Regulation of glucogenesis by thyroid hormones in fetal sheep during late gestation

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Abstract

The effects of thyroid hormone deficiency in utero on the fetal glucogenic capacity were investigated by measuring glucose production and hepatic levels of glycogen and gluconeogenic enzymes in normal sheep fetuses in the fed and fasted states during late gestation and in those made thyroid hormone deficient by fetal thyroidectomy (TX). In the fed state, fetal TX had no effect on glucose uptake, utilisation or production by the fetus. It also had no apparent effect on the glycogen content or activities of the key gluconeogenic enzymes in the fetal liver. In addition, fetal plasma concentrations of insulin, cortisol, adrenaline or noradrenaline were unaffected by fetal TX in the fed state. In contrast, the rates of fetal O2 consumption and CO2 production per kilogram fetal bodyweight were significantly lower in TX than in intact fetuses in the fed state (P<0.05). TX prevented fetal glucose production in response to maternal fasting for 48 h. It also abolished the normal decreases in the fetal glucose carbon oxidation fraction, the rate of CO2 production from glucose carbon and in the fraction of the umbilical O2 uptake used for glucose carbon oxidation that occur during fasting in intact fetuses. At the end of the fast, plasma noradrenaline concentrations and hepatic levels of glycogen, glucose 6-phosphatase, fructose diphosphatase and alanine aminotransferase were significantly lower in TX than in intact fetuses. These observations show that thyroid hormones are essential for glucogenesis in the sheep fetus during late gestation and suggest that these hormones act both on the hepatic gluconeogenic pathways and on the mechanisms activating glucogenesis in utero.

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Introduction

In normal well-fed pregnant ewes, the fetal requirement for glucose is met almost entirely by transplacental transport of glucose from the mother with little, if any, glucogenesis by the fetus itself (Hay 1995). However, during adverse intrauterine conditions such as undernutrition, hypoxia or placental insufficiency, glucogenesis has been shown to occur in the sheep fetus, particularly in animals close to term (Jones et al. 1983, Hay et al. 1984, Owens et al. 1989, Dalinghaus et al. 1991, Fowden et al. 1998b). Glucogenesis is also a major source of circulating glucose at birth during the period between placental separation and the establishment of nutritive suckling (Fowden et al. 1998a). The ability of the fetus to produce glucose endogenously depends on the hepatic glycogen and gluconeogenic enzyme levels and on the functional capacity of the mechanisms that normally activate the gluconeogenic pathways, such as catecholamine release (see Silver 1990). In turn, deposition of hepatic glycogen, upregulation of gluconeogenic enzymes and induction of hepatic adrenoreceptors all depend on the prepartum increase in fetal plasma cortisol (Barnes et al. 1978, Fowden et al. 1993, Barnes 1997).

In adult animals, glucogenesis is also influenced by thyroid hormones (see Dauncey 1990). Hyperthyroidism enhances gluconeogenesis and glucose utilisation, while conversely, hypothyroidism reduces the capacity for gluconeogenesis, in part, by lowering hepatic glycogen synthesis (Malbon & Campbell 1984, Bollen & Stalman 1988). Much less is known about the effects of thyroid hormones on glucogenesis in utero, although thyroid hormones are known to mediate some of the maturational effects of cortisol close to term (Fowden et al. 1998b). In the lungs, thyroid hormones synergise with cortisol in regulating lung liquid reabsorption and the structural changes which enable pulmonary gas exchange to occur at birth (Barker et al. 1988, Liggins 1994). Similarly, in fetal liver, thyroid hormones contribute to the switch in somatotrophic gene expression which activates the endocrine synthesis of insulin-like growth factor-I essential for postnatal growth (Forhead et al. 1998, 2000). Administration of thyroxine (T4) to neonatal rats has been shown to enhance the hepatic activity of glucose 6-phosphatase (G6P), the
final rate-limiting enzyme in the glucogenic pathways (Greengard 1971). However, there have been no studies of the effects of thyroid hormone deficiency in utero on the glucogenic capacity of the fetus. Hence, in the present study, fetal glucose production, and hepatic glycogen and gluconeogenic enzyme levels have been measured in normal sheep fetuses in the fed and fasted states during late gestation and in those made thyroid hormone deficient by fetal thyroidectomy (TX).

Materials and Methods

Animals

A total of 18 Welsh Mountain ewes carrying single fetuses of known gestational age were used in this study. All the fetuses were alive at delivery. The ewes were housed in individual pens and maintained on concentrates (200 g/day; Beart Ltd, Stowbridge, Suffolk, UK) with hay and water freely available. Food but not water was withdrawn for 18–24 h before surgery.

Surgical procedures

Between 102 and 111 days (term 145 ± 2 days), nine fetuses underwent TX under halothane anaesthesia (1-5% in O₂/N₂O) using surgical procedures described previously (Hopkins & Thorburn 1972). Sham operations in which the fetal head was exteriorised were carried out on four fetuses at the same range of gestational ages. The TX and sham operated fetuses were catheterised at a second operation 20–27 days later together with five additional intact fetuses aged 124–130 days which had had no previous surgery. Catheters were inserted into the maternal artery, uterine vein, umbilical vein, fetal aorta and caudal vena cava of all the animals, as described by Comline & Silver (1972). At the end of the experimental period, the fetuses were delivered by Caesarean section under general anaesthesia (10 mg/kg i.v. sodium pentobarbitone, 200 mg/kg i.v. into the ewe). The remaining four TX and four intact fetuses (two sham operated, two not sham operated) were sampled daily for hormone measurement until delivery by Caesarean section in the fed state at gestational ages similar to those of the fasted ewes. Samples of liver were collected from all the fetuses after administration of a lethal dose of anaesthetic (sodium pentobarbitone, 200 mg/kg i.v. into the fetus). The tissue was frozen immediately in liquid nitrogen and stored at −80 °C until assay for glycogen content and gluconeogenic enzyme activities. Morphometric measurements of the fetus were also made at delivery. No thyroid remnants were found in any of the TX fetuses at post mortem after delivery.

Biochemical analysis

The blood gas status, O₂ content and whole blood concentrations of glucose, [¹⁴C]glucose, ³H₂O and ¹⁴CO₂ were measured in all four sets of simultaneous samples. Blood O₂ content was calculated from the percentage O₂ saturation and the haemoglobin concentration measured with a Radiometer OSM2 Hemoximeter (Radiometer, Copenhagen, Denmark) that had been calibrated with ovine blood (Owens et al. 1987). Blood pH, pO₂ and pCO₂ were measured using ABL 330 Radiometer equipment and corrected for a fetal body temperature at 39 °C. The concentration of whole blood glucose was determined enzymatically with glucose oxidase (Sigma Chemical Co., Poole, Dorset, UK) while ³H₂O concentrations were measured in plasma using a scintillation counter. The latter values were corrected for quenching and converted
to blood concentrations as described by Bell et al. (1987). Labelled glucose and CO₂ concentrations were determined using chemical methods described previously (Hay et al. 1981, 1983). The mean recovery of labelled glucose from the anion exchange columns was 97·9 ± 0·9% (s.e.m.) (n = 20) and, therefore, no corrections for glucose recovery were made. The mean recovery of 14CO₂ from blood was 71·8 ± 6·0% (n = 20) and hence, all blood 14CO₂ values have been corrected for recovery.

Plasma T₄, triiodothyronine (T₃), cortisol and insulin concentrations were measured using RIAs validated for use with ovine plasma. Plasma concentrations of total T₄ concentrations were measured using diagnostic kits (ICN Biochemicals). The interassay coefficients of variations were 10% in each case and the minimum detectable quantity of hormone was 7 ng/ml for T₄ and 0·1 ng/ml for T₃. Plasma cortisol and insulin concentrations were measured as described previously (Fowden 1980, Robinson et al. 1983). The interassay coefficients of variation for the cortisol and insulin assays were 10·0 and 13·7% respectively, and the minimum detectable quantity of hormone was 1·0 ng/ml for cortisol and 5·0 µU/ml for insulin.

Plasma catecholamine concentrations were determined by HPLC using electrochemical detection (Silver et al. 1987). The samples were prepared by absorption of 300–500 µl plasma onto acid-washed alumina and 20 µl aliquots of the 100 µl perchloric acid eluates were injected into the column. Isoproterenol was added as the internal standard to each plasma sample before absorption. Recovery ranged from 63 to 97% and all catecholamine values have been corrected for their respective recoveries. The limits of sensitivity of the method were 50 pg/ml for adrenaline and 40 pg/ml for noradrenaline. The interassay coefficients of variation for adrenaline and noradrenaline were 7·3 and 6·2% respectively.

Hepatic levels of glycogen, G6P (EC 3·1·3·9), fructose diphosphatase (FDP, EC 3·1·3·11), phosphoenolpyruvate carboxykinase (PEPCK, EC 4·1·1·32), alanine aminotransferase (AAsAT, EC 2·6·1·2) and aspartate aminotransferase (AspAT, EC 2·6·1·1) were measured as described previously (Barnes et al. 1978, Fowden et al. 1993).

Calculations

All calculations were made by using equations derived for steady state kinetics. Umbilical flows were measured using the ³H₂O steady state diffusion technique (Meschia et al. 1967, Bell et al. 1987). Net umbilical uptake rates of glucose and O₂ and net umbilical excretion rates of [14C]glucose and 14CO₂ were calculated by the Fick principle as the product of umbilical blood flow times the venous–arterial (uptake) or arterio–venous concentration difference (excretion) across the umbilical circulation. The net umbilical O₂ uptake rate (µmol/min) was used to oxidise fetal glucose and fractional utilisation of glucose was calculated from the Fick principle as the product of fetal arterial (O₂ uptake) or arterio–venous concentration difference (excretion) across the umbilical circulation.

The fetal rates of utilisation and production of glucose, CO₂ production from glucose carbon, and the fraction of the net umbilical O₂ uptake used for oxidation of glucose carbon by the fetus were calculated according to the following equations (Hay et al. 1981, 1983).

Fetal glucose utilisation (µmol/min) = [net fetal tracer uptake (d.p.m./min)]/[fetal arterial glucose specific activity (d.p.m./µmol glucose)], where net fetal tracer uptake (d.p.m./min) = [tracer glucose infusion rate (d.p.m./min)] – [net umbilical tracer glucose excretion rate (d.p.m./min)].

Oxidation of fetal glucose carbon was calculated as the rate of 14CO₂ production (µmol/min) = [net umbilical 14CO₂ excretion rate (d.p.m./min)]/[fetal arterial blood glucose specific activity (d.p.m./µmol glucose carbon)].

The glucose carbon oxidation fraction and the fraction of umbilical O₂ uptake consumption used to oxidise glucose carbon in the fetus were then calculated as follows. Glucose carbon oxidation fraction (fraction of fetal glucose carbon utilisation rate that is oxidised) = [net umbilical 14CO₂ excretion rate (d.p.m./min)]/[net fetal tracer glucose uptake (d.p.m./min)]. Fraction of O₂ uptake used for oxidation of glucose carbon = [amount of O₂ used to oxidise fetal glucose carbon (µmol/min)]/[net umbilical O₂ uptake rate (µmol/min)], where the amount of O₂ used to oxidise fetal glucose = the amount of CO₂ produced by this oxidative process (from equation for oxidation of fetal glucose carbon above).

Endogenous glucose production (µmol/min) = [fetal glucose utilisation (µmol/min)] – [umbilical glucose uptake (µmol/min)].

In the second tracer study, the values for [14C]glucose and 14CO₂ in the 0 min arterial and umbilical venous samples of the second study were subtracted from the values in the subsequent corresponding samples before calculation of the glucose metabolic rates. All metabolic rates have been expressed per kilogram fetal bodyweight. No increase in fetal bodyweight was assumed to occur during the 48 h period of maternal food withdrawal.

Statistical analyses

Steady state was defined as <10% variation of values around the mean for each sampling period with no consistent trend for the absolute values or venous–arterial concentration differences to increase or decrease with time. Mean values ± s.e.m. have been used throughout. Statistical analyses were made according to the methods of Armitage (1971). Statistical significance between groups was assessed using one–way ANOVA with Fisher’s projected least significant differences, Student’s paired and unpaired t-tests, where appropriate. Simple correlation coefficients were calculated by regression analyses and assessed for significance using Fisher’s t-test. Differences at the 5% level were considered significant. In common with previous studies (Fowden & Silver 1995), there was no
apparent difference between intact fetuses that had and had not been sham operated. Hence, all data from the intact fetuses in this study have been combined for subsequent analyses.

Results

Basal values in the fed state

Hormone and metabolite concentrations Fetal TX reduced plasma T₄ and T₃ levels to the limits of sensitivity of the assays throughout the experimental period. Mean plasma concentrations of T₄ and T₃ in the TX fetuses were therefore significantly less than those in intact fetuses (Table 1). No significant differences in fetal or maternal concentrations of plasma glucose were observed between the two groups of animals (Table 2). The fetal arterial concentrations of plasma cortisol, insulin, adrenaline and noradrenaline were also not significantly different in the intact and TX fetuses in the fed state (Table 3); mean values were similar to those published previously for intact and TX sheep fetuses during late gestation (Fowden & Silver 1995). Nor were there any significant differences in the glucose oxidation fraction and the fraction of umbilical O₂ uptake used to oxidise glucose carbon (Fig. 1). However, the rates of umbilical O₂ uptake and CO₂ production from glucose carbon were significantly less in TX than in intact fetuses even when the differences in fetal bodyweight were taken into account (Fig. 1). No endogenous glucose production was observed in either group of fetuses in the fed state (Table 2).

Hepatic glycogen and gluconeogenic enzyme levels There were no significant differences in hepatic glycogen or gluconeogenic enzyme levels between the two groups of fetuses in the fed state (Table 4).

The effects of maternal fasting

Hormone and metabolite concentrations Fasting reduced fetal and maternal concentrations of blood glucose in both TX and intact fetuses; the mean decrements in blood glucose during fasting and the degree of hypoglycaemia observed at the end of the fast were similar in the two groups of animals (Table 2). Fasting had no effect on fetal plasma T₄ or T₃ levels in the two groups; mean concentrations at the end of the fast remained undetectable in the TX fetuses and were not significantly different between fed andfasted states in intact animals (Table 1). However, there were significant decreases in plasma insulin and significant increases in plasma cortisol during fasting in both the TX and intact fetuses (Table 3). The absolute levels of plasma insulin and cortisol at the end of

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Table 1 Mean ± S.E.M. plasma concentrations of T₄, T₃ and cortisol and bodyweights, crown rump lengths and limb lengths of intact and TX fetuses at delivery in the fed state or at the end of a 48 h period of maternal fasting before delivery

<table>
<thead>
<tr>
<th></th>
<th>Intact Fed</th>
<th>Intact Fasted</th>
<th>TX Fed</th>
<th>TX Fasted</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma (ng/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T₄</td>
<td>133 ± 19</td>
<td>121 ± 13</td>
<td>7 ± 2**</td>
<td>6 ± 2**</td>
</tr>
<tr>
<td>T₃</td>
<td>0.21 ± 0.07</td>
<td>0.43 ± 0.13</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Cortisol</td>
<td>24.6 ± 8.4</td>
<td>41.0 ± 10.8</td>
<td>26.7 ± 6.7</td>
<td>34.9 ± 7.4</td>
</tr>
<tr>
<td><strong>Bodyweight (g)</strong></td>
<td>3664 ± 200</td>
<td>3435 ± 182</td>
<td>2958 ± 77*</td>
<td>2721 ± 210*</td>
</tr>
<tr>
<td><strong>Length (cm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crown rump</td>
<td>51.1 ± 1.0</td>
<td>50.6 ± 0.7</td>
<td>45.8 ± 1.3**</td>
<td>45.4 ± 108**</td>
</tr>
<tr>
<td>Forelimb</td>
<td>26.8 ± 0.7</td>
<td>26.0 ± 0.4</td>
<td>23.6 ± 0.4**</td>
<td>22.6 ± 0.8**</td>
</tr>
<tr>
<td>Hind limb</td>
<td>32.4 ± 0.9</td>
<td>32.5 ± 0.5</td>
<td>28.1 ± 0.8*</td>
<td>27.8 ± 1.0*</td>
</tr>
<tr>
<td><strong>Number of animals</strong></td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

ND=not detected.
Significantly less than values observed in intact fetuses in a similar nutritional state, *P<0.05, **P<0.01 (ANOVA).
the fast and the mean changes in concentration during fasting were similar in the two groups of fetuses (Table 3).

