Experimental Physiology (1999), **84**, 979–987
Printed in Great Britain

INFLUENCE OF THYROTROPHIN-RELEASING HORMONE ON THERMOREGULATORY ADAPTATION AFTER BIRTH IN NEAR-TERM LAMBS DELIVERED BY CAESAREAN SECTION

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(MANUSCRIPT RECEIVED 3 DECEMBER 1998, ACCEPTED 29 JUNE 1999)

SUMMARY

We investigated the hypothesis that exogenous stimulation with thyrotrophin-releasing hormone (TRH) immediately prior to umbilical cord clamping can improve thermoregulatory adaptation after birth in near-term lambs delivered by Caesarean section. Lambs received an umbilical vein injection of saline $\pm$ TRH (8 μg) prior to cord clamping. The rate of change in colonic temperature and oxygen consumption after birth were not influenced by TRH, but TRH-treated lambs exhibited a greater incidence of shivering compared with controls over the first hour of neonatal life. Two and a half hours after birth, TRH-treated lambs possessed brown adipose tissue (BAT) with a higher thermogenic activity (i.e. GDP binding to mitochondrial protein), but their BAT had a reduced DNA content and they had less hepatic glycogen than control lambs. TRH administration had no effect on iodothyronine 5′ deiodinase activity in BAT and liver, or on plasma concentrations of total triiodothyronine, thyroxine, cortisol or free fatty acids. Three TRH-treated but no control lambs, failed to establish continuous breathing, so tissues from these treated lambs together with time-matched controls were sampled 25 min after birth. These 'non-surviving' TRH-treated lambs had very high plasma catecholamine concentrations, but their lung weights were similar to controls. 'Surviving' TRH-treated lambs possessed lungs with less DNA than non-surviving TRH-treated lambs. It is concluded that umbilical vein injection of TRH prior to umbilical cord clamping increases the recruitment of both shivering and non-shivering thermogenesis after birth.

INTRODUCTION

The metabolic responses that occur during the transition from fetal to neonatal life represent a change from a thermoregulatory quiescent state in which inhibitory stimuli dominate (Gunn & Gluckman, 1995) to one of near-maximal rates of heat production that are rarely matched again during postnatal or adult life. Route of delivery is known to have a critical role in determining thermoregulatory adaptation after birth in both infants and lambs (Christensson *et al.* 1993; Clarke *et al.* 1997*a*). Lambs born vaginally at term are able to maintain a normothermic body temperature (Cabello, 1983; Clarke *et al.* 1997*a*), whilst near-term lambs delivered by Caesarean section rapidly become hypothermic (Sack *et al.* 1976) even when delivered into a warm ambient temperature of 30 °C (Clarke *et al.* 1997*a*). It has been proposed that Caesarean section delivery compromises thermoregulation due to an

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impairment of heat production by non-shivering thermogenesis in brown adipose tissue (BAT) (Christensson *et al*. 1993; Clarke *et al*. 1997*a*). Lambs born near term by Caesarean section are characterised as having low plasma thyroid hormone concentrations and reduced sympathetic activity (Symonds *et al*. 1994; Clarke *et al*. 1997*a*). As a consequence of inadequate endocrine stimulation at birth there is a rapid decline in colonic temperature over the first 30 min of neonatal life, which is not observed in lambs born vaginally (Clarke *et al*. 1997*a*).

