Regulation of gluconeogenesis 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 gluconeogenesis in the sheep fetus during late gestation and suggest that these hormones act both on the hepatic glucogenic pathways and on the mechanisms activating gluconeogenesis *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, gluconeogenesis by the fetus itself (Hay 1995). However, during adverse intrauterine conditions such as under-nutrition, hypoxia or placental insufficiency, gluconeogenesis 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.* 1998*b*). Gluconeogenesis 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.* 1998*a*). 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 glucogenic 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, gluconeogenesis 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 gluconeogenesis *in utero*, although thyroid hormones are known to mediate some of the maturational effects of cortisol close to term (Fowden *et al.* 1998*b*). 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 O_2/N_2O) 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).

Experimental procedures

Blood samples of 1.0 ml were taken daily from the fetus and mother to monitor fetal well being. At least 7 days after catheterisation, measurement of glucose metabolism was made in both TX fetuses ($n=5$) and intact ($n=5$; $n=2$ sham operated, $n=3$ not sham operated). Tritiated water (3H_2O , 8 $\mu Ci/ml$; Amersham International, Amersham, Bucks, UK) and universally labelled [^{14}C]glucose (10 $\mu Ci/ml$ in 0.09% NaCl w/v; ICN Biochemicals, Thame, Oxon, UK) were infused together into the fetal caudal vein for 2–4 h at known rates of between 0.08 and 0.09 ml/min after an initial priming dose (3–4 ml). Blood samples were taken simultaneously from the umbilical

vein, fetal artery, maternal artery and uterine vein before (0 min) and when steady state had been established, at known times approximately 120, 140, 160, 180 min after beginning the infusion. A second study was carried out in these animals 48 h later after withdrawing food but not water from the ewes for the intervening period.

The blood samples were analysed immediately for blood pH, gas tension, packed cell volume and O_2 content (0.5 ml) and $^{14}CO_2$ where appropriate (1.0 ml). The remainder of the blood sample (2.0 ml) was added to a chilled tube containing EDTA for subsequent analysis. An aliquot (0.5 ml) of EDTA-treated blood was deproteinised with zinc sulphate and barium hydroxide and the supernatant used for determination of both labelled and unlabelled whole blood glucose concentrations. The rest of the EDTA sample was centrifuged 4 °C and the plasma stored at -20 °C until required for 3H_2O and hormone measurements. An additional aliquot of fetal arterial blood (1 ml) was taken at 0 min and placed in a chilled heparinised tube containing EGTA and glutathione for catecholamine assay. These samples were centrifuged immediately at 4 °C and the supernatant stored at -80 °C until analysis. At the end of the study in the fasted state, the fetuses were delivered by Caesarean section under general anaesthesia (sodium pentobarbitone, 20 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 assayed 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, O_2 content and whole blood concentrations of glucose, [^{14}C]glucose, 3H_2O and $^{14}CO_2$ were measured in all four sets of simultaneous samples. Blood O_2 content was calculated from the percentage O_2 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, pO_2 and pCO_2 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 3H_2O 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 CO_2 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 $97.9 \pm 0.9\%$ (S.E.M.) ($n=20$) and, therefore, no corrections for glucose recovery were made. The mean recovery of $^{14}\text{CO}_2$ from blood was $71.8 \pm 0.6\%$ ($n=20$) and hence, all blood $^{14}\text{CO}_2$ values have been corrected for recovery.

Plasma T_4 , triiodothyronine (T_3), cortisol and insulin concentrations were measured using RIAs validated for use with ovine plasma. Plasma concentrations of total T_4 and T_3 were determined 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 T_4 and 0.1 ng/ml for T_3 . 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 $\mu\text{U}/\text{ml}$ 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 (AlaAT, 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 $^3\text{H}_2\text{O}$ steady state diffusion technique (Meschia *et al.* 1967, Bell *et al.* 1987). Net umbilical uptake rates of glucose and O_2 and net umbilical excretion rates of ^{14}C glucose and $^{14}\text{CO}_2$ 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 fetal rates of utilisation and production of glucose, CO_2 production from glucose carbon, and the fraction of the net umbilical O_2 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 ($\mu\text{mol}/\text{min}$) = [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 $^{14}\text{CO}_2$ production ($\mu\text{mol}/\text{min}$) = [net umbilical $^{14}\text{CO}_2$ 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 O_2 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 $^{14}\text{CO}_2$ excretion rate (d.p.m./min)]/[net fetal tracer glucose uptake (d.p.m./min)]. Fraction of O_2 uptake used for oxidation of glucose carbon = [amount of O_2 used to oxidise fetal glucose carbon ($\mu\text{mol}/\text{min}$)]/[net umbilical O_2 uptake rate ($\mu\text{mol}/\text{min}$)], where the amount of O_2 used to oxidise fetal glucose = the amount of CO_2 produced by this oxidative process (from equation for oxidation of fetal glucose carbon above).

Endogenous glucose production ($\mu\text{mol}/\text{min}$) = [fetal glucose utilisation ($\mu\text{mol}/\text{min}$)] – [umbilical glucose uptake ($\mu\text{mol}/\text{min}$)].