By contrast, there were no significant changes in the individual or total catecholamine concentrations during fasting in either the TX or intact fetuses (Table 3). However, at the end of the fast, plasma noradrenaline and total catecholamine levels were significantly less in TX than in intact fetuses (Table 3).

**Metabolic rates** Maternal fasting induced significant reductions in umbilical glucose uptake in both the intact and TX fetuses which were of similar magnitude (Table 2). These changes were accompanied by a significant reduction in fetal glucose utilisation in the TX, but not in the intact fetuses (Table 2). Consequently at the end of the fast, there was a significant rate of endogenous glucose production in the intact fetuses but not in the TX animals (Table 2).

Fasting had no effect on umbilical blood flow (Table 2) or on umbilical O₂ uptake in either the TX or intact fetuses (Fig. 1). However, it did reduce the glucose oxidation fraction, the rate of CO₂ production from glucose carbon and the fraction of O₂ used for glucose carbon oxidation in the intact fetuses (Fig. 1). None of these parameters changed significantly in response to maternal fasting in the TX fetuses (Fig. 1).

**Hepatic glycogen and gluconeogenic enzyme levels**

In both TX and intact fetuses, hepatic glycogen levels were significantly lower after 48 h of maternal fasting than in fed animals (Table 4). Mean hepatic glycogen content in the fasted state was also significantly less in TX than in intact fetuses (Table 4). Hepatic activities of G6P, FDP and AlaAT were significantly higher in the fasted than in fed state in the intact fetuses but not in the TX fetuses (Table 4). The mean values of these three enzymes were therefore significantly greater in intact than in TX fetuses after 48 h of maternal fasting (Table 4). No significant differences in the hepatic activities of PEPCK and AspAT were observed between the fed and fasted states in either group of fetuses (Table 4). When the data from the fed and fasted animals were combined, there were significant positive correlations between the plasma cortisol concentration and the hepatic activities of G6P, FDP and PEPCK in the intact fetuses (G6P, \( r = 0.715, n = 9, P < 0.02 \); FDP, \( r = 0.703, n = 9, P < 0.05 \); PEPCK, \( r = 0.798, n = 9, P < 0.01 \)) but not in the TX fetuses (G6P, \( r = 0.015, n = 9 \); FDP, \( r = 0.397, n = 9 \); PEPCK, \( r = 0.525, n = 9 \)). No significant correlations were observed between fetal plasma cortisol and the hepatic activities of AspAT or AlaAT in either group of fetuses. The range of cortisol values observed in utero was similar in the TX (10.0–65.5 ng/ml) and intact fetuses (12.5–73.0 ng/ml). There were also no significant correlations between the plasma T₃ concentrations and the hepatic activities of any of the five enzymes in either the TX or intact fetuses.
Table 3 Mean ± S.E.M. plasma concentrations of insulin, cortisol, adrenaline, noradrenaline and total catecholamines in intact (n=5) and TX (n=5) fetuses in the fed state and at the end of maternal fasting for 48 h, and the mean change in values (Δ) during fasting.

