Maternal manipulation of brown adipose tissue and liver development in the ovine fetus during late gestation

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We examined the effect of maternal chronic cold exposure, induced by winter-shearing ewes 4 weeks before their predicted lambing date, on brown adipose tissue (BAT) and liver development in lambs. Fetuses were sampled from under-fed (60% of energy requirements for maintenance and pregnancy of an unshorn ewe) shorn or unshorn ewes at 126, 140 and 145 d of gestation. Lambs were sampled from ewes within 2 h of birth. Throughout gestation fetal body, BAT and liver weights were similar in shorn and unshorn groups. The level of GDP binding to mitochondrial uncoupling protein remained low throughout gestation, but increased dramatically after birth. Lambs born to shorn ewes possessed more mitochondrial protein and exhibited a significantly higher total thermogenic activity in BAT. Type I iodothyronine 5′deiodinase (*EC* 3.8.1.4) activity in BAT peaked at birth, as did hepatic iodothyronine 5′deiodinase activity and was significantly greater in lambs born to under-fed shorn ewes, which exhibited a higher plasma triiodothyronine concentration. Chronic maternal adaptations to prolonged cold exposure appear to enable pregnant ewes to compensate for the negative effects of under-feeding on fetal growth and development.

Brown adipose tissue: Fetus: Liver

Newborn lambs must possess sufficient energy reserves in the form of lipid within adipose-tissue depots and hepatic glycogen since a large proportion of the energy requirement for heat production immediately after birth must come from the mobilization of these stores (Mellor & Cockburn, 1986). In precocious thermoregulators, of which lambs are a typical example, the ability to utilize non-shivering thermogenesis in brown adipose tissue (BAT) is vital to prevent hypothermia in the perinatal period (Symonds *et al.* 1992). Insufficient BAT, in conjunction with starvation and exposure to a cold extra-uterine environment, is therefore a major cause of lamb death. The generation of heat via non-shivering thermogenesis in BAT is due to the presence of a unique uncoupling protein (UCP) which uncouples oxidative phosphorylation from ATP synthesis and releases energy as heat (Casteilla *et al.* 1987). The development of perirenal BAT, which constitutes up to 80% of adipose tissue depots in the ovine fetus, begins with the appearance of lipid locules formed in the endoplasmic reticulum of pre-adipocytes (Gemmell & Alexander, 1978). An increase in BAT weight plus specific enzymic and morphological changes occur predominantly between 120 d of gestation and term. These include development of the sympathetic nervous system (Gemmell & Alexander, 1978), an increase in the activity of

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iodothyronine 5′deiodinase (*EC* 3.8.1.4; I5′D) (Wu *et al.* 1990), expression of the gene for UCP and appearance of UCP (Casteilla *et al.* 1987, 1989). During fetal life there is a low abundance of UCP mRNA and BAT exhibits a low thermogenic activity. Within 12 h of birth, this situation is reversed following an increase in both the thermogenic activity of BAT and mRNA for UCP (Casteilla *et al.* 1987, 1989). Changes in the ability of BAT to generate heat therefore appear to be linked to developmental adaptations in BAT function, either close to term or after birth.

Fetal BAT growth can be limited as a result of maternal undernutrition in species where placental transport of fatty acids is low (i.e. sheep and rats), independently of effects on fetal growth (Alexander, 1978; Higham *et al.* 1984). In contrast, maternal cold exposure during late gestation increases the amount of BAT present in neonatal rats (Hyvarien *et al.* 1976), and increases birth weight and adipose tissue depots in lambs (Symonds *et al.* 1992). Furthermore, maternal cold exposure also stimulates the *in vivo* capacity for non-shivering thermogenesis in newborn lambs (Symonds *et al.* 1992). This response may be mediated via an increase in glucose supply to the late-gestation fetus in conjunction with a rise in fetal insulin levels (Thompson *et al.* 1982). The exact time course of such events, or effects on the development of sympathetic nervous system and I5′D activity, in BAT or liver have yet to be elucidated. The present study therefore investigated the effect of chronic maternal cold exposure, induced by winter-shearing, on fetal BAT and liver development up to the period immediately after birth in chronically under-fed (60 % of energy requirements for maintenance and pregnancy of an unshorn ewe) shorn or unshorn ewes. Preliminary communications of some of this work have been published as summarized by Symonds *et al.* (1995).

METHODS

Animals and diet

Thirty-one Bluefaced Leicester cross Swaledale ewes of known mating date and number of fetuses diagnosed using real-time ultrasound echograph were used in the present study. From 5 weeks before lambing thirty-one ewes were individually housed at ambient temperature (minimum 5·2 (SD 3·9), maximum 9·0 (SD 3·9)°) and 1 week later, fifteen ewes were randomly selected and shorn. All ewes were fed daily, at 08.30 hours, with a diet consisting of barley-based concentrate and chopped hay. They were under-fed by only feeding sufficient diet to meet 60 % of the energy requirements for maintenance and pregnancy of an unshorn ewe for the final 4 weeks of gestation, i.e. 0·15–0·25 kg concentrate and 1·0–1·25 kg hay (Agricultural Research Council, 1980).

Experimental design

Maternal. At an average of 3 weeks before predicted lambing date (i.e. at 126 d of gestation) catheters were inserted into the jugular vein of six shorn and six unshorn ewes. On the following day, 10 ml blood samples were taken at intervals of 1 h between 08.30 and 15.30 hours, and the plasma collected and stored at − 20°. This procedure was repeated at 7 d before lambing (i.e. at 140 d of gestation) in the same ewes. The mean ambient temperature over the sampling periods was 8·4 ± 4·2°.

Tissue sampling. Fetal and neonatal tissues were sampled over the 3 weeks of gestation (i.e. at 126, 140 and 145 d of gestation) and within 2 h of birth as outlined in Table 1. Ewes or lambs were humanely slaughtered by intravenous administration of barbiturate (50–100 mg/kg sodium pentobarbitone; Euthatal, RMB, Animal Health, supplied by National

Table 1. *Schedule of experimentation on shorn and unshorn ewes*

Treatment	Stage of pregnancy* (d)			
	126	140	145	Birth
Unshorn				
	3 singles	3 twins	3 twins	1 twin
	2 twins			4 twins†
Shorn				
	3 singles	3 twins	2 twins	1 twin
	2 twins		1 triplet	3 twins†

*Estimated from known mating date.
†Only one lamb per twin sampled.

Veterinary Supplies, Stoke-on-Trent, Staffs.). The fetuses were rapidly removed, injected with an overdose of barbiturate and the liver and both perirenal depots of adipose tissue sampled. One perirenal adipose tissue depot was stored at $-20°$ for measurement of GDP binding and lipid content as described by Symonds *et al.* (1992). The remaining perirenal adipose tissue depot plus 20 g liver were placed in liquid N_2 and stored at $-70°$ until assayed for I5′D activity and catecholamine content.

Laboratory procedures. Mitochondria were prepared from frozen tissue based on the method of Casteilla *et al.* (1987) as described by Symonds *et al.* (1992). The protein contents of homogenates and mitochondria were measured by the method of Lowry *et al.* (1951) and cytochrome c oxidase (*EC* 1.9.3.1) activity (Wharton & Tzagaloff, 1967) measured in order to assess the recovery of mitochondrial protein. BAT thermogenic activity was then assessed from the *in vitro* activity of the mitochondrial conductance pathway using GDP at a concentration of 2 μM, with non-specific binding measured using a 200 μM concentration of GDP. The amount of [^{3}H]GDP trapped in extra-mitochondrial spaces was corrected for by measuring the trapping of [^{14}C]sucrose (Symonds *et al.* 1992). The lipid content of BAT was measured by ether extraction of a 5 g sample of tissue (Symonds *et al.* 1992). Hepatic glycogen content was assayed using the method of Keppler & Decker (1984). The activities of type I (BAT and liver) and type II I5′D (BAT) were determined by measuring the release of $^{125}I^-$ from [^{125}I]reverse triiodothyronine (rT_3; Amersham International plc, Amersham, Bucks.) based on the method of Wu *et al.* (1990), as described by Clarke *et al.* (1994). Type I I5′D activity can be distinguished from type II activity, since type I I5′D has a higher affinity for rT_3, and is sensitive to propylthiouracil (PTU) whereas type II I5′D activity has a greater affinity for thyroxine (T_4) and is insensitive to PTU inhibition. Total catecholamine content of perirenal adipose tissue was determined using HPLC as described by Clarke *et al.* (1996).

Plasma concentrations of glucose, lactate, 3-hydroxybutyrate and non-esterified fatty acids (NEFA) were measured enzymically, and cortisol, insulin, T_3, and T_4 were measured using radioimmunoassays as described by Symonds *et al.* (1986) and Clarke *et al.* (1994). Blood haemoglobin was measured using the cyanmethaemoglobin method using the Boehringer Mannheim test kit no. 124-7429 (Boehringer Mannheim UK Ltd, Lewes, East Sussex).

Statistical analysis

Two-way ANOVA was used for statistical analysis of the effects of age and treatment in shorn and unshorn ewes. This analysis also indicated that there were no significant influences of individuals at any time point in any treatment group.

RESULTS

Ewe body weight and feed intake

There were no differences in ewe body weight or condition score (an index of body fat distribution; Russel, 1984) between groups or in the quantity of hay consumed (Table 2).

Concentration of hormones and metabolites in maternal plasma

The packed cell volume (PCV) and blood haemoglobin concentrations were both higher ($P < 0.05$), in shorn ewes at 126 d of gestation compared with unshorn ewes, but these findings were not extended to 140 d of gestation (Table 3). Plasma glucose concentrations were higher ($P < 0.05$) at 126 and 140 d of gestation in shorn ewes compared with unshorn ewes. Plasma concentrations of 3-hydroxybutyrate were similar in both groups of ewes at 126 d of gestation, but increased at 140 d of gestation in all ewes, although this response was only significant in the shorn group. Plasma T_3 concentrations were greater ($P < 0.05$) in the shorn group at 126 d of gestation than in the unshorn group. At 140 d of gestation, although T_3 levels were similar between groups, plasma T_4 concentration was higher ($P < 0.05$) in the shorn group. Plasma concentrations of lactate, cortisol and insulin were similar in both groups of ewes at both sampling dates.

Fetal growth and development

In shorn and unshorn groups fetal body weights increased ($P < 0.01$) up to 140 d of gestation (Table 4). No differences in fetal weights were observed between these groups, but lamb birth weight was significantly higher ($P < 0.05$) in the shorn group. Perirenal adipose tissue weight and its protein and lipid content all increased ($P < 0.01$) between 112 and 140 d of gestation (Table 4), after which stage no significant changes occurred with the exception of the shorn group in which protein content continued to rise ($P < 0.05$) between 140 and 145 d of gestation. The change in perirenal adipose tissue weight in fetuses sampled from under-fed ewes was associated with increases ($P < 0.01$) in protein, lipid and DNA content (Table 4). At birth lambs born to under-fed shorn ewes possessed more ($P < 0.05$) perirenal adipose tissue than those born to under-fed unshorn ewes. This tissue also had more ($P < 0.01$) protein than adipose tissue sampled from unshorn ewes, but the same amount of lipid.

Table 2. *Body weights, condition score and feed intake of shorn and unshorn pregnant ewes*
(Values are means with their standard errors)

		Body weight (kg)		Body condition score*		Hay intake (g)		Concentrate intake (g)		Estimated ME intake (MJ/d)	
Treatment	*n*	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Unshorn	16	76	4	2·4	0·2	1075	23	221	3	11·1	0·2
Shorn	15	78	2	2·3	0·2	1062	33	219	1	11·2	0·4

ME, metabolizable energy.
* Assessed as described by Russel (1984).

Table 3. *Mean maternal jugular venous packed cell volume (PCV) and concentration of blood haemoglobin (Hb) and plasma concentrations of glucose, lactate, 3-hydroxybutyrate (3-OHB), non-esterified fatty acids (NEFA), triiodothyronine (T_3), thyroxine (T_4), insulin and cortisol from shorn (S) (n 6) and unshorn (US) (n 6) twin-bearing pregnant ewes*

(Values are means with their standard errors)

	Gestational age (d)							
	126				140			
	US		S		US		S	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
PCV (%)	27·6**	1·3	34·3	1·0	28·6	1·5	27·8	1·0
Hb (g/l)	10·6*	0·5	12·8	0·7	11·3	2·0	11·4	0·4
Glucose (mmol/l)	2·58*	0·06	2·81	0·04	2·33*	0·08	3·01	0·23
Lactate (mmol/l)	0·52	0·04	0·62	0·05	0·50	0·08	0·49	0·05
3-OHB (mmol/l)	0·65	0·1	0·69	0·16	0·96	0·34	1·16	0·12
NEFA (mmol/l)	0·47	0·04	0·98	0·27	0·63	0·10	0·90	0·06
T_3 (nmol/l)	1·21*	0·20	2·09	0·25	1·38	0·10	1·66	0·18
T_4 (nmol/l)	23·8	1·5	58·5	14	30·2*	3·6	45·0	4·4
Insulin (pmol/l)	8·0	1·6	10·4	4·0	9·2	0·3	10·1	1·4
Cortisol (nmol/l)	37·2	8·8	35·9	3·0	32·6	1·4	29·0	3·9

Mean values were significantly different from those for S ewes: * $P < 0.05$, ** $P < 0.01$ (ANOVA).

Table 4. *Mean fetal or neonatal body weight and perirenal adipose tissue weight, protein and lipid content in shorn (S) and unshorn (US) ewes*

(Values are means with their standard errors)

		Gestational age (d)							
		126 (*n* 7)		140 (*n* 6)		145 (*n* 6)†		Birth (*n* 6)‡	
	Treatment	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Body wt (kg)	US	3·18	0·23	4·78	0·17	5·02	0·18	4·60*	0·44
	S	3·15	0·32	5·12	0·23	5·40	0·45	5·41	0·22
Perirenal adipose tissue (g)	US	19·0	1·6	23·4	2·4	25·8	1·6	22·5*	2·2
	S	17·5	1·5	25·8	2·7	25·2	1·8	28·1	1·2
Protein (mg)	US	922	127	1911	207	2223	140	1502	195
	S	674	95	1531	106	2007	146	2140	101
Lipid (g)	US	5·3	0·6	6·2	0·7	5·9	0·8	6·9	1·0
	S	5·4	0·7	7·1	0·8	7·1	0·7	8·2	1·4

* Mean values were significantly different from those for S ewes at the same stage of gestation, $P < 0.05$ (ANOVA).
† For the S group, *n* 7.
‡ For the S group, *n* 5.

Development of brown adipose tissue

In fetuses sampled from unshorn ewes, thermogenic activity of perirenal adipose tissue (i.e. GDP-binding to mitochondria) increased ($P < 0.01$) between 126 and 140 d of gestation (Fig. 1), but remained unchanged in fetuses sampled from shorn ewes and was significantly lower ($P < 0.05$) than in the unshorn group at 140 d of gestation. Total mitochondrial protein content of fetal perirenal adipose tissue increased ($P < 0.01$) between 126 and 145 d of gestation, although in fetuses sampled from shorn ewes significantly more ($P < 0.01$)

mitochondrial protein was present at 140 d of gestation. As a result of these differential changes in the amount of mitochondrial protein and GDP-binding, total GDP-binding per lamb was similar in the two groups of ewes throughout gestation. A decrease in total mitochondrial protein occurred in both groups between late gestation and birth, but this was

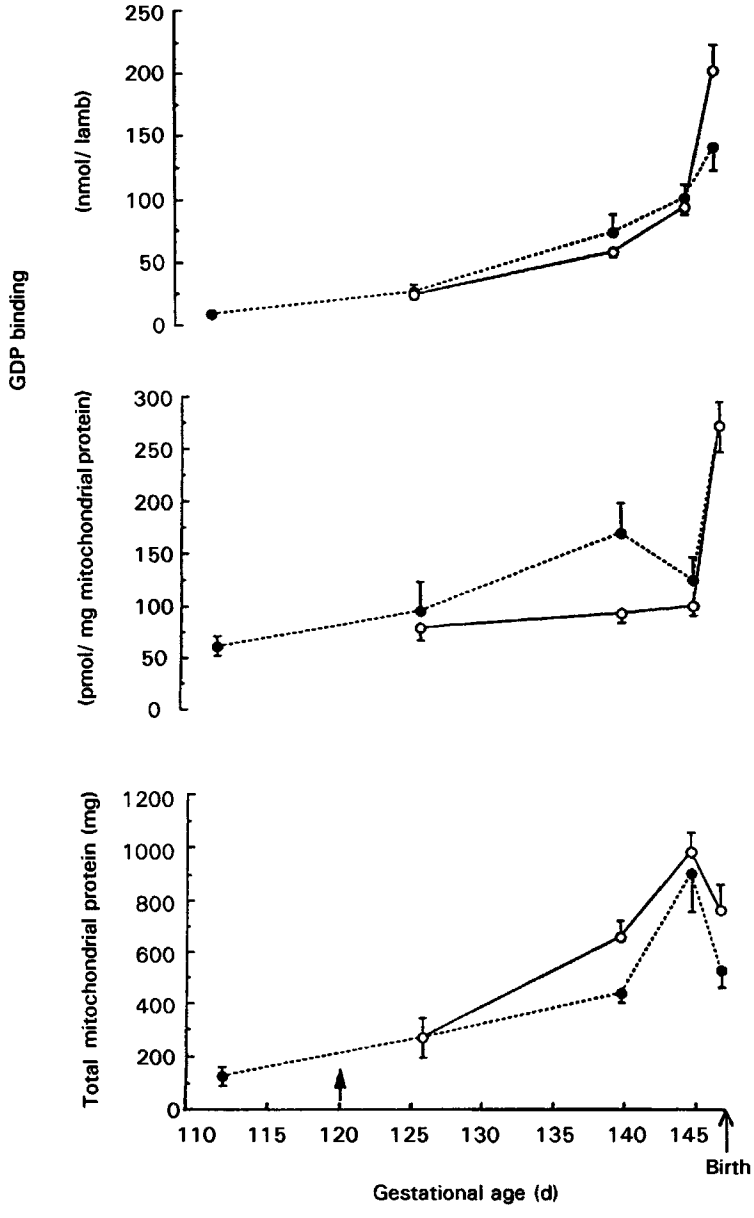

Fig. 1. Developmental changes in ovine perirenal adipose tissue between late gestation and birth. Perirenal adipose tissue was obtained from fetal or neonatal lambs born to under-fed unshorn (●) or shorn (○) ewes and then analysed as described on p. 873. Values are means for five to seven sheep with their standard errors represented by vertical bars. ↟, Stage of gestation when ewes were shorn.

Table 5. *Catecholamine content of fetal or neonatal perirenal adipose tissue in shorn (S) or unshorn (US) ewes*

(Values are means with their standard errors)

		Gestational age (d)							
		126 (*n* 7)		140 (*n* 6)		145 (*n* 6)†		Birth (*n* 6)‡	
	Treatment	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Noradrenaline (mmol)	US	132*	22	227	52	121*	38	152	58
	S	240	62	274	32	196	30	183	68
Adrenaline (mmol)	US	113*	30	122*	50	345*	94	68	28
	S	288	99	504	177	85	34	107	79
Dopamine (mmol)	US	43	15	91	31	132	33	40	20
	S	52	7	127	22	77	10	35	10

* Mean values were significantly different from those for the S group at the same stage of gestation, $P < 0.05$ (ANOVA).
† For the S group, *n* 7.
‡ For the S group, *n* 5.

only significant ($P < 0.05$) in the unshorn group with the result that lambs born to shorn ewes possessed more ($P < 0.01$) perirenal adipose tissue mitochondrial protein. Between 145 d of gestation and birth, perirenal adipose tissue thermogenic activity increased ($P < 0.01$) 2–3-fold above fetal values in shorn and unshorn groups. However, between late gestation and birth there was a greater ($P < 0.05$) increase in total GDP-binding in animals sampled from shorn ewes, with the result that lambs born to these ewes possessed perirenal adipose tissue with a greater ($P < 0.01$) total thermogenic activity than those lambs born to unshorn ewes.

Catecholamine content of brown adipose tissue

No significant changes in catecholamine content were observed in either the under-fed shorn or the unshorn group between 126 d of gestation and birth (Table 5). The total noradrenaline content of perirenal adipose tissue was higher ($P < 0.05$) in fetuses sampled from shorn ewes at 126 and 145 d of gestation, as was the adrenaline content at 126 and 140 d of gestation. In contrast at 145 d the total adrenaline was higher ($P < 0.05$) in fetuses sampled from unshorn ewes. There were no differences in total dopamine content of perirenal adipose tissue between fetal groups. No significant differences in mean catecholamine content of perirenal adipose tissue were observed between lambs born to shorn and unshorn ewes.

Iodothyronine 5′deiodinase activity

There was an increase ($P < 0.05$) in type I and II I5′D activity in fetal perirenal adipose tissue throughout gestation up to 140 d of gestation in both groups (Fig. 2). Between 140 and 145 d of gestation this activity continued to increase in fetuses sampled from shorn ewes but declined ($P < 0.05$) in the unshorn group. In both groups type I I5′D activity increased rapidly between 145 d of gestation and birth but this change was not observed for type II activity. No differences in I5′D activity were observed between lambs born to shorn and unshorn ewes.

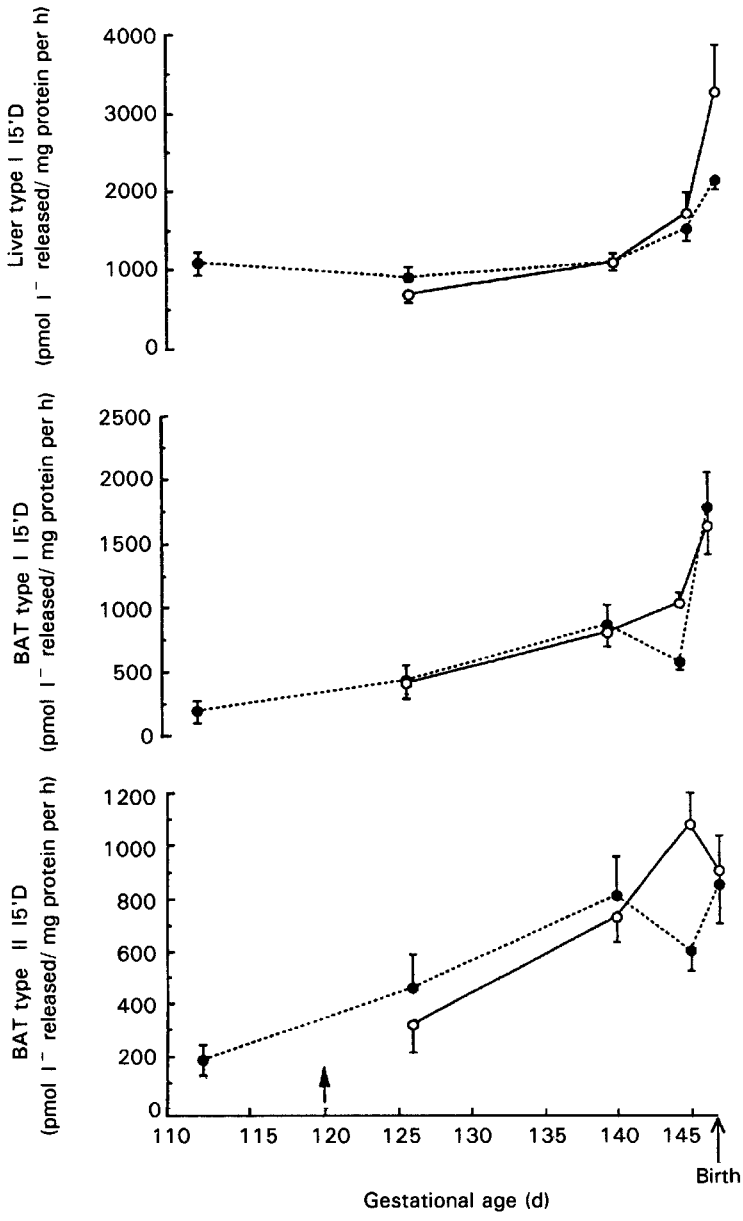

Fig. 2. Developmental changes in ovine hepatic or brown adipose tissue (BAT) iodothyronine 5′deiodinase (I5′D) activity between gestation and birth. Hepatic and perirenal adipose tissues were obtained from fetal or neonatal lambs born to under-fed unshorn (●) or shorn (○) ewes and then analysed as described on p. 873. Values are means for five to seven sheep with their standard errors represented by vertical bars. ↑, Stage of gestation when ewes were shorn.

Fetal hepatic I5′D activity was similar in both shorn and unshorn groups throughout gestation and increased ($P < 0.05$) with gestational age in both groups, but after birth this rise was greater ($P < 0.05$) in lambs born to shorn ewes (Fig. 2). The plasma concentration of T_3 in jugular venous blood samples taken 2 h post-partum was greater ($P < 0.05$) in

Table 6. *Fetal or neonatal liver composition in shorn (S) or unshorn (US) ewes*
(Values are means with their standard errors)

		Gestational age (d)							
		126 (*n* 7)		140 (*n* 6)		145 (*n* 6)†		Birth (*n* 6)‡	
	Treatment	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Liver wt (g)	US	97	9	115	7	105	7	88**	9
	S	93	10	112	5	104	6	118	7
Total hepatic glycogen (mmol)§	US	7·8	0·9	29·8	3·9	27·1	4·1	12·1**	2·1
	S	8·2	1·5	23·7	2·2	28·8	1·6	21·5	5·3

** Mean values were significantly different from those for the S group at the same stage of gestation, $P < 0.01$ (ANOVA).
† For the S group, *n* 7.
‡ For the S group, *n* 5.
§ mmol glucose stored as glycogen.

lambs born to shorn ewes (shorn 6·45 (SE 0·06) nmol/l, *n* 5, unshorn 4·68 (SE 0·11) nmol/l, *n* 6) but there were no differences in plasma T_4 concentrations (shorn 150 (SE 13) nmol/l, unshorn 144 (SE 15) nmol/l).