Thyrotrophin-releasing hormone (TRH) content of the hypothalamus peaks immediately before birth (Polk *et al*. 1991) and plasma 3,5,3′-triiodothyronine (T_3) concentrations increase rapidly from fetal values of less than 2 nmol l^{-1} (Fraser & Liggins, 1988) to 6 nmol l^{-1} within 0·5 h of birth (Symonds *et al*. 1994). Chronic TRH and corticosteroid treatment of fetal lambs results in improved lung mechanics and surfactant production (Schellenberg *et al*. 1993), as well as improved cardiovascular adaptation in artificially ventilated, and warmed lambs delivered prematurely by Caesarean section (Stein *et al*. 1994). TRH not only has cardiorespiratory effects but also enhances the thermogenic activity of BAT following acute administration to adult rats (Griffiths *et al*. 1988). We have previously shown that intravenous TRH injection into hypothermic lambs 60–80 min after Caesarean section delivery increases the rate of fat oxidation (Bird *et al*. 1998). TRH does not, however, appear to have any thermoregulatory effects in normothermic lambs born vaginally. The current study was therefore designed to examine the hypothesis that exogenous stimulation with TRH immediately prior to umbilical cord clamping can improve thermoregulatory adaptation after birth in near-term lambs delivered by Caesarean section. It was conducted in unanaesthetised lambs that were not provided with any ventilatory support or additional warming. Changes in colonic temperature after birth, in conjunction with incidence of shivering, rate of oxygen consumption and carbon dioxide production were measured during the first 2·5 h of neonatal life in TRH- and saline-treated lambs. This was combined with measurements of the primary hormones (i.e. thyroid hormones, cortisol, catecholamines) and metabolites (i.e. free fatty acids (FFA) and glucose) involved in thermoregulatory adaptation at birth plus the thermogenic activity and capacity of BAT.

METHODS

Animals and diet

Eight singleton-bearing and four twin-bearing Welsh Mountain ewes of similar age, known mating date, and confirmed as being pregnant using a real-time ultrasound echograph were entered into the study. Six weeks prior to the predicted lambing date each ewe was individually housed and fed daily 0·4–0·6 kg barley-based concentrate and 1·2 kg hay, the combination of which contained sufficient energy and nitrogen to fully meet requirements for maintenance and pregnancy over this final period of gestation. Mean ewe body weight was $60·0 \pm 3·0$ kg (S.E.M.; $n = 12$) and condition score (an index of body energy reserves) as assessed by the physical characteristics in the lumbar region, on and around the backbone in the loin area immediately behind the first rib was $2·6 \pm 0·2$ arbitrary units (a.u., $n = 12$). The mean daily minimum and maximum temperatures recorded at 09.00 h were $3·1 \pm 2·8$ (S.D.) and $10·2 \pm 3·8$ °C, respectively. Mean ewe colonic temperature immediately before Caesarean section was $39·70 \pm 0·09$ °C ($n = 12$).

Experimental design

Caesarean section delivery was performed as described by Clarke *et al*. (1997*a*) between 144–146 days of gestation (term, 147 days), whilst ewes were maintained in a purpose-built constant temperature control room at 15 °C (±1 °C). Paravertebral anaesthesia was administered by inserting spinal needles to allow blockage of the T13, L1, and L2 spinal nerves by surrounding them with 2 % Xylocaine, as the dorsal and ventral branches of these nerves pass above and below the transverse

processes of the vertebrae. This was followed by jugular venous injection of 4–6 ml ketamine (100 mg ml^{-1} in saline) into the ewe. A flank incision was made, the fetus delivered and placed onto the ewe's flank. A bolus injection of 0·5 ml of saline ± TRH was then injected into the umbilical vein at a dose of 8 μg per lamb, i.e. 2 μg kg^{-1} (Wrutniak & Cabello, 1985). This dose is established to increase plasma thyroid hormone concentrations to those seen in vaginally delivered lambs over the first day of life (Bird *et al*. 1998). Five seconds later all remaining blood in the cord was squeezed into the fetus to ensure all saline entered the lamb. The cord was clamped, sutured and cut, and the lamb immediately placed into a warm ambient temperature of 30 °C. In the case of twin-bearing ewes this process was then immediately repeated for its twin with only one lamb from each pair receiving TRH.

All lambs were monitored to ensure that continuous breathing was established, which normally occurred within 2–4 min of birth. Colonic temperature was continuously recorded using an electronic thermometer (Type 3GID, Light Laboratories, Brighton, UK) and the lambs dried with a towel. Three TRH-treated lambs (2 twins and 1 singleton) failed to establish continuous breathing by 25 min of life and death was ensured at this point by intracardiac injection of barbiturate (100 mg kg^{-1} pentobarbitone sodium, Euthatal; RMB Animal Health, UK). Immediately before this procedure, a 10 ml blood sample was taken. In order to obtain time-matched controls for these lambs, either their saline-treated twin or a saline-treated singleton lamb that had established normal breathing was sampled at an identical age. In the remaining five TRH-treated (2 twins and 3 singletons) and 5 control (2 twins and 3 singletons) lambs, a jugular vein catheter was inserted 25–30 min after birth, to allow blood sampling (Clarke *et al*. 1996). Each catheterised lamb was subsequently placed in an indirect calorimeter maintained at 30 °C. Continuous measurements of colonic temperature, plus breathing frequency and pattern using inductance plethysmography (Symonds *et al*. 1992) were made until lambs were 2–2·5 h old. Sleep state was determined from these respiratory pattern measurements. The occurrence of interference on respitrace patterns was also used to assess the incidence of shivering, with intensity of shivering being classified from its amplitude and duration (Clarke *et al*. 1997*a*). A maximum of four, 10 ml blood samples were taken every 30 min up to 2 h of age. Oxygen consumption and carbon dioxide production were measured continuously using indirect open-circuit calorimetry. Mean values presented represent values obtained during periods of non-rapid-eye-movement sleep in order to minimise variations due to animal movement, and were recorded using two identical indirect-calorimetry systems based on that described by Symonds *et al*. (1992), with the modification that air flow was measured using a differential flow indicator (Perflow Instruments Ltd, Willesdon, UK). Lambs were then humanely killed 2·5 h after birth by intravenous administration of barbiturate. Both perirenal adipose tissue depots plus a liver and lung sample were rapidly removed, placed in liquid nitrogen and stored at −70 °C until analysed. All operative procedures and experimental protocols had the required Home Office approval as designated by the Animals (Scientific Procedures) Act of 1986.

Laboratory procedures

Mitochondria were prepared from frozen perirenal adipose tissue 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 activity was measured in order to assess the recovery of mitochondrial protein. The thermogenic activity of perirenal adipose tissue was 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). Uncoupling protein-1 (UCP1) was detected in mitochondrial preparations following separation by sodium dodecyl polyacrylamide gel electrophoresis using immunoblotting and enhanced chemiluminescence (ECL, Amersham International). Antibodies used were raised against purified ovine UCP1 as described by Schermer *et al*. (1996). All gels were run in duplicate. Densitometric analysis was then performed on each gel using an Ultroscan XL densitometer and GelScan XL software package (Pharmacia LKB Biotechnology, Uppsala, Sweden). All values were expressed as a percentage of reference samples (i.e. from a 1-day-old lamb born vaginally) run on all gels. The activities of type I (BAT and liver) and type II (BAT and lungs) iodothyronine 5′ deiodinase (I5′D) were determined by measuring the release of $^{125}I^{-}$ from [^{125}I] reverse triiodothyronine (Amersham International plc, Bucks, UK) as described by Clarke *et al*. (1994). Tissue DNA content was measured fluorometrically (Hinegardner, 1971). Hepatic glycogen was assayed using the method of Keppler & Decker (1984).

Plasma concentrations of FFA were measured enzymatically, whilst total T_3 and thyroxine (T_4) were measured using radioimmunoassays as described by Clarke *et al*. (1994). Plasma cortisol and free T_3

Table 1. *Time to commence breathing, shivering and changes in colonic temperature*

	Time to breath (min)	Time to shiver (min)	Initial colonic temperature (°C)	Rate of decline in colonic temperature (°C min^{-1})	Trough colonic temperature (°C)	Time to restore colonic temperature (min)	Plateau colonic temperature (°C)
Controls	1·25 ± 0·95	17·8 ± 3·3	40·45 ± 0·26	0·069 ± 0·012	37·79 ± 0·77	27·4 ± 8·7	38·77 ± 0·96
TRH	0·25 ± 0·25	17·3 ± 5·3	40·27 ± 0·15	0·087 ± 0·015	37·69 ± 0·80	36·8 ± 13·3	39·46 ± 0·31

The values are means ± S.E.M., $n = 5$ for control and TRH-treated lambs.

Table 2. *Mean oxygen consumption, carbon dioxide production and breathing frequency*

	Time after birth (min)	Incidence of shivering (a.u.)	Oxygen consumption (ml min^{-1} kg)	Carbon dioxide production (ml min^{-1} kg)	Breathing frequency (breaths min^{-1})
Controls					
	40–60	0·5 ± 0·1 [a]	22·1 ± 4·4	20·5 ± 4·0	77 ± 7
	70–110	0·6 ± 0·1	15·3 ± 3·6***	14·3 ± 3·1***	65 ± 8***
TRH					
	40–60	1·3 ± 0·1 [b]	29·7 ± 3·7	27·2 ± 3·6	82 ± 8
	70–110	0·6 ± 0·1***	19·6 ± 1·9***	18·7 ± 1·9***	57 ± 4***

The values are means ± S.E.M., $n = 5$ for control and TRH-treated lambs. Significant differences between control and TRH-treated groups: [ab]$P < 0·001$. Significant differences between 40–60 and 70–110 min: *** $P < 0·001$.

Table 3. *Mean jugular venous plasma concentrations of thyroid hormones, cortisol, catecholamines, FFA, glucose and lactate as measured between 25 and 120 min after birth*

	[T_4] (nmol l^{-1})	[T_3] (nmol l^{-1})	[Free T_3] (pmol l^{-1})	[Cortisol] (nmol l^{-1})	[NA] (ng ml^{-1})	[Adr] (ng ml^{-1})	[FFA] (mmol l^{-1})	[Glucose] (mmol l^{-1})	[Lactate] (nmol l^{-1})
Controls	135±16	4·46±0·74	3·14±0·55	245±47	3·07±0·46	2·37±0·56	0·59±0·11	3·01±0·50	6·09±1·10
TRH	129±11	4·50±0·636	8·48±3·09	154±35	2·48±0·54*	1·19±0·19*	0·41±0·14	3·42±0·90	6·38±2·13

The values are means ± S.E.M., $n = 8$ for control and TRH-treated groups except where indicated with asterisks when $n = 5$ for the TRH-treated group. Adr, adrenaline; NA, noradrenaline.

were measured using magnetic solid phase immunoenzymatic assay kits (Bird *et al*. 1996). The intra- and interassay coefficients of variation for free T_3 analysis were 3·5 and 3·9 % ($n = 5$). Plasma for measuring catecholamine content was prepared from heparinised blood to which was added 20 μl ml^{-1} of a 9·5 % (w/v) solution of EGTA plus 6 % reduced glutathione. The concentrations of noradrenaline and adrenaline were then determined as described by Clarke *et al*. (1996).

Statistical analysis

Statistical analysis of treatment effects was assessed by analysis of variance. For all tissue and plasma measurements made, an assessment of any effects of sampling time and TRH treatment was initially performed. This indicated there were no significant differences between saline-treated lambs sampled at 25 min or 2·5 h so these animals were considered together as one group. A similar relationship was observed for TRH-treated lambs with the exception of lung weights and plasma catecholamine concentrations, so for these measurements only TRH-treated lambs sampled at 25 min and 2·5 h were considered separately. In the case of *in vivo* measurements taken from lambs sampled at 2·5 h of age, analysis of variance with correction for repeated measures (i.e. between 40–60 and 70–100 min) was used.

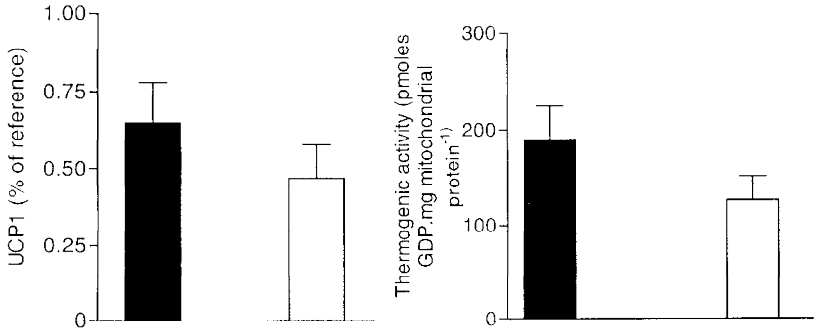

Fig. 1. Effect of TRH treatment on uncoupling protein-1 (UCP1) abundance and thermogenic activity of brown adipose tissue. Lambs were injected either with TRH (8 μg; ■) or saline (□). Results are given as means + S.E.M. (bars) and $n = 8$ per group.

RESULTS

Thermoregulation after birth

There was no effect of TRH on time taken to commence continuous breathing or shivering between lambs studied up to 2·5 h after birth (Table 1). Lamb birth weight was similar between groups (controls, $n = 8$: 4·07 ± 0·5 kg (mean ± S.E.M.); TRH treated, $n = 8$: 3·67 ± 0·4 kg). The rate of decline in colonic temperature, as well as its rate of restoration and plateau values, were not influenced by TRH. It should be noted that all five TRH-treated lambs sampled at 2·5 h were able to restore colonic temperature but one control lamb remained hypothermic with a body temperature of 35·11 °C. Incidence of shivering was greater ($P < 0·01$) in TRH-treated lambs compared with controls at 1 h but not 2 h of age (Table 2), as the intensity of shivering declined with age in treated lambs but not controls. Oxygen consumption, carbon dioxide production and breathing frequency were not altered by TRH, and in both groups these all declined with time after birth.

Plasma hormone and metabolite concentrations

Plasma T_3, T_4, cortisol, FFA, glucose and lactate concentrations were not influenced by TRH treatment (Table 3). The mean plasma concentration of free T_3 was 170 % higher in TRH-treated lambs, but there was a large variation between individuals. TRH treatment had no effect on plasma catecholamine concentrations, with the exception of those of non-surviving TRH-treated lambs that exhibited significantly higher catecholamine levels (noradrenaline, 13·50 ± 4·08 ng ml^{-1} ($P < 0·001$); adrenaline, 39·84 ± 0·29 ng ml^{-1} ($P < 0·001$) ($n = 3$)).

Perirenal adipose tissue, liver and lung composition

There was no influence of TRH treatment on BAT weight (controls, $n = 8$: 23·15 ± 2·43 g; TRH treated, $n = 8$: 20·36 ± 1·08 g), protein content (controls: 2·85 ± 0·54 g; TRH treated: 2·57 ± 0·43 g) or mitochondrial protein content (controls: 1·12 ± 0·19 g; TRH treated: 1·08 ± 0·13 g), but DNA content was significantly ($P < 0·05$) lower in TRH-treated lambs (controls: 129 ± 20 mg; TRH treated: 80 ± 6 mg). BAT from the TRH-treated group had more UCP1 and a higher thermogenic activity (Fig. 1), although only this latter difference approached significance ($P = 0·06$). I5′D activity in BAT (Type I – controls: 203 ± 31 pmol I$^-$

Table 4. *Mean lung weight and composition*

	Weight (g)	Protein content (g)	DNA content (mg)	I5′D – Type I (fmol I^- released (mg protein)$^{-1}$ h^{-1})
Controls	135 ± 16	6·2 ± 1·1	2029 ± 421	437 ± 101
TRH				
Surviving	106 ± 12	4·5 ± 1·3	812 ± 241	891 ± 181
Non-surviving	147 ± 10	7·4 ± 2·3	2980 ± 474 *	400 ± 207

The values are means ± S.E.M., $n = 8$ for control group, $n = 5$ for surviving and $n = 3$ for non-surviving TRH-treated groups. Significant differences between surviving and non-surviving TRH-treated groups: * $P < 0{\cdot}05$.