<table>
<thead>
<tr>
<th></th>
<th>Intact Fed</th>
<th>Fasted</th>
<th>Δ</th>
<th>TX Fed</th>
<th>Fasted</th>
<th>Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (µU/ml)</td>
<td>21.6 ± 2.1</td>
<td>14.0 ± 1.6</td>
<td>-7.6 ± 1.4**</td>
<td>21.3 ± 3.8</td>
<td>11.8 ± 1.5</td>
<td>-9.9 ± 2.9**</td>
</tr>
<tr>
<td>Cortisol (ng/ml)</td>
<td>164 ± 2.7</td>
<td>41.0 ± 10.8</td>
<td>+246 ± 9.9*</td>
<td>190 ± 2.5</td>
<td>34.9 ± 7.4</td>
<td>+155 ± 6.8*</td>
</tr>
<tr>
<td>Adrenaline (pg/ml)</td>
<td>45 ± 31</td>
<td>65 ± 41</td>
<td>+23 ± 13</td>
<td>0</td>
<td>50 ± 29</td>
<td>+50 ± 29</td>
</tr>
<tr>
<td>Noradrenaline (pg/ml)</td>
<td>410 ± 140</td>
<td>822 ± 306</td>
<td>+413 ± 351</td>
<td>192 ± 54</td>
<td>282 ± 64*</td>
<td>+90 ± 56</td>
</tr>
<tr>
<td>Total catecholamines (pg/ml)</td>
<td>455 ± 140</td>
<td>887 ± 276</td>
<td>+433 ± 342</td>
<td>192 ± 54</td>
<td>332 ± 78*</td>
<td>+140 ± 80</td>
</tr>
</tbody>
</table>

Significant change during fasting, *P<0.05, **P<0.01 (paired t-test).
Significantly different from values in intact fetuses in the same nutritional state, *P<0.02, (unpaired t-test).

Discussion

The results demonstrate that thyroid hormones are essential for the sheep fetus to be able to produce glucose endogenously in response to short-term undernutrition during late gestation. In normal sheep fetuses, undernutrition in late gestation has been shown to induce glucogenesis in this and previous studies (Hay et al. 1983, Dalinghaus et al. 1991, Fowden et al. 1998b). The rise in fetal glucose production in these circumstances helps to ameliorate the fall in umbilical glucose supply and, in the present study, prevented a significant fall in the rate of fetal glucose utilisation. In contrast, short-term undernutrition did not stimulate glucogenesis in the TX fetuses in the present study despite a degree of hypoglycaemia similar to that seen in the intact animals. Consequently, there were significant reductions in both the rates of umbilical uptake and utilisation of glucose in the TX fetuses during maternal fasting. The absence of significant glucogenesis in these fetuses was accompanied by reduced circulating catecholamine concentrations and by low levels of hepatic glycogen and key gluconeogenic enzymes at the end of the 48 h period of maternal fasting. Thyroid hormone deficiency in utero therefore appears to prevent fetal glucose production in response to undernutrition at least, in part, by effects on both the gluconeogenic pathways and the mechanisms that activate them.

In TX fetuses, unlike intact animals, there were no significant increases in the hepatic activities of G6P, FDP and AlaAT in response to maternal fasting. Hepatic PEPCK activities tended to be higher in the fasted than the fed state in both groups of fetuses but were not significantly so. There were also no significant differences in PEPCK activity between the intact and TX fetuses in either nutritional state. These observations suggest that either PEPCK is unlikely to be the rate-limiting enzyme in fetal glucogenesis or that glucose production is occurring primarily via glycogenolysis in the intact fetuses during maternal fasting. During normal conditions, the activities of many of the gluconeogenic enzymes are dependent on the fetal cortisol concentration (Fowden et al. 1993). Certainly, in the present study, hepatic activities of G6P, FDP and PEPCK in the intact fetuses were positively correlated with the plasma cortisol concentration, irrespective of nutritional state. Plasma cortisol levels are known to rise normally toward term in TX fetuses (Hopkins & Thornburn 1972) and increased to
Table 4 Mean ± s.e.m. values of hepatic glycogen, G6P, FDP, PEPCK, AspAT and AlaAT in intact and TX fetuses delivered at 138–141 days either in the fed state or at the end of 48 h of maternal fasting, number of fetuses in parentheses

<table>
<thead>
<tr>
<th>State</th>
<th>Glycogen (mg/g)</th>
<th>G6P (U/g)</th>
<th>FDP (U/g)</th>
<th>PEPCK (U/g)</th>
<th>AspAT (U/g)</th>
<th>AlaAT (U/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fed (4)</td>
<td>73.0 ± 11.0</td>
<td>3.82 ± 1.1</td>
<td>2.65 ± 0.73</td>
<td>1.56 ± 0.40</td>
<td>26.3 ± 4.2</td>
<td>2.13 ± 0.62</td>
</tr>
<tr>
<td>Fasted (5)</td>
<td>41.6 ± 7.9*</td>
<td>8.14 ± 1.2*</td>
<td>8.05 ± 0.77**</td>
<td>2.13 ± 0.35</td>
<td>33.4 ± 1.7</td>
<td>12.31 ± 0.80**</td>
</tr>
<tr>
<td>TX</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fed (4)</td>
<td>64.9 ± 8.5</td>
<td>3.12 ± 0.25</td>
<td>1.93 ± 0.17</td>
<td>1.51 ± 0.23</td>
<td>39.9 ± 3.5</td>
<td>1.86 ± 0.36</td>
</tr>
<tr>
<td>Fasted (5)</td>
<td>20.7 ± 3.3**</td>
<td>3.77 ± 0.40**</td>
<td>1.75 ± 0.21**</td>
<td>2.65 ± 0.41</td>
<td>32.8 ± 3.0</td>
<td>2.34 ± 0.10**</td>
</tr>
</tbody>
</table>

Significantly different from values in fed state in the same endocrine state, *p<0.05, **p<0.01.
Significantly different from values in intact fetuses in the same nutritional state, *p<0.05, **p<0.01.

a similar extent in TX and intact fetuses in response to maternal fasting in the present study. However, when all the data from the TX fetuses were combined irrespective of nutritional state, none of the gluconeogenic enzyme activities measured in this study were significantly correlated with fetal plasma cortisol. These observations suggest that, in common with other maturational processes (Forhead et al. 1998, 2000), thyroid hormones are essential for the cortisol-induced upregulation of gluconeogenic enzyme activities in the fetal liver. Intrauterine thyroid hormone deficiency may therefore limit glucose production in response to undernutrition, in part, by preventing the stimulatory effect of cortisol on the activities of key gluconeogenic enzymes in the fetal liver.

Undernutrition reduced the hepatic glycogen content in both the intact and TX fetuses but appeared to have a more pronounced effect after fetal TX. Hepatic glycogen content is determined by the net rate of synthesis and utilisation of glycogen and can be reduced by enhanced glycogenolysis, impaired glycogen deposition or by a combination of both processes. Enhanced hepatic glycogenolysis is unlikely to explain the low hepatic glycogen content of the TX fetus during fasting as no endogenous glucose production was observed by the fetus as a whole in these circumstances. In normal fetal sheep, hepatic glycogen deposition depends on gluconeogenesis and the activity of glycogen synthase (Levitsky et al. 1988). In adult rats, thyroid hormones enhance total glycogen synthase activity in the liver and the percentage of synthase in the active form (Malbon & Campbell 1984, Bollen & Stalmann 1988). Reduced glycogen synthase in the liver of the TX fetus could therefore account, in part, for the low level of hepatic glycogen in these fetuses during maternal fasting. However, any action of thyroid hormone deficiency on glycogen synthase does not appear to effect glycogen deposition when glucose is more abundant as hepatic glycogen levels were similar in TX and intact fetuses in the fed state. The low hepatic glycogen levels in the TX fetus during fasting are, therefore, more likely to be due to the low hepatic FDP and AlaAT activities in these circumstances and/or reduced availability of gluconeogenic precursors, such as lactate and amino acids. These metabolite concentrations were not measured in the present study but lactate levels have been shown to be within the normal range in TX fetuses in previous studies of well-fed ewes (Bhakthavathsalan et al. 1977).

The low catecholamine concentrations at the end of the fast may also have contributed to the lack of glucose production in the TX fetuses during fasting. Fetal glucose production has been shown to occur in response to catecholamines but only when the total catecholamine concentration in fetal plasma exceeds 500 pg/ml (Apatu & Barnes 1991, Menon et al. 1990, Milley 1997, Fowden et al. 1998b). Since this value is higher than the concentrations observed in the TX fetuses during maternal fasting, the lack of gluconeogenesis in these fetuses was not entirely unexpected. In the TX fetuses, the fasting-induced rise in fetal catecholamine levels, particularly of noradrenaline, was small compared with that in the controls, despite the same degree of fetal hypoglycaemia. These observations are consistent with previous findings that showed a reduced noradrenaline response to hypoxaemia after TX of the sheep fetus (Walker & Schuijers 1989). Thyroid hormone deficiency in utero therefore appears to impair the functional development of the sympathetic nervous system in the sheep fetus although it has little apparent effect on the catecholamine content of the fetal adrenal medulla itself (Walker & Schuijers 1989). In addition, if thyroid hormones have similar effects on adrenoreceptor development in the liver as seen in other fetal tissues (Padbury et al. 1986, Barker et al. 1988, Warburton et al. 1988, Birk et al. 1992, Barnes 1997), catecholamines may be less effective at stimulating glycogenolysis in TX fetuses, even at concentrations above the 500 mg/ml threshold. Furthermore, since thyroid hormones are known to influence development of the central nervous system in sheep and other species (Erenberg et al. 1974, Bhakthavathsalan et al. 1977, Timiras & Nzekwe 1989), there may be changes in the central as well as the peripheral responses to undernutrition in the hypothyroid fetus. Consequently, thyroid hormones may enhance the capacity for sympathetic activation of hepatic glucose production both via the splanchnic nerves and via

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endocrine release of catecholamines and other glucogenic hormones such as glucagon.

In addition to abolishing fetal glucose production in response to maternal fasting, fetal TX prevented the decrements in the glucose carbon oxidation fraction, the rate of CO₂ production from glucose carbon and in the fraction of umbilical O₂ uptake used for glucose carbon oxidation that occurred in the intact fetuses during undernutrition. Previous studies of normal sheep fetuses have shown that these variables are controlled, in part, by the fetal levels of glucose and insulin and fall as the fetus becomes hypoglycaemic and hypoinsulinaemic during maternal fasting (Hay et al. 1983, 1989, Fowden et al. 1998b). However, differences in the fetal glucose and insulin concentrations are unlikely to explain the absence of any significant change in glucose carbon oxidation in the TX fetuses as the degree of hypoglycaemia and hypoinsulinaemia in these fetuses was similar to that seen in intact animals during fasting. Since the weight-specific rate of CO₂ production from glucose carbon was low in the TX fetuses even in the fed state, oxidative glucose metabolism may already have been at or near the minimum value before the induction of fetal hypoglycaemia and hypoinsulinaemia by maternal fasting. Since thyroid hormones regulate the rate of O₂ consumption by the fetal tissues (Fowden & Silver 1995), oxidation of glucose carbon may be more dependent on the tissue availability of O₂ than of glucose after fetal TX. Indeed, the low rate of CO₂ production in the TX fetus may account, in part, for the lack of glucogenesis in response to maternal fasting as activity of the glucogenic pathways has been linked to tissue oxygenation in neonatal animals (Warnes et al. 1977, Girard et al. 1992).

In summary, thyroid hormones act to ensure that glucose production is activated by adverse intrauterine conditions, such as undernutrition, in the sheep fetus during late gestation. This action is consistent with the other known developmental effects of the thyroid hormones (Liggins 1994) although the extent to which it is direct or secondary to metabolic and other changes remains unclear. Thyroid hormone response elements have been identified on many glucoregulatory genes (Dauncey 1990) but thyroid hormones also induce changes in fetal oxidative metabolism, catecholamine concentrations and in the development of the sympathetic and central nervous systems, all of which could influence activation of the glucogenic pathways in utero. Whatever the mechanisms involved, the thyroid hormone dependence of fetal glucogenesis has important implications for fetal and neonatal viability. Hypothyroid fetuses are less likely to survive adverse intrauterine conditions or to adapt successfully to extrauterine life. Indeed, the hypothyroidism observed in clinical conditions, such as growth retardation and placental insufficiency, may contribute to the poor perinatal viability observed in these circumstances.

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