Liver development

Fetal liver weight and glycogen content were similar in shorn and unshorn groups throughout gestation and both increased ($P < 0.01$) until 140 d (Table 6). Then between 140 d of gestation and 2 h post-partum a decline ($P < 0.05$) in liver weight and amount of glycogen was observed only in lambs born to unshorn ewes. This resulted in lambs born to shorn ewes possessing larger ($P < 0.05$) livers and therefore more glycogen after birth.

DISCUSSION

The aim of the present study was to investigate the extent to which chronic maternal cold exposure, induced by winter-shearing of under-fed pregnant ewes, influences perirenal adipose tissue and liver development over the final month of gestation. Long-term adaptations in the maternal metabolic and hormonal environment are known to have significant effects on lamb birth weight and the *in vivo* capacity for non-shivering thermogenesis in the newborn lamb (Symonds *et al.* 1986, 1988*a*, 1992), but the precise time courses of these events during fetal life have not yet been elucidated. The present study demonstrated that: (1) pronounced alterations in perirenal adipose tissue and liver development occur between 145 d of gestation and 2 h post-partum; (2) the maternal metabolic and hormonal environment influences this adaptation, and is likely to contribute to the increased ability of lambs born to shorn ewes to increase heat production by non-shivering thermogenesis (Symonds *et al.* 1992).

Maternal nutrition, cold exposure and fetal growth

Between mid and late gestation fetal glucose utilization gradually rises, which can result in a one-third decline in fetal plasma glucose concentration thereby increasing the maternal–fetal glucose gradient (Bell *et al.* 1986; Molina *et al.* 1991). This adaptation occurs in

conjunction with a 5-fold increase in placental glucose capacity (Molina *et al.* 1991), which ensures that abrupt changes in fetal growth during the final month of gestation are uncommon (Mellor, 1983). In the present study ewes were only fed at 60% of their estimated energy requirements for the final month of pregnancy, which resulted in a cessation of fetal growth over the last days of gestation. Chronic maternal cold exposure limited this effect and resulted in increased lamb perirenal adipose tissue and liver weights at birth. Examination of the observed fetal weight changes during late gestation demonstrates that whole-body, adipose tissue and liver weights were similar up to 145 d of gestation and only diverged after birth, when there was a trend for body and tissue weights to decrease in the unshorn but not in the shorn group. These findings suggest that the mechanism by which winter shearing stimulates lamb birth weight is by preventing the loss of fetal tissues at about the time of parturition rather than any sustained change in fetal growth as previously proposed (Symonds *et al.* 1988*b*). Birth results in a dramatic reduction in placental transfer of nutrients and the fetus becomes rapidly dependent on endogenous energy stores including hepatic glycogen and adipose tissue lipid (Mellor & Cockburn, 1986). In both groups studied there was a decrease in total hepatic glycogen between 145 d of gestation and birth but this decline was lowest in lambs born to shorn ewes.

The degree of metabolic stress experienced by the fetus could explain effects of maternal nutrition and cold exposure on the adrenaline content of perirenal adipose tissue, which is likely to be derived from the adrenal medulla via the circulation in response to stress (Slotkin & Seidler, 1988). At 145 d of gestation adrenaline levels were higher in perirenal adipose tissue sampled from unshorn, compared with shorn ewes, despite being lower at 126 and 140 d of gestation. These differences in perirenal adipose tissue adrenaline content support the proposal that the magnitude of metabolic stress experienced by fetuses was attenuated by maternal cold exposure.

The mechanism by which under-fed shorn ewes were able to maintain fetal nutrition is likely to be a direct consequence of the metabolic adaptations to cold stress in these animals (Symonds *et al.* 1986, 1988*a*). On 126 and 140 d of gestation maternal plasma glucose concentration was significantly higher in under-fed shorn ewes compared with under-fed unshorn controls, and at 140 d glucose levels remained similar to those observed in well-fed unshorn ewes, which were 30% higher than in the under-fed unshorn group (Symonds, 1995). Maternal plasma glucose concentration is known to be the main determinant of fetal glucose supply (Hay *et al.* 1984), so it may be predicted that this should be maintained in the shorn group, thereby preventing any catabolism of fetal tissues that appeared to be occurring over the final 2 d of gestation in the unshorn group. Higher plasma glucose concentrations in shorn ewes are due to an increased rate of maternal glucose synthesis (Symonds *et al.* 1988*b*) and this phenomenon is one of a series of maternal metabolic and hormonal adaptations to chronic cold exposure. Confirmation that shorn ewes were effectively adapting to chronic cold exposure is provided by the observation of higher PCV, haemoglobin, glucose and thyroid hormone concentrations in shorn compared with unshorn ewes in the absence of any differences in circulating 3-hydroxybutyrate, NEFA, lactate, insulin or cortisol concentrations.