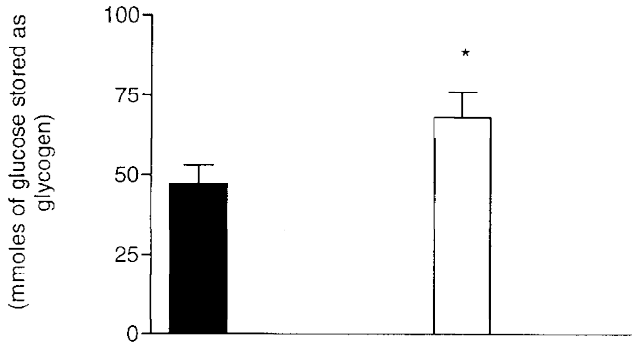

Fig. 2. Effect of TRH treatment on hepatic glycogen content. Lambs were either injected with TRH (8 μg; ■) or saline (□). Results are given as means + S.E.M. (bars) and $n = 8$ per group. Significant difference between groups: * $P < 0{\cdot}05$.

released (mg protein)$^{-1}$ h^{-1}; TRH treated: 186 ± 29 pmol I^- released (mg protein)$^{-1}$ h^{-1}; Type II – controls, $n = 8$: 249 ± 47 fmol I^- released (mg protein)$^{-1}$ h^{-1}; TRH treated: 297 ± 57 fmol I^- released (mg protein)$^{-1}$ h^{-1}) and liver (Type I – controls: 2279 ± 357 pmol I^- released (mg protein)$^{-1}$ h^{-1}: TRH treated: 1771 ± 388 pmol I^- released (mg protein)$^{-1}$ h^{-1}) was unaffected by TRH treatment. Liver weight (controls: 115 ± 17 g; TRH treated: 101 ± 12 g), protein (controls: 17·1 ± 3·4 g; TRH treated: 15·5 ± 2·9 g) and DNA content of liver (controls: 1693 ± 374 mg; TRH treated: 1687 ± 388 mg) were not influenced by TRH, but glycogen content was significantly ($P < 0{\cdot}05$) lower in controls (Fig. 2).

There was no difference in lung weight between controls and non-surviving TRH-treated lambs (Table 4), but when expressed as a percentage of control values, lung weight was lower in surviving TRH-treated lambs (surviving, $n = 5$: 78·5 ± 9·1 %; non-surviving, $n = 3$: 108·8 ± 7·6 %; $P = 0{\cdot}06$). No differences between groups were recorded for lung protein content, but DNA content was lower in surviving TRH-treated lambs. I5′D activity in lung was higher in TRH-treated lambs surviving to 2·5 h of age, but there was a large variation between individuals and this difference was not significant (Table 4).

DISCUSSION

The major finding of the present study is that an umbilical vein injection of TRH can influence adaptation after birth in near-term lambs delivered by Caesarean section. TRH treatment

resulted in either marked changes in thermoregulation or was associated with a failure to establish continuous breathing in a small subgroup of lambs. It is therefore necessary to consider these divergent responses separately. Over the first hour of neonatal life, although TRH-treated lambs exhibited an increased incidence of shivering, it was not accompanied by a higher colonic temperature. This response can be explained by the fact that shivering is an inefficient method of thermoregulation as it results in an increased rate of air movement around the animal, thereby reducing external insulation and increasing heat loss (Alexander, 1979). A higher incidence of shivering can increase the rate of depletion of endogenous energy stores (Mellor & Cockburn, 1986), which could explain why hepatic glycogen stores were reduced in TRH-treated lambs of this study. The stimulatory effect of TRH on shivering was not maintained during the second hour of neonatal life as oxygen consumption continued to decline. By this time, TRH-treated lambs benefited from possessing BAT with a higher thermogenic activity and were all fully able to restore body temperature. In TRH-treated lambs, however, both the amount and activity of UCP1 remained below values observed in vaginally delivered lambs (Clarke *et al*. 1997*a*). It is, therefore, not unexpected that in contrast to vaginally delivered lambs colonic temperature was not maintained after birth. Further evidence of the potential responsiveness of BAT to TRH stimulation is provided by the appreciably lower quantity of DNA in BAT from TRH-treated lambs. We have previously observed a decline in DNA content in BAT over the first 6 h of life in fed Caesarean section delivered lambs (Clarke *et al*. 1997*b*) that could indicate apoptosis.

