

## The role of leptin in the transition from fetus to neonate

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Leptin is a 16 kDa hormone which has been shown to have a major physiological role in the control of energy balance. Leptin is produced primarily in white adipose tissue, although there is evidence for its production in brown adipose tissue (BAT) and the placenta. BAT is critically important for the initiation of non-shivering thermogenesis in the newborn through the BAT-specific uncoupling protein (UCP), UCP1. This factor is particularly important in lambs in which levels of UCP1 peak at birth, concomitant with a rapid decline in plasma leptin levels. Our studies have examined the effect of acute and chronic administration of leptin to neonatal lambs, investigating effects on colonic temperature, UCP1 and thermogenic potential of BAT. Administration of leptin in sequential physiological doses of 10, 100 and 100 µg to neonatal lambs caused a modest increase in colonic temperature which was not observed in weight-matched vehicle-treated controls. This increase in colonic temperature was not mediated by an increase in either abundance or thermogenic potential of UCP1, as previously shown in adult rodents. UCP1 mRNA levels were 30 % lower in leptin-treated lambs, which is also contradictory to findings in adult rodents. Leptin treatment resulted in a dose-dependent rise in plasma leptin, with levels at the end of the study being almost twenty times greater in leptin-treated animals. To determine whether these findings in neonatal lambs were transient due to the complex milieu of hormones present after birth, we examined the effect of chronic leptin treatment over 6 d. Pairs of lambs were treated daily, from the second to seventh day of life with 100 µg leptin or vehicle. Colonic temperatures of leptin- and vehicle-treated animals remained similar throughout the study. UCP1 abundance was significantly lower in the leptin-treated animals, suggesting that the drop in UCP1 mRNA seen in the previous study had been translated to protein levels. In conclusion, the decline in plasma leptin levels at birth may be a signal to initiate enteral feeding. In lambs, the rapid loss of UCP1 mRNA, which occurs within the first few days of life, appears to be accelerated by leptin administration, possibly stimulating the development of white adipose tissue and generation of body heat through mechanisms other than non-shivering thermogenesis by UCP1 in BAT.

### Leptin: Brown adipose tissue: Neonate: Uncoupling protein

Since the discovery of leptin in 1994 (Zhang *et al.* 1994) there has been a great deal of interest in its role in metabolic regulation and the possibility of using leptin for promoting weight loss. Evidence from human studies suggests that there are dynamic changes in leptin over the period of transition from fetus to neonate (Matsuda *et al.* 1999; Fig. 1).

### Leptin and adipose tissue

Leptin is a 16 kDa protein and a member of the cytokine superfamily (Madej *et al.* 1995). It is produced mainly in white adipose tissue, although there is evidence for its production in brown adipose tissue (BAT; Cinti *et al.* 1997; Dessolin *et al.* 1997), the placenta (Hassink *et al.* 1997; Hoggard *et al.* 1997a, 2000) and also the stomach (Bado

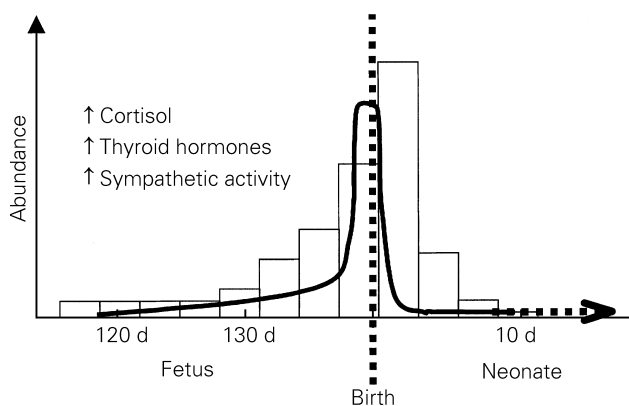
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**Abbreviations:** BAT, brown adipose tissue; OB-R, leptin receptor; UCP, uncoupling protein.

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*et al.* 1998). BAT is derived from stem cell precursors with the potential to become brown or white adipose tissue (Gregoire *et al.* 1998; Wu *et al.* 1999) and is present at birth in most mammalian species studied to date. BAT contains the unique uncoupling protein (UCP), UCP1, that is responsible for the rapid production of large amounts of heat after birth in precocial newborns, including the lamb (Clarke *et al.* 1997b) and human infant. Adult rodent studies have shown that administration of leptin to *ob/ob* mice, who do not produce leptin and are thus hypothermic, hyperphagic and obese, restores a normal body temperature, despite a 50% reduction in food intake (Pellemounter *et al.* 1995). These changes in body temperature were linked to an increased abundance of UCP1 in BAT (Scarpace *et al.* 1997). Circulating leptin concentrations are known to be regulated by many factors, including cortisol (De Vos *et al.* 1995), thyroid hormones (Flier *et al.* 2000) and the sympathetic nervous system (Mostyn *et al.* 1998), all of which are critically important at parturition and for the onset of breathing and thermoregulation at birth (Fig. 1; Symonds, 1995).

Leptin acts through six alternatively spliced receptors (OB-R), OB-Ra, b, c, d and e (Tartaglia *et al.* 1995; Lee *et al.* 1996; Fei *et al.* 1997; Hoggard *et al.* 1997b), which are encoded by the diabetes (*db*) gene (Chen *et al.* 1996) and expressed in selected tissues. OB-Rb is the long and signalling form of the receptor and has a high level of expression in the regions of the brain involved in energy balance and appetite regulation, including the ventromedial nucleus, arcuate nucleus and paraventricular nucleus (Mercer *et al.* 1996). OB-Rb is also expressed in the placenta (Hoggard *et al.* 1997a). OB-Ra is thought to be the most widely expressed form of receptor and has been found in all tissues studied to date. OB-Ra functions as a transporter and OB-Re is a soluble binding protein. Fig. 2 shows the distribution and relative abundance of OB-R in a selection of fetal and postnatal ovine tissues, including lung, kidney, hypothalamus, testis and BAT. Leptin acts through its receptors to regulate many physiological functions, including the onset of puberty, reproduction (Messinis & Milingos, 1999), appetite and energy regulation (Friedman & Halaas, 1998). Leptin is also thought to act in an autocrine



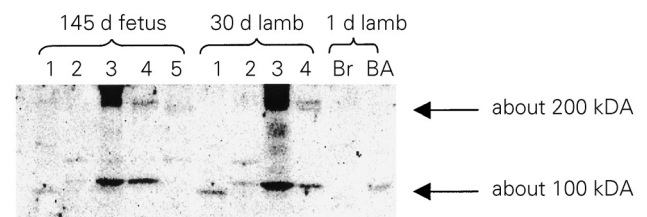
**Fig. 1.** The ontogeny of uncoupling protein-1 (□) and leptin mRNA (—) from late gestation to early neonatal life. -.-, Stimulation by factors indicated. (From Clarke *et al.* 1997a; Yuen *et al.* 1999.)

way, feeding back to the adipose tissue site of production to modify expression of leptin (Zhang *et al.* 1997). In this way leptin may influence BAT function and UCP1 production.

### Leptin and the fetus

Leptin mRNA is detectable in fetal adipose tissue of sheep by about 90 d of gestation, with levels increasing up to term (Yuen *et al.* 1999), which is about 147 d in the sheep. Leptin mRNA levels are closely correlated with fetal weight. The slope of the relationship between leptin mRNA abundance and fetal weight is steeper at about 90 d of gestation compared with 125–140 d of gestation (Yuen *et al.* 1999). This finding suggests that production of leptin mRNA is modulated by the increase in size and number of adipocytes which occurs during late gestation in the ovine fetus (Lonnqvist *et al.* 1997), but is also sensitive to the rapid increase in body weight at this period. The increase in leptin mRNA may also be attributed to the rise in circulating corticosteroids during late gestation, since glucocorticoids have been shown to stimulate leptin expression both *in vitro* and *in vivo* (De Vos *et al.* 1995; Mostyn *et al.* 2001). OB-R mRNA is present in a variety of murine fetal tissues (Hoggard *et al.* 1997a, 2000), although its physiological relevance is as yet unknown.

It has been suggested that leptin may act as a growth factor in the fetus, directing growth and development via central or peripheral actions (Hassink *et al.* 1997; Stepan & Swick, 1999; Udagawa *et al.* 2000). Many studies have measured plasma leptin concentrations in newborn infants; however, the results from these studies have been highly inconsistent, as shown in Table 1. These contrasting findings may be due to a number of confounding factors, including geographical location, maternal and social differences, as well as the clinical status of the infant at the time of blood sampling. Also, there are known to be sensitivity problems with the radioimmunoassay kit used (Linco Multi-Species; Linco Research Inc., St Charles, MO, USA), with regard to the detection of low-range values, and cross-reactivity between non-specific plasma proteins and the 'multi-species' antibody (Imagawa *et al.* 1998; Delavaud *et al.* 2000).



**Fig. 2.** Western blot of leptin receptor protein in ovine tissues. Protein (30 µg) was loaded onto each lane. Samples were as follows: 1, hypothalamus; 2, adrenal; 3, kidney; 4, lung; 5, testis; Br, brain; BA, brown adipose tissue (plasma membranes). Major bands were observed at about 100 and 200 kDa, as determined by molecular-weight markers.

**Table 1.** Summary of the studies investigating fetal and neonatal leptin concentrations in human subjects

Country of study	Gestation	Cord leptin (ng/ml)		Neonatal leptin (ng/ml)		Main finding	Reference
		Mean	SE	Mean	SE		
Finland	Term	9.7	5.2			Leptin concentrations correlate with adiposity in female but not male newborns	Hytinantti <i>et al.</i> (1999)
Sweden	Term	7.3(median)				Cord leptin correlates with adipose tissue mass	Marchini <i>et al.</i> (1998)
UK	Term	5.9	3.0			Fetal leptin levels increase towards term	Geary <i>et al.</i> (1999)
Germany	Term	3.6	1.4	0.4	0.3	Cord leptin correlates with adiposity and levels decline after birth	Schubring <i>et al.</i> (1999)
Turkey	Term			2.29	0.52	Plasma leptin levels correlate with adiposity and are related to nutritional status	Cinaz <i>et al.</i> (1999)
USA	Term	4.1	3.5			Cord leptin levels are correlated with adiposity with no gender differences	Shekhawat <i>et al.</i> (1998)
USA	Term	4.2(median)				Cord leptin levels are correlated with birth weight and are reduced in premature infants	Tarquini <i>et al.</i> (1999)
Japan	Term	19.6	14.3	1.9	1.1	Leptin concentrations are reduced after birth	Matsuda <i>et al.</i> (1999)
Japan	Term	Artery: 9.8 Vein: 12.9	1.2 1.8			Leptin levels are higher in umbilical veins than arteries; leptin concentrations fall rapidly after birth	Yura <i>et al.</i> (1998)

Results from human clinical studies suggest that leptin values remain low during early gestation, are detectable by about 35 weeks of gestation, and increase towards term in response to the increased abundance of adipose tissue (Cinaz *et al.* 1999; Matsuda *et al.* 1999). Leptin levels peak around birth in the infant, then rapidly decline by day 3 of postnatal life (Hytinantti *et al.* 1999; Schubring *et al.* 1999). There is also evidence that leptin is expressed in rodent adipose tissue at birth (Dessolin *et al.* 1997) and may play a role in energy regulation of the neonate (Yuen *et al.* 2000). These postnatal changes are likely to occur in response to the dramatic alterations in energy balance experienced by the neonate during the transition to enteral feeding, since fasting has been shown to reduce circulating leptin levels (Ahima *et al.* 1996). Initially there may be some delay before the mother's milk is produced and colostrum secreted during the first few days has a low energy content. There is evidence that leptin levels are higher in female infants and lambs compared with the corresponding males (Mostyn *et al.* 1999), as leptin production is inhibited by testosterone (Behre *et al.* 1997). Leptin is also present in the placenta (Hassink *et al.* 1997; Hoggard *et al.* 1997a, 2000; Ashworth *et al.* 2000). This additional source may increase both fetal and maternal circulating concentrations of leptin (Dandrea *et al.* 2000), and has been shown to be nutritionally sensitive (Wilson *et al.* 2000).

### The uncoupling proteins and leptin

UCP1 is the UCP responsible for non-shivering thermogenesis in the neonate (Cannon & Nedergaard, 1985). UCP1 acts by 'uncoupling' normal respiration and allowing the protons produced by the respiratory chain to flow back into the mitochondrial matrix rather than through ATPase. The chemical energy from the proton motive force is dissipated

as heat by the animal (Nedergaard & Cannon, 1992). The ontogeny of UCP1 mRNA in BAT is very similar to that of leptin (Fig. 1). In the ovine fetus UCP1 mRNA appears at about 110 d of gestation, and levels remain low, increasing towards term, since non-shivering thermogenesis does not occur *in utero* under normal circumstances (Symonds *et al.* 1995). In man and other large mammals UCP1 mRNA disappears within a few days after birth (for example, see Clarke *et al.* 1997a). UCP1 protein disappears in the first months after life, and is not expressed during adulthood except under extreme pathological conditions, e.g. pheochromocytoma, a tumour of the adrenal gland which produces high circulating concentrations of catecholamines (Ricquier *et al.* 1982; Lean *et al.* 1986a,b). In rodents UCP1 can be reactivated by a variety of factors, including cold exposure (Denjean *et al.* 1999) and cafeteria-diet feeding (Denjean *et al.* 1999; Rippe *et al.* 2000).

When leptin is administered in physiological doses (e.g. 1 mg) it has been shown to stimulate UCP1 expression in the adult rodent (Scarpace *et al.* 1997), by increasing sympathetic activity and noradrenaline turnover in BAT (Collins *et al.* 1996; Haynes *et al.* 1997). However, these studies are not suitable for comparison with large mammals or neonates.

UCP1 belongs to a family of UCP, other members including UCP3, which is found selectively in adipose tissue and muscle (Vidal-Puig *et al.* 1997), and UCP2 which is expressed in a large number of tissues (Fleury *et al.* 1997). Both UCP2 and UCP3 are thought to have roles in energy regulation, and leptin has been shown to regulate their abundance (Scarpace *et al.* 1998). UCP3 is not expressed during fetal life in rodents, but mRNA levels surge at birth (Carmona *et al.* 1998). The factors responsible for this postnatal rise are unknown. UCP2 and UCP3 mRNA have been shown to be present in piglet muscle (Damon *et al.* 2000), a

species that does not express UCP1. It has therefore been suggested that UCP2 and UCP3 are involved in producing heat by non-shivering thermogenesis in muscle.

Rodent models of over- and underexpression of UCP3 have shown contrasting effects. Mice which overexpress human UCP3 in skeletal muscle have been shown to be hyperphagic, consuming about 40 % more than wild-type mice from 4 to 12 weeks of age, but were leaner (Clapham *et al.* 2000). Fat mass in the UCP3-overexpression group was also significantly lower than wild type ( $P < 0.05$ ), suggesting increased fat turnover and metabolism in the mice overexpressing UCP3. However, lack of UCP3 expression is not associated with obesity, nor a reduced ability to maintain body temperature during cold exposure (Vidal-Puig *et al.* 2000). Problems of interpreting findings from transgenic mice have been further compounded by the contrasting effects on obesity seen in UCP1-ablated mice. The first study on UCP1-ablated mice (Lowell *et al.* 1993) found the animals became moderately hypothermic (i.e. body temperature about 35°C) during cold exposure (i.e. 2 h at 5°C) and developed obesity, although UCP1 protein was still detectable at very low levels. A more recent study (Enerback *et al.* 1997) found that mice lacking UCP1 became extremely hypothermic (i.e. body temperature decreased by 10°C to about 27°C during cold exposure (at 4°C). These mice did not become obese; the compensation for loss of UCP1 in this case has been attributed to an increase in UCP2 mRNA in BAT (Enerback *et al.* 1997).

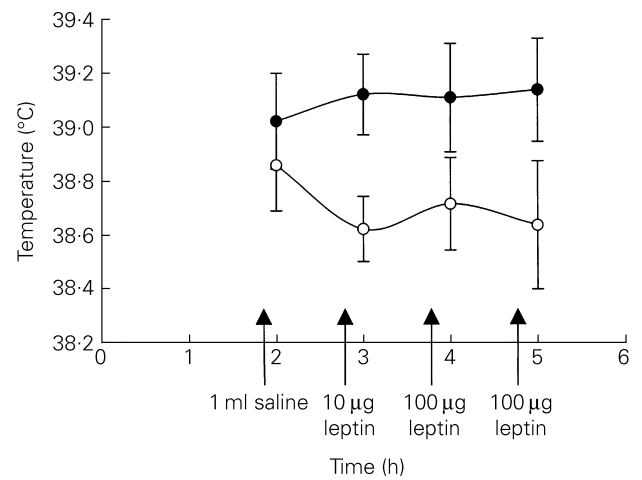
#### Effect of acute leptin treatment on neonatal lambs

Given the rapid decline in plasma leptin concentrations after birth (Fig. 1), we investigated the role of acute leptin administration during the early neonatal period in a precocial species with thermoregulatory and hormonal responses at birth similar to those in human subjects, i.e. lambs. Lambs (1-d-old) were treated sequentially with 10, 100 and 100 µg leptin or vehicle (sterile water) while colonic temperature was measured continuously. Blood samples were taken throughout the study to allow measurement of plasma leptin. Administration of leptin in sequential doses of 10, 100 and 100 µg promoted maintenance of colonic temperature in the absence of feeding, which was not observed in weight-matched vehicle-treated controls (Fig. 3; Mostyn *et al.* 2000a).

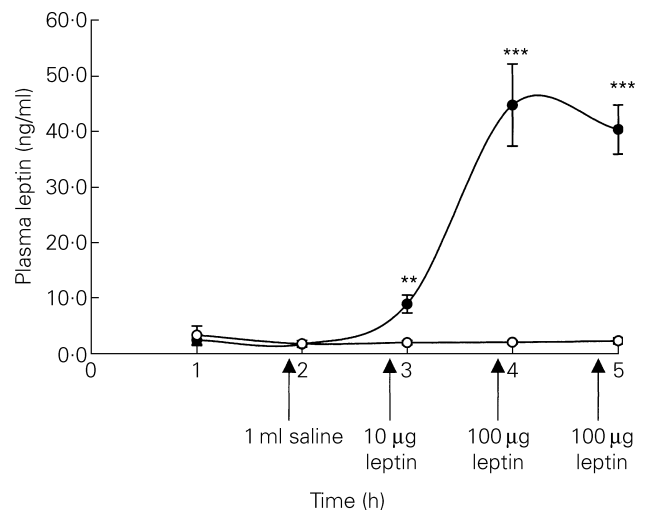
As a consequence, colonic temperature was significantly lower ( $P < 0.05$ ) in controls compared with leptin-treated animals at the end of the study. All lambs in both groups shivered, but leptin-treated lambs shivered for half as much time as controls. Lamb body and perirenal adipose tissue weights were similar between groups (Mostyn *et al.* 2000a). In contrast, abundance of UCP1 mRNA, when expressed as a ratio of r18S, was lower in six of eight leptin-treated lambs compared with controls. On average, UCP1 mRNA expression was 30 % lower in the leptin-treated group (Bispham *et al.* 1999). Despite this decline in UCP1 mRNA expression there was no difference between groups in UCP1 protein abundance, or the thermogenic potential, as assessed by the guanosine diphosphate-binding assay (Mostyn *et al.* 1999). This outcome was as expected, since the half-life of UCP1 is 7 d (Clarke *et al.* 1997a).

There was no difference in plasma leptin concentrations at the start of the study (Fig. 4), although leptin concentrations were significantly higher in female lambs compared with male lambs ( $P < 0.05$ ; Mostyn *et al.* 1999). Leptin administration resulted in a significant ( $P < 0.01$ ) dose-dependent increase in plasma leptin concentrations, irrespective of gender.

These findings show that administration of leptin to 1-d-old lambs causes maintenance of colonic temperature, despite a reduction in UCP1 mRNA and no change in thermogenic potential. The loss of UCP1 mRNA suggests that leptin may be one of the factors involved in initiating the rapid loss of UCP1 after birth. These results are therefore contradictory to those of adult rodent studies (Scarpace *et al.* 1997) which used higher doses of



**Fig. 3.** Colonic temperatures of leptin- (●) and vehicle (sterile water; ○)-treated 1-d-old lambs. Values are means with their standard errors represented by vertical bars. (From Mostyn *et al.* 2000a.)



**Fig. 4.** Plasma leptin concentrations in leptin- (●) and vehicle (sterile water; ○)-treated 1-d-old lambs. Values are means with their standard errors represented by vertical bars. Mean values were significantly different from those of lambs treated with vehicle: \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . (From Mostyn *et al.* 1999.)

leptin and possibly observed pharmacological effects (Scarpace *et al.* 1997).

**Effect of chronic leptin administration to neonatal lambs**

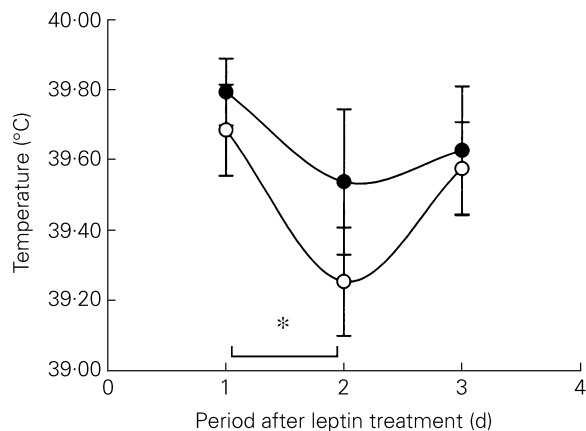
In order to determine whether the earlier findings in neonatal lambs were transient due in part to the complex milieu of hormones present after birth, we decided to carry out a longer-term study. Pairs of 1-d-old lambs were treated daily with either 100 µg leptin or vehicle for 6 d (days 2–7 of life; Mostyn *et al.* 2000b). Colonic temperature and blood samples were taken daily before treatment. There was no difference in colonic temperature between the two groups on any day (Fig. 5), but leptin-treated animals were better able to maintain body temperature between days 1 and 2 when the vehicle group exhibited a significant decline in colonic temperature ( $P < 0.05$ ).

Despite this maintenance of colonic temperature, the leptin-treated group had significantly lower UCP1 abundance in BAT than controls ( $P < 0.05$ ; Fig. 6), but similar thermogenic potential, as measured by guanosine diphosphate binding, after 6 d of treatment (Mostyn *et al.* 2000b). Body weight, BAT weight and growth rates were similar between groups.

Chronic leptin administration to neonatal lambs therefore causes loss of UCP1 protein, with no change in thermogenic potential or temperature. This loss of UCP1 may be compensated for by modification of expression of one or more of the other UCP, although this possibility has not yet been investigated.

**Future perspectives**

Leptin has the potential to play a role in the transition from fetal to neonatal life, although its exact function remains unknown. Leptin concentrations decline rapidly after birth, as the infant moves from a state of passive nutrient uptake to

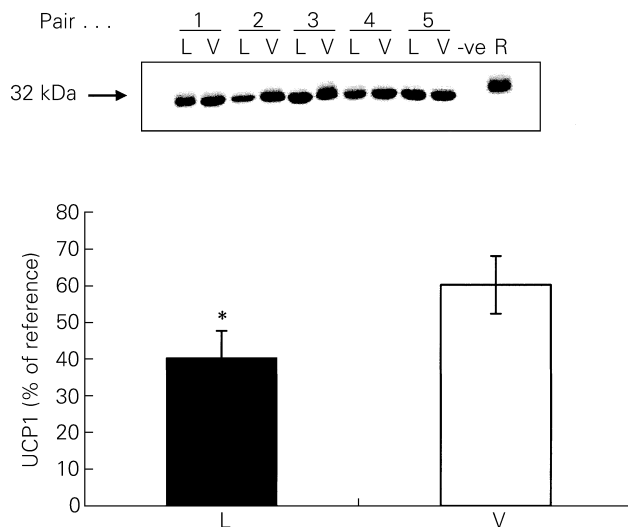


**Fig. 5.** Effect of chronic leptin treatment (100 µg/d) on colonic temperature in pairs of 1-d-old lambs treated daily with either leptin (●) or vehicle (sterile water; ○) for 6 d (days 2–7 of life). Values are means with their standard errors represented by vertical bars. There was a significant decline in temperature in vehicle-treated lambs between days 1 and 2 that was not observed in leptin-treated lambs: \* $P < 0.05$ . (From Mostyn *et al.* 2000b.)

one of active enteral feeding. Leptin levels are known to decrease in response to starvation (Ahima *et al.* 1996), and this decline may be one way of facilitating the infant's intake of milk. As the infant or lamb commences feeding and increases food intake, fat deposition will occur, since less energy is needed for maintaining body temperature. The neonate will therefore have less requirement for non-shivering thermogenesis in BAT, since heat production from dietary-induced and shivering thermogenesis in muscle plus increased insulation act to maintain body temperature (Symonds *et al.* 1989a,b). It is hypothesised that with increasing age plasma leptin increases in response to feeding and at the same time promotes loss of UCP1. Concurrently, abundance of UCP2 and UCP3 may be increased, the activity of which could be stimulated during periods of nutritional stress (e.g. starvation; Ahima *et al.* 1996). This process will enable the neonate to maintain a BMR and prevent hypothermia despite the loss of UCP1. More studies are required to fully elucidate the role of leptin in the fetus and neonate. The emergence of sheep transgenic models (McCreath *et al.* 2000) in which under- and over-expression of UCP can be demonstrated are likely to provide more information regarding the effect of leptin on thermogenesis at birth. Both acute and chronic leptin treatment reduce UCP1; whether a lack of leptin at birth will allow UCP1 to function for longer than the usual first 2 weeks of life remains to be established. The potential therapeutic use of leptin to promote thermoregulation and postnatal adaptation remains an intriguing possibility.

**Acknowledgements**

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**Fig. 6.** Uncoupling protein (UCP)-1 abundance in chronic leptin-treated (100 µg/d for 6 d; ■) and vehicle-treated (sterile water; □) neonatal lambs. Values are means with their standard errors represented by vertical bars. L, leptin-treated; V, vehicle-treated; -ve liver mitochondria; R, reference sample from 4-h-old lamb. Mean value was significantly different from that of vehicle-treated lambs: \* $P < 0.05$ .

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