Regulation of perirenal adipose tissue development

The thermogenic activity of perirenal adipose tissue, as measured from GDP-binding to mitochondria remained low throughout fetal life despite a 3–4-fold increase in mitochondrial protein between 126 and 145 d of gestation. This resulted in a 4-fold

increase in total perirenal adipose tissue thermogenic activity (nmol/lamb) in both groups. UCP constitutes a large proportion of mitochondrial protein in BAT, which suggests that there is a gradual increase in UCP synthesis during late gestation but it is not in a thermogenically active state. In rat fetuses, the increase in mRNA for UCP following cold exposure after birth is dependent on the prenatal rise in I5′D activity (Giralt *et al.* 1990). An increase in BAT I5′D activity may stimulate UCP gene expression due to local T_3 production, an effect mediated primarily by the action of noradrenaline on α-adrenergic adrenoreceptors (Himms-Hagen, 1989). Both type I and type II I5′D activities in perirenal adipose tissue continued to increase between 140 and 145 d of gestation in fetuses sampled from shorn ewes, whilst in the unshorn group I5′D activity decreased over this period. This decline occurred at the same time as perirenal adipose tissue lipid and protein content reached a plateau suggesting that the timing of these events may be inter-related.

Between 145 d of gestation and 2 h after birth the thermogenic activity of perirenal adipose tissue increased 2–3-fold. This increased thermogenic activity is likely to represent "unmasking" of GDP binding sites in response to cold exposure of the extra-uterine environment. Therefore it is likely that this pattern of BAT recruitment is more rapid than that observed in altricial species such as rats in which the post-partum increase in mRNA for UCP can take several hours to occur (Giralt *et al.* 1990). Although there was no influence of maternal cold exposure on thermogenic activity, lambs born to shorn ewes possessed more mitochondrial protein which resulted in a 40% higher total thermogenic activity. The amount of mitochondrial protein was greater in fetuses sampled from shorn ewes between 140 d of gestation and birth, as was the noradrenaline content of perirenal adipose tissue between 126 and 145 d of gestation. An increase in the rate of noradrenaline production could have contributed to the higher mitochondrial protein content of perirenal adipose tissue sampled from shorn ewes, as noradrenaline is known to stimulate mitochondrial protein synthesis in rats (Desautels & Himms-Hagen, 1978). It is possible that differences in noradrenaline content between shorn and unshorn groups reflect the contrasting nutritional status of the ewes, since intravenous administration of glucose to rats can enhance sympathetic efferent activity (Niijima, 1986) and chronic maternal glucose infusion increases the catecholamine content of ovine perirenal adipose tissue (Clarke *et al.* 1996).

A decrease in BAT type I I5′D activity has been observed 2–3 weeks before birth in fetal calves (Giralt *et al.* 1989) and a fall in I5′D type II activity occurs immediately before birth in rats (Giralt *et al.* 1990). It has been proposed by Wu *et al.* (1990) that in ovine BAT type I I5′D is important in increasing circulating T_3 levels at birth and that type II I5′D activity regulates the overall thermogenic activity of BAT. Results from the present study do not fully support this proposal as there were no consistent changes in type I or II I5′D activity in perirenal adipose tissue for which an increase in type I and no change in type II was observed between 145 d of gestation and birth. Hepatic I5′D activity peaked after birth, with this response being greatest in lambs born to shorn ewes and may have contributed to their higher plasma T_3 concentrations.

In summary, chronic maternal cold exposure, induced by winter-shearing, over the final month of gestation appears to have little effect on fetal growth. Lambs born to unshorn ewes do, however, possess less mitochondrial protein in perirenal adipose tissue and have lower amounts of hepatic glycogen than those born to shorn ewes. These lambs are therefore increasingly reliant on shivering thermogenesis in order to increase heat production during acute cold exposure on the first day of life (Symonds *et al.* 1992). In addition a greater amount of adrenaline is present in perirenal adipose tissue sampled from fetuses of under-fed unshorn ewes at 145 d of gestation which is indicative of chronic

metabolic stress in response to a reduction in fetal nutrient supply. Chronic maternal cold exposure induced by winter shearing may act to maintain fetal glucose supply as a result of subtle changes in maternal metabolism which promote fetal development and thermoregulation in the newborn.

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