Plasma Leptin Concentration in Fetal Sheep during Late Gestation: Ontogeny and Effect of Glucocorticoids


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The ontogeny and developmental control of plasma leptin concentration in the fetus are poorly understood. The present study investigated plasma leptin concentration in chronically catheterized sheep fetuses near term, and in neonatal and adult sheep. The effect of glucocorticoids on plasma leptin in utero was examined by fetal adrenalectomy and exogenous cortisol or dexamethasone infusion. In intact, untreated fetuses studied between 130 and 140 d (term, 145 ± 2 d), plasma leptin concentration increased in association with the preparatum cortisol surge. Positive relationships were observed between plasma leptin in utero and both gestational age and plasma cortisol. Plasma leptin was also inversely correlated with fetal pO2. The ontogenic rise in plasma leptin was abolished by fetal adrenalectomy. In intact fetuses at 123–127 d, plasma leptin was increased by infusions of cortisol (3–5 mg kg⁻¹ d⁻¹, +127 ± 21%) for 5 d and dexamethasone (45–80 µg kg⁻¹ d⁻¹, +268 ± 61%) for 2 d. However, the cortisol-induced rise in plasma leptin was transient; by the fifth day of infusion, plasma leptin was restored to within the baseline range. These findings show that, in the sheep fetus, an intact adrenal gland is required for the normal ontogenic rise in plasma leptin near term. Furthermore, fetal treatment with exogenous and endogenous glucocorticoids increases circulating leptin concentration in utero. (Endocrinology 143: 1166–1173, 2002

Leptin IS A polypeptide hormone synthesized and secreted primarily by adipose tissue in both fetal and adult animals (1, 2). Since discovery of the ob gene and its protein product, the physiological functions and regulation of leptin in adult life have become increasingly well characterized (1, 3). In adