose from the anion exchange column was 99.7 ± 1.3% (n = 32). No corrections for glucose recovery were therefore made.

Plasma catecholamine concentrations were determined by HPLC using electrochemical detection (15). Recovery of isoprenaline added to the samples ranged from 63 to 97%, and hence, all samples have been corrected for their respective recoveries. The limits of sensitivity were 0.26 nm for epinephrine and 0.18 nm for norepinephrine. The interassay coefficients of variation for epinephrine and norepinephrine were 7.3 and 6.2%, respectively. Plasma concentrations of insulin and cortisol were measured by RIA validated for use with ovine plasma (10, 25). The interassay coefficients of variation for these two assays were 13.7 and 10.0%, respectively, whereas the minimum detectable quantity of hormone was 4 nm for cortisol and 35 pm for insulin.

Hepatic glycogen content and the activities of glucose-6-phosphatase (G6Pase; EC 3.1.3.9) and phosphoenolpyruvate carboxykinase (PEPCK; EC 4.1.1.32) in liver and kidney were assayed using established methods described in detail elsewhere (26–28). Hormone concentrations and enzyme activities were measured in duplicate, whereas all other biochemical analyses were measured in triplicate.

Calculations

All calculations were made using equations derived for steady-state kinetics (21, 24, 29, 30). Umbilical blood flow was measured using the ³H₂O steady-state diffusion technique corrected for fetal accumulation and non-placental removal of ³H₂O in calculating umbilical flow and also for uteroplacental ³H₂O accumulation in calculating uterine blood flow, where appropriate, using the following equations (21, 29, 30):

Umbilical blood flow (milliliters per minute)

\[ = \text{net rate of } ³\text{H}_2\text{O uptake by the umbilical circulation} \]

\[ \text{arteriovenous concentration difference in} \]

\[ \text{blood } ³\text{H}_2\text{O (dpm · milliliter)} \quad (1) \]

Uterine blood flow (milliliters per minute)

\[ = \text{rate of } \text{³H}_2\text{O uptake by the uterine circulation} \]

\[ \text{uterine venous-arterial concentration difference in blood } ³\text{H}_2\text{O} \]

\[ \text{(dpm · milliliter)} \quad (2) \]

Net umbilical uptake of glucose and oxygen and net umbilical excretion rates of [¹⁴C]glucose and ¹⁴CO₂ were calculated by the Fick principle as the product of umbilical blood flow (milliliters per minute) and the umbilical venous-arterial (uptake) or arteriovenous (excretion) blood concentration difference across the umbilical circulation (micromoles per liter). The net uterine uptake of glucose and oxygen and the uterine output of tracer glucose were measured in a similar manner using uterine blood flow and the corresponding blood concentration differences across the uterine circulation. Net uteroplacental consumption of glucose and oxygen was calculated as the differences between the uterine and umbilical uptakes.

The fetal rates of use and production of glucose, CO₂ production from glucose carbon and the fraction of the net umbilical O₂ uptake used for glucose carbon oxidation by the fetus were calculated according to the following equations (22, 24):

Fetal glucose utilization (micromoles per minute)

\[ = \text{net fetal tracer glucose uptake} \]

\[ \text{(dpm · minute)/fetal arterial glucose specific activity (dpm · micromoles glucose)} \quad (3) \]

where

Net fetal tracer glucose uptake (dpm · minute per minute) = tracer glucose infusion rate (dpm · minute) − net umbilical tracer glucose excretion rate (dpm · milliliter) \quad (4)

Oxidation of glucose carbon was calculated as the rate of ¹⁴CO₂ production:

CO₂ production from the oxidation of glucose

\[ \text{carbon (micromoles per minute)} = \text{net umbilical } ¹⁴\text{CO}_2 \text{excretion rate} \]

\[ \text{(dpm · minute)/fetal arterial glucose specific activity (dpm · micromoles glucose carbon)} \quad (5) \]

The glucose carbon oxidation fraction and the fraction of O₂ consumption used to oxidize glucose carbon in the fetus were then calculated as follows:

Glucose carbon oxidation fraction

\[ \text{(fraction of fetal glucose carbon utilization used for oxidation)} = \text{net umbilical } ¹⁴\text{CO}_2 \]
excretion rate (disintegrations per minute)/net fetal tracer glucose infusion uptake (disintegrations per minute) (6)
Fraction of O\textsuperscript{2} uptake used for
oxidation of glucose carbon = amount of O\textsubscript{2}
used to oxidize fetal glucose carbon
(micromoles per minute)/net umbilical O\textsubscript{2}
uptake rate (micromoles per minute) (7)
where the amount of O\textsubscript{2} used to oxidize fetal glucose carbon
equals the amount of CO\textsubscript{2} produced by this oxidative process
(see above).

Endogenous glucose production by the fetus was calculated as follows:

Endogenous glucose production (micromoles per minute) = fetal glucose utilization (micromoles per minute)
− umbilical glucose uptake (micromoles per minute) (8)

Finally, glucose use by nonuterine maternal tissues was calculated in the ewes by measuring the labeled and unlabeled glucose concentrations in maternal arterial and uterine venous blood.

Glucose utilization by maternal nonuterine tissues (micromoles per minute) = net maternal tracer glucose uptake (disintegrations per minute per micromole)/
maternal arterial specific activity
(disintegrations per minute per micromoles) (9)

Total maternal glucose utilization (micromoles per minute) = net uterine glucose uptake (micromoles per minute) + glucose utilization
by maternal nonuterine tissues (micromoles per minute) (10)

The total rate of glucose utilization by the maternal tissues is equivalent to the rate of glucose production by the ewe (19).

Because studies were carried out in both the fed and fasted states, the values for [\textsuperscript{14}C]glucose, \textsuperscript{3}H\textsubscript{2}O and \textsuperscript{14}CO\textsubscript{2} in the 0 min samples of the fasted study were subtracted from the subsequent samples before calculation of blood flow and glucose metabolic rates. All fetal metabolic rates have been expressed per kilogram fetal body weight. No increase in fetal body weight was assumed to occur during the 48-h period of maternal food withdrawal.

**Statistical analyses**

Steady state was defined as less than 10% variation of values around the mean for each sampling period with no consistent trend for the absolute values to increase or decrease with time. Mean values ± SEM have been used throughout. Statistical analyses were made using SigmaStat (Jandel Scientific, Chicago, IL). Comparison of metabolic rates between treatments and nutritional states were made using two-way ANOVA and unpaired and paired *t* tests, as appropriate. For all statistical analyses, significance was accepted when *P* < 0.05. Some of the data on the hormone and metabolite concentrations of the sham-operated, intact fetuses have been published previously (18).

**Results**

**Biometry**

Pancreatectomy reduced the crown-rump and hind limb lengths of the fetus at delivery in the fasted state at 139–141 d (Table 1). Body weight also tended to be less in PX than intact fetuses, but this did not reach statistical significance (*P* = 0.06, Table 1). There were no significant differences in placentome weight or in the total weight of all uteroplacental tissues between PX and intact fetuses at delivery in fasted conditions (Table 1). There were no significant differences in blood pH or gases between intact and PX fetuses in either nutritional state (Table 2).

**Metabolite and hormone concentrations**

**Fed state**

In common with previous studies (10, 11), arterial concentrations of plasma insulin were significantly lower and concentrations of both plasma and blood glucose were significantly higher in PX than intact fetuses, but this did not reach statistical significance (*P* = 0.06, Table 1). There were no significant differences in placentome weight or in the total weight of all uteroplacental tissues between PX and intact fetuses at delivery in fasted conditions (Table 1). There were no significant differences in blood pH or gases between intact and PX fetuses in either nutritional state (Table 2).