The extent to which changes in free T_3 levels in TRH-treated lambs was responsible for stimulating BAT function remains to be established. Failure to detect any significant differences in plasma thyroid hormone concentrations between TRH-treated lambs and controls may relate to the short time period over which the study was conducted. We have found a significant increase in total and free plasma thyroid hormone concentrations following an umbilical vein injection of TRH to lambs delivered by Caesarean section 3–4 h after birth (Heasman *et al*. 1998). Any effects of TRH on free T_3 production did not appear to be mediated by changes in peripheral deiodination as I5′D activity was similar between control and TRH-treated lambs. The extent to which TRH may alter thyroid binding globulin activity remains to be clarified. TRH has been shown to be a very potent stimulator of fetal prolactin secretion (Thomas *et al*. 1975). The extent to which prolactin can directly influence BAT function is not known. High abundance of mRNA for both the long and short forms of the prolactin receptor has recently been described in ovine fetal BAT (Symonds *et al*. 1998) for which the amount of receptor protein can be correlated to UCP1 abundance (Symonds & Stephenson, 1999).

TRH treatment not only altered thermoregulation but also had a marked effect on lung weight. In TRH-treated lambs sampled at 2·5 h lung weight was 21–28 % lighter than in either controls or TRH-treated lambs sampled at 25 min of age and is indicative of increased clearance of lung liquid (Stein *et al*. 1994; Clarke *et al*. 1996). The extent to which lung function may have been acutely altered following TRH administration remains to be determined. TRH has been shown to elicit an immediate stimulatory effect on fetal breathing movements (Bennet *et al*. 1988). A 60 h period of fetal exposure to TRH plus corticosteroids results in improved ventilatory function following Caesarean section delivery at 130 days of gestation (Stein *et al*. 1994). In the present study, surviving TRH-treated lambs were characterised as possessing lungs with a greatly reduced DNA content. A decline in DNA content could be indicative of apoptosis, a process implicated in fetal lung airway branching (Catlin *et al*. 1997). During fetal development, enhanced apoptosis has been associated with a

decrease in total lung bud number (Catlin *et al*. 1997) which increases dramatically at birth (Kresch *et al*. 1998). T_3 stimulates apoptosis *in vitro* (Suzuki *et al*. 1997) but has yet to be shown to promote similar effects *in vivo*.

It is intriguing that three of eight TRH-treated lambs failed to establish continuous breathing. This problem did not appear to be due to a failure to clear lung liquid fluid as lung weights were similar to controls, but higher than surviving TRH-treated lambs. Non-surviving TRH-treated lambs were characterised as having very high plasma catecholamine concentrations at the time of death, which could be a response to failing to establish continuous breathing. Alternatively, it could reflect an initial hyper-responsiveness to TRH treatment as TRH can enhance sympathetic activity (Mattila & Bunag, 1986). It remains to be determined whether TRH treatment may actually inhibit the establishment of continuous breathing at birth.

In conclusion, TRH treatment of near-term lambs at the time of Caesarean section delivery increases the recruitment of both shivering and non-shivering thermogenesis.

This work was funded by The Wellcome Trust and Lindsay Heasman was supported by a Ministry of Agriculture Fisheries and Food Studentship.

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