In the second tracer study, the values for ^{14}C glucose and $^{14}\text{CO}_2$ 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 $\pm$ 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

Table 1 Mean $\pm$ S.E.M. plasma concentrations of T_4 , T_3 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

	Intact		TX	
	Fed	Fasted	Fed	Fasted
Plasma (ng/ml)				
T_4	133 $\pm$ 19	121 $\pm$ 13	7 $\pm$ 2**	6 $\pm$ 2**
T_3	0.21 $\pm$ 0.07	0.43 $\pm$ 0.13	ND	ND
Cortisol	24.6 $\pm$ 8.4	41.0 $\pm$ 10.8	26.7 $\pm$ 6.7	34.9 $\pm$ 7.4
Bodyweight (g)	3664 $\pm$ 200	3435 $\pm$ 182	2958 $\pm$ 77*	2721 $\pm$ 210*
Length (cm)				
Crown rump	51.1 $\pm$ 1.0	50.6 $\pm$ 0.7	45.8 $\pm$ 1.3**	45.4 $\pm$ 108**
Forelimb	26.8 $\pm$ 0.7	26.0 $\pm$ 0.4	23.6 $\pm$ 0.4**	22.6 $\pm$ 0.8**
Hind limb	32.4 $\pm$ 0.9	32.5 $\pm$ 0.5	28.1 $\pm$ 0.8*	27.8 $\pm$ 1.0*
Number of animals	4	5	4	5

ND=not detected.

Significantly less than values observed in intact fetuses in a similar nutritional state, * $P < 0.05$, ** $P < 0.01$ (ANOVA).

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 T_4 and T_3 levels to the limits of sensitivity of the assays throughout the experimental period. Mean plasma concentrations of T_4 and T_3 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 reported previously for intact fetuses of well-fed ewes during late gestation (Fowden & Silver 1995, Fowden *et al.* 1998b). In common with previous findings (Hopkins & Thorburn 1972, Erenberg *et al.* 1974), fetal TX retarded fetal growth in the present study. At delivery, bodyweight, crown rump length and limb lengths were all significantly less in TX than in intact fetuses, irrespective of nutritional state (Table 1).

Metabolic rates The rates of umbilical blood flow and of umbilical uptake and fetal utilisation of glucose were not significantly different in the TX and intact fetuses when values were expressed on a weight-specific basis (Table 2);

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 O_2 uptake used to oxidise glucose carbon (Fig. 1). However, the rates of umbilical O_2 uptake and CO_2 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 T_4 or T_3 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 and fasted 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

Table 2 Mean $\pm$ s.e.m. umbilical blood flows, fetal and maternal arterial concentrations of blood glucose, and fetal rates of umbilical uptake, utilisation and production of glucose 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 changes in value (Δ) during fasting

		Blood glucose (mmol/l)		Mother		Umbilical blood flow (ml/min per kg)	
	Fetus	Fed	Fasted	Δ	Fed	Fasted	Δ
Intact		0.82 $\pm$ 0.09	0.52 $\pm$ 0.04	-0.30 $\pm$ 0.06 ⁺⁺	2.34 $\pm$ 0.14	1.18 $\pm$ 0.11	-1.15 $\pm$ 0.19 ⁺⁺
TX		0.93 $\pm$ 0.04	0.55 $\pm$ 0.06	-0.38 $\pm$ 0.04 ⁺⁺	2.22 $\pm$ 0.10	1.28 $\pm$ 0.11	-0.95 $\pm$ 0.16 ⁺⁺
		Fetal glucose metabolism (μ mol/min per kg)		Utilisation		Production	
	Fetus	Fed	Fasted	Δ	Fed	Fasted	Δ
Intact		27.2 $\pm$ 2.4	9.7 $\pm$ 2.13	-17.5 $\pm$ 3.6 ⁺	27.8 $\pm$ 2.6	0.1 $\pm$ 2.1	+8.5 $\pm$ 2.4 ⁺
TX		24.6 $\pm$ 2.0	14.0 $\pm$ 1.7	-10.6 $\pm$ 1.5 ⁺	23.8 $\pm$ 1.5	-0.9 $\pm$ 1.3	+1.5 $\pm$ 1.1 [*]

Significant change during fasting, ⁺ $P<0.05$, ⁺⁺ $P<0.01$ (paired *t*-test).
Significantly different from values in intact fetuses, ^{*} $P<0.05$, ^{**} $P<0.01$ (unpaired *t*-test).

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 the 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

	Intact			TX		
	Fed	Fasted	Δ	Fed	Fasted	Δ
Insulin (μU/ml)	21.6 ± 2.1	14.0 ± 1.6	−7.6 ± 1.4 ⁺⁺	21.3 ± 3.8	11.8 ± 1.5	−9.9 ± 2.9 ⁺⁺
Cortisol (ng/ml)	16.4 ± 2.7	41.0 ± 10.8	+24.6 ± 9.9 ⁺	16.0 ± 2.5	34.9 ± 7.4	+19.0 ± 6.8 ⁺
Adrenaline (pg/ml)	45 ± 31	65 ± 41	+23 ± 13	0	50 ± 29	+50 ± 29
Noradrenaline (pg/ml)	410 ± 140	822 ± 306	+413 ± 351	192 ± 54	282 ± 64*	+90 ± 56
Total catecholamines (pg/ml)	455 ± 140	887 ± 276	+433 ± 342	192 ± 54	332 ± 78*	+140 ± 80

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, under-

nutrition 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 gluconeogenesis 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

Figure 1 Mean ± S.E.M. values of (A) umbilical O₂ uptake, (B) the glucose carbon oxidation fraction, (C) CO₂ production from glucose carbon and (D) the fraction of umbilical O₂ used to oxidise glucose carbon in intact (I, n=5) and TX (n=5) fetuses in the fed state (open bars) and at the end of a 48 h period of maternal fasting (striped bars) and the mean changes in value during fasting (stippled bars). *Significantly different (P<0.05, unpaired t-test) from the values in intact fetuses in the same nutritional state. †Significant change (P<0.05, paired t-test) during fasting.

Table 4 Mean $\pm$ 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

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

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. Intra-uterine 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 & Stalman 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 pre-

cursors, 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 (Bhakhavathsalan *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 glucogenesis 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 adrenoceptor 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, Bhakhavathsalan *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

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 under-nutrition. 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 O₂ consumption 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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