**TABLE 1.** Morphometric data

<table>
<thead>
<tr>
<th>Weight</th>
<th>Uteroplacental tissues g</th>
<th>Placentomes g</th>
<th>Fetus g</th>
<th>Pancreas g</th>
<th>Hind limb length (cm)</th>
<th>CRL (cm)</th>
<th>Ponderal index (kg/m\textsuperscript{3})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>1641 ± 199</td>
<td>262 ± 23</td>
<td>3435 ± 216</td>
<td>2.60 ± 0.25</td>
<td>45.7 ± 0.5</td>
<td>50.6 ± 0.7</td>
<td>24.9 ± 2.8</td>
</tr>
<tr>
<td>Pancreateomized</td>
<td>1423 ± 130</td>
<td>250 ± 31</td>
<td>2773 ± 233</td>
<td>ND</td>
<td>41.4 ± 0.9\textsuperscript{b}</td>
<td>45.8 ± 0.9\textsuperscript{b}</td>
<td>28.6 ± 1.5</td>
</tr>
</tbody>
</table>

Mean (±ss) crown rump length (CRL), ponderal index (fetal body weight/CRL\textsuperscript{3}), and weights of the total uteroplacental tissues, placentomes, fetus, and pancreas of intact and PX sheep fetuses at delivery in the fasted state at 139–141 d of gestation (n = 5 in each group). ND, Not detected.

\textsuperscript{a} Combined weight of uterus, placentomes, and fetal membranes.

\textsuperscript{b} Significantly different from the intact value (*P* < 0.01, *t* test).
TABLE 2. Metabolite and hormone concentrations

<table>
<thead>
<tr>
<th></th>
<th>Intact Fed</th>
<th>Fasted Fed</th>
<th>Intact Pancreatectomized Fed</th>
<th>Fasted Pancreatectomized Fed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pH</td>
<td>7.368 ± 0.005</td>
<td>7.376 ± 0.008</td>
<td>7.377 ± 0.009</td>
<td>7.362 ± 0.010</td>
</tr>
<tr>
<td>Blood gases (kPa)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(pO_2)</td>
<td>2.87 ± 0.21</td>
<td>2.72 ± 0.04</td>
<td>3.01 ± 0.20</td>
<td>2.89 ± 0.08</td>
</tr>
<tr>
<td>(pCO_2)</td>
<td>6.81 ± 0.28</td>
<td>6.42 ± 0.31</td>
<td>6.12 ± 0.17</td>
<td>6.17 ± 0.19</td>
</tr>
<tr>
<td>Blood glucose (mM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fetus</td>
<td>0.86 ± 0.08</td>
<td>0.53 ± 0.04a</td>
<td>1.19 ± 0.11b</td>
<td>0.60 ± 0.05a</td>
</tr>
<tr>
<td>Mother</td>
<td>2.34 ± 0.13</td>
<td>1.18 ± 0.12a</td>
<td>2.02 ± 0.10</td>
<td>1.27 ± 0.24a</td>
</tr>
<tr>
<td>Plasma glucose (mM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fetus</td>
<td>0.99 ± 0.09</td>
<td>0.64 ± 0.05a</td>
<td>1.35 ± 0.15b</td>
<td>0.70 ± 0.05a</td>
</tr>
<tr>
<td>Mother</td>
<td>3.07 ± 0.12</td>
<td>1.83 ± 0.13a</td>
<td>3.05 ± 0.10</td>
<td>1.87 ± 0.38a</td>
</tr>
<tr>
<td>Transplacental</td>
<td>2.10 ± 0.14</td>
<td>1.17 ± 0.10a</td>
<td>1.70 ± 0.15b</td>
<td>1.18 ± 0.30a</td>
</tr>
<tr>
<td>Insulin (pm)</td>
<td>152 ± 17</td>
<td>96 ± 11a</td>
<td>51 ± 9b</td>
<td>45 ± 10b</td>
</tr>
<tr>
<td>Cortisol (nmol)</td>
<td>35.3 ± 7.4</td>
<td>113.1 ± 27.5a</td>
<td>41.4 ± 7.7</td>
<td>43.9 ± 4.1b</td>
</tr>
<tr>
<td>Epinephrine (nmol)</td>
<td>0.23 ± 0.01</td>
<td>0.34 ± 0.02</td>
<td>0.63 ± 0.17</td>
<td>1.10 ± 0.15a,b</td>
</tr>
<tr>
<td>Norepinephrine (nmol)</td>
<td>1.16 ± 0.32</td>
<td>5.11 ± 1.25a</td>
<td>1.66 ± 0.23</td>
<td>2.59 ± 0.72</td>
</tr>
<tr>
<td>Total catecholamines (nmol)</td>
<td>1.38 ± 0.43</td>
<td>5.44 ± 1.11a</td>
<td>2.08 ± 0.43</td>
<td>3.68 ± 1.18a</td>
</tr>
</tbody>
</table>

Mean (± SE) values of fetal blood pH, fetal blood gases, maternal and fetal arterial concentrations of blood and plasma glucose, and fetal arterial concentrations of plasma cortisol, insulin, epinephrine, norepinephrine, and total catecholamines together with the transplacental plasma glucose concentration gradient in intact \(n = 4–5\) and PX fetuses \(n = 5\) in fed conditions and after maternal fasting for 48 h.

a Significantly different from the value in the fed state in the same group of fetuses (\(P < 0.05\), paired \(t\) test).
b Significantly different from the value in intact fetuses in the same nutritional state (\(P < 0.05\), ANOVA).

Createctomy (Table 2). Consequently, the transplacental concentration gradient in plasma glucose was significantly less in ewes with PX than intact fetuses in fed conditions (Table 2). There were no significant differences in the plasma concentrations of cortisol, epinephrine, norepinephrine, and total catecholamines between PX and intact fetuses in fed conditions (Table 2).

Fasted state

Fasting significantly reduced maternal and fetal glucose levels in both groups of animals (Table 2). In contrast to the fed state, glucose concentrations were not significantly different in PX and intact fetuses after maternal fasting for 48 h. There were also no differences in the maternal glucose concentrations between the two groups of fasted ewes (Table 2). The transplacental concentration gradient in plasma glucose decreased during fasting in both groups of animals and was not significantly different between the two groups after fasting for 48 h (Table 2).

Fasting had no effect on insulin concentrations in PX fetuses (Table 2). In contrast, insulin concentrations of intact fetuses fell during maternal fasting, although levels were still significantly higher than PX values after fasting for 48 h (Table 2). Fasting increased plasma cortisol concentrations in intact but not PX fetuses (Table 2). Consequently, cortisol concentrations were lower in PX than intact fetuses in fasted conditions (Table 2). Plasma concentrations of norepinephrine but not epinephrine rose in intact fetuses during fasting, whereas the reverse was observed in PX fetuses. Thus, plasma concentrations of epinephrine but not norepinephrine were higher in PX than intact fetuses after maternal fasting for 48 h (Table 2).

Total catecholamine concentrations rose significantly in both groups of fetuses during maternal fasting and were similar in the two groups after 48 h of fasting (Table 2).

Glucose metabolism

Fed state

In common with previous findings (11), the rate of umbilical glucose uptake was significantly lower in PX than intact fetuses in fed conditions (Table 3) and when expressed per kilogram fetal body weight (Fig. 1). Weight-specific rates of fetal glucose utilization and of \(CO_2\) production from glucose carbon were also significantly less in PX than intact fetuses of fed ewes (Fig. 1). However, the fraction of glucose used that was oxidized did not differ between intact and PX fetuses (Table 4). There was no endogenous production of glucose by either group of fetuses in fed conditions (Fig. 1). There were also no significant differences in the total rate of maternal glucose utilization or in the individual rates of glucose utilization by the uteroplacental tissues and the nonuterine maternal tissues between ewes with PX or intact fetuses in fed conditions (Table 3). Hepatic glycogen content was also similar in PX and intact fetuses at delivery in fed conditions at 139–141 d of gestation (Fig. 2). In contrast, hepatic activities of G6Pase and PEPCK were significantly higher in PX than intact fetuses in fed conditions (Fig. 2). There was no significant difference in the...
plasma cortisol concentration between the intact (70.3 ± 12.6 nM, n = 6) and the PX fetuses (57.1 ± 7.4 nM, n = 6, P > 0.05) at delivery in fed conditions at 139–141 d.

**TABLE 3. Glucose metabolism**

<table>
<thead>
<tr>
<th>Glucose use (μmol/min)</th>
<th>Intact Fed</th>
<th>Intact Fasted</th>
<th>Pancreatectomized Fed</th>
<th>Pancreatectomized Fasted</th>
</tr>
</thead>
<tbody>
<tr>
<td>All maternal tissues</td>
<td>521 ± 63</td>
<td>247 ± 26</td>
<td>398 ± 54</td>
<td>181 ± 37</td>
</tr>
<tr>
<td>Nonuterine maternal tissues</td>
<td>291 ± 48</td>
<td>121 ± 14</td>
<td>209 ± 53</td>
<td>88 ± 27</td>
</tr>
<tr>
<td>Uterine tissues</td>
<td>230 ± 26</td>
<td>127 ± 30</td>
<td>189 ± 26</td>
<td>93 ± 11</td>
</tr>
<tr>
<td>Uteroplacental tissues</td>
<td>135 ± 22</td>
<td>95 ± 30</td>
<td>141 ± 28</td>
<td>82 ± 8</td>
</tr>
<tr>
<td>Fetal tissues</td>
<td>95 ± 13</td>
<td>32 ± 9</td>
<td>47 ± 5</td>
<td>11 ± 4</td>
</tr>
<tr>
<td>Total maternal use (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonuterine tissues</td>
<td>54.5 ± 5.1</td>
<td>50.5 ± 7.4</td>
<td>50.1 ± 7.6</td>
<td>46.3 ± 4.9</td>
</tr>
<tr>
<td>Uteroplacental tissues</td>
<td>27.3 ± 5.5</td>
<td>36.5 ± 8.6</td>
<td>37.4 ± 7.8</td>
<td>47.8 ± 4.5</td>
</tr>
<tr>
<td>Fetal tissues</td>
<td>18.2 ± 0.8</td>
<td>13.0 ± 3.5</td>
<td>12.5 ± 1.6</td>
<td>6.4 ± 2.0</td>
</tr>
</tbody>
</table>

Mean (±SE) rates of total maternal glucose use and its distribution to the nonuterine and uterine tissues of ewes with intact and PX fetuses in fed conditions and after maternal fasting for 48 h (n = 5 in each group). 

\( ^{a} \) Significantly different from the value in the fed state in the same group of fetuses (P < 0.05, paired t test).

\( ^{b} \) Significantly different from the value in intact fetuses in the same nutritional state (P < 0.05, ANOVA).

**Fasted state**

Fasting reduced the rates of umbilical glucose uptake to a similar extent in both groups of fetuses, whether expressed in absolute terms (Table 3) or per kilogram fetal body weight (Fig. 1). This was accompanied by a significant fall in the weight specific rate of CO₂ production from glucose carbon in intact but not PX fetuses (Fig. 1). The rate of fetal glucose utilization tended to be less in fasted than fed animals but was not significantly reduced by maternal fasting in either group of fetuses (Fig. 1). Mean weight-specific rates of umbilical uptake and utilization of glucose, and of CO₂ production from glucose carbon, did not differ between PX and intact fetuses in the fasted state (Fig. 1). Fasting also had no effect on the fraction of fetal glucose use that was oxidized in either group of animals (Table 4). However, this fraction was significantly greater in PX than intact fetuses in fasted conditions (Table 4). The total rate of maternal glucose utilization and the rates of glucose utilization by the nonuterine and uteroplacental tissues decreased to the same extent in both groups of ewes and were not significantly different between the two groups after fasting for 48 h (Table 3). The percentage distribution of maternal glucose use between uterine and nonuterine tissues was also unaffected by fasting in both groups of ewes (Table 4).

Endogenous glucose production was activated by maternal fasting in both groups of fetuses; mean rates of glucogenesis were similar in PX and intact fetuses after maternal fasting for 48 h (Fig. 1). Fasting decreased the hepatic glycogen content and increased the hepatic activities of G6Pase and PEPCK in the intact fetuses but had no apparent effect on these variables in the PX fetuses (Fig. 2). Thus, there were no significant differ-

![FIG. 1. Mean (±SE) of the rates of umbilical glucose uptake (A), glucose use (B), endogenous glucose production (C), and CO₂ production (D) from glucose carbon in intact and PX fetuses (n = 5 in both groups) in the fed (white columns) and the fasted states (gray columns) and the mean change in these values during the 48-h period of maternal fasting (black columns). * Significant change during fasting (P < 0.02, paired t test); †, significantly different from the value in the intact fetuses in the same nutritional state (P < 0.01, Student’s t test); ‡, significant rate of endogenous glucose production (P < 0.05, paired t test).](https://academic.oup.com/endo/article-abstract/153/8/4008/2424233)
TABLE 4. Oxygen metabolism

<table>
<thead>
<tr>
<th></th>
<th>Intact</th>
<th>Pancreatectomized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fed</td>
<td>Fasted</td>
</tr>
<tr>
<td>Blood flow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Umbilical (ml/min)</td>
<td>592 ± 38</td>
<td>570 ± 47</td>
</tr>
<tr>
<td>Umbilical (ml/min/kg)</td>
<td>174 ± 13</td>
<td>170 ± 21</td>
</tr>
<tr>
<td>Uterine (ml/min)</td>
<td>1225 ± 151</td>
<td>1206 ± 119</td>
</tr>
<tr>
<td>Uterine (ml/min/kg)</td>
<td>795 ± 128</td>
<td>792 ± 135</td>
</tr>
<tr>
<td>Fraction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose oxidized</td>
<td>0.469 ± 0.077</td>
<td>0.398 ± 0.039</td>
</tr>
<tr>
<td>Oxygen for glucose oxidation</td>
<td>0.294 ± 0.023</td>
<td>0.179 ± 0.034</td>
</tr>
<tr>
<td>Total O₂ uptake (μmol/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uterus</td>
<td>1344 ± 61</td>
<td>1221 ± 20</td>
</tr>
<tr>
<td>Uteroplacental tissues</td>
<td>502 ± 67</td>
<td>418 ± 100</td>
</tr>
<tr>
<td>Fetus</td>
<td>842 ± 56</td>
<td>803 ± 92</td>
</tr>
<tr>
<td>Uterine O₂ uptake (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uteroplacental tissues</td>
<td>38.5 ± 3.6</td>
<td>33.8 ± 7.4</td>
</tr>
<tr>
<td>Fetus</td>
<td>61.5 ± 3.6</td>
<td>66.2 ± 6.8</td>
</tr>
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</table>

Mean (±SEM) values of absolute and weight-specific umbilical and uterine blood flow, the fraction of umbilical oxygen uptake used for glucose carbon oxidation by the fetus, the fraction of glucose used that is oxidized, the total uterine, uteroplacental, and fetal uptake of oxygen, and the distribution of the total uterine oxygen uptake between the uteroplacental and fetal tissues in intact and pancreatectomized fetuses (n = 5 in each group) in fed conditions and after maternal fasting for 48 h.

- Per kilogram fetal body weight.
- Per kilogram total weight of uterus, placentomes, and fetal membranes.
- Significantly different from the value in intact fetuses in the same nutritional state (P < 0.05, ANOVA).
- Significantly different from the value in the fed state in the same group of fetuses (P < 0.05, paired t test).

Oxygen metabolism

Fed state

Uterine O₂ uptake did not differ between ewes with PX and intact fetuses in fed conditions (Table 4). There was also no significant difference in the rate of umbilical O₂ uptake between PX and intact fetuses either in absolute terms (Table 4) or when expressed per kilogram fetal body weight (Fig. 3). Absolute and weight-specific rates of O₂ consumption by the uteroplacental tissues were also similar in the two groups of fed animals (Table 4 and Fig. 3). Distribution of uterine O₂ uptake between the fetus and uteroplacental tissues was also similar in the two groups of fed ewes (Table 4). However, the fraction of the umbilical O₂ uptake that was used to oxidize glucose carbon was significantly less in PX than intact fetuses in the fed state (Table 4).

Fasted state

In ewes with intact fetuses, absolute rates of uterine, uteroplacental, and umbilical uptake of O₂ were unaffected by maternal fasting for 48 h (Table 4). There were also no changes in fetal and uteroplacental O₂ consumption during maternal fasting in these animals when values were expressed on a weight-specific basis (Fig. 3). In contrast, fasting reduced the rate of umbilical O₂ uptake by PX fetuses both in absolute terms (Table 4) and on a weight-specific basis (Fig. 3). Because there was no change in uterine O₂ uptake during fasting (Table 4), uteroplacental O₂ consumption was increased in response to fasting of ewes with PX fetuses, both in absolute value (Table 4) and when expressed per kilogram total uteroplacental weight (Fig. 3). Distribution of uterine O₂ uptake between fetal and uteroplacental tissues therefore differed with fasting in PX but not intact fetuses (Table 4).

Discussion

This study demonstrates that insulin deficiency alters the endocrine and metabolic responses of fetal sheep to short-term maternal fasting. In contrast to intact fetuses, there was no increase in the plasma concentrations of cortisol or norepinephrine in PX fetuses during maternal fasting, despite a similar degree of fetal hypoglycemia. Instead, there was a significant fasting-induced rise in the plasma epinephrine concentration in PX fetuses, which was not seen in intact fetuses. Umbilical glucose uptake decreased to a similar extent in both groups of fasted animals but was associated with a significant fall in rate of glucose carbon oxidation only in intact fetuses. Pancreatectomized fetuses, on the other hand, lowered their rate of oxidative metabolism by 15–20%
during maternal fasting, unlike intact fetuses. Both groups of fetuses produced glucose endogenously after maternal fasting for 48 h, which prevented any significant fall in the rate of fetal glucose utilization. However, although intact fetuses appeared to activate both the glycogenolysis and gluconeogenesis, PX fetuses appeared more dependent on gluconeogenesis as their hepatic glycogen content did not fall with maternal fasting as occurred in intact fetuses. Intact and insulin deficient PX fetuses therefore have different strategies in responding to the reduced glucose availability induced by maternal fasting.

The lack of a fetal cortisol response to maternal fasting after PX suggests that sensitivity of the hypothalamic-pituitary-adrenal (HPA) axis to hypoglycemia is reduced in PX fetuses. This may be the consequence of the chronic fetal hyperglycemia and hypoinsulinemia preceding maternal fasting as the sensitivity of both the pituitary and adrenal glands to insulin-induced hypoglycemia is diminished in adult diabetic rats (31). Alternatively, the changes in HPA function may be associated with the reduced growth of PX fetuses. The adrenal cortex is known to be less sensitive to both exogenous ACTH and endogenous stresses, such as hypoxemia, in twin than larger singleton sheep fetuses (32, 33). Because the cortisol response to hypoglycemia normally increases in fetal sheep toward term (34), the current findings may reflect a delay in HPA axis maturation in the more slowly growing PX fetus, as occurs in twins (33).

Total catecholamine concentrations increased in PX fetuses during maternal fasting so the hypothalamus is sensitive to fetal hypoglycemia and can initiate a sympathetic and/or sympathoadrenal medullary response in these fetuses despite the lack of an adrenocortical response. However, the contribution of epinephrine and norepinephrine to the total catecholaminergic response differed in PX and intact fetuses. The epinephrine increment was significant in PX fetuses, whereas norepinephrine was the predominant component of the response in intact fetuses. Because plasma epinephrine is secreted from the adrenal medulla whereas circulating norepinephrine is derived from both the adrenal medulla and the sympathetic nerve terminals (35), the fetal catecholaminergic response to fasting induced-hypoglycemia may be more dependent on the sympathoadrenal medullary than sympathetic contribution in PX fetuses, whereas the reverse dependence appears to occur in intact fetuses. Alternatively, the adrenal medulla of PX fetuses may contain proportionately more epinephrine than norepinephrine than in intact fetuses.

During fasting, umbilical glucose uptake fell to a similar extent in both groups of fetuses and resulted in a similar degree of fetal hypoglycemia. However, the metabolic response to this decrease in glucose availability differed in the two groups of fetuses. Intact fetuses reduced their rate of glucose carbon oxidation significantly, whereas PX fetuses did not. Thus, the fraction of glucose used by the fetal tissues that was oxidized was lower in intact than PX fetuses during
fasting. Because CO₂ production from glucose carbon depends on both the glucose and insulin concentrations in the fetus (5), these differences probably reflect the differing insulin response to fasting in the two groups, with a fall in insulin concentrations in the intact but not the PX fetuses in response to the fetal hypoglycemia. This is consistent with the finding that the absolute rate of CO₂ production in hypoinsulinemic intact fetuses in the fasted state was similar to that of insulin deficient PX fetuses in fed conditions despite the differences in fetal glycemia. The current findings also indicate that, under physiological conditions, CO₂ production from glucose carbon by fetal sheep may be more dependent on insulin than on glucose concentrations.

Despite oxidizing less glucose carbon, intact fetuses maintained their overall rate of oxygen consumption during maternal fasting. They must therefore have been using oxidative substrates other than glucose such as lactate, volatile fatty acids, ketones, and/or amino acids. The most likely alternative fuels for oxidative metabolism are the amino acids as leucine oxidation and production of urea, the deamination product of amino acid catabolism, are known to increase in fetal sheep in response to maternal undernutrition (36, 37). In contrast, PX fetuses reduced their rate of oxygen consumption and thereby lowered their total demand for oxidative substrates during maternal fasting. Amino acid concentrations are already higher than normal in PX fetuses in the fed state (10), consistent with a greater use of amino acids for oxidative purposes when glucose uptake by fetal issues is impaired by insulin deficiency (11). The growth-restricted PX fetus may therefore be unable to mobilize any further reserves to maintain glucose endogenously to help maintain the fetal rate of glucose utilization when the umbilical glucose supply was reduced by maternal fasting. In adults, hepatic glucose production is activated overnight by a decrease in the ratio of the insulin to glucagon concentrations and, in longer-term fasting, by increased sympathetic activity (35, 38). Previous studies in fetal sheep have shown that fetal glucogenesis occurs both during experimental reductions in the fetal insulin to glucagon ratio and in response to maternal fasting when there are increases in the fetal cortisol and catecholamine concentrations (9, 15, 18, 39). In the current study, glucogenesis occurred in both groups of fetuses, despite the differences in their endocrine response to maternal fasting. Because total catecholamine concentrations were elevated in both groups of fetuses and hepatic glucose production can be stimulated by both catecholamines in fetal sheep (39), activation of fetal glucogenesis during fasting in the present study was probably due to increased sympathetic activity either directly via the splanchnic nerves to the liver or indirectly by adrenomedullary release of circulating catecholamines. Certainly neither a change in the insulin level nor an elevated cortisol level was essential for inducing glucogenesis in PX fetuses during maternal fasting.

Cortisol is known to increase hepatic activities of G6Pase and PEPCK in fetal sheep during late gestation, which may explain the increase in these enzyme activities in response to fasting and hence the cortisol dependence of fetal glucogenesis in intact fetuses in this and previous studies of maternal fasting (15, 18, 27). Hepatic activities of G6Pase and PEPCK were already greater in PX relative to intact fetuses, suggesting a cortisol-mediated induction in PX fetuses, but the relative contribution of cortisol to the increase in hepatic glucogenesis during fasting remains to be determined.
endocrine and metabolic consequences of chronic insulin deficiency *in utero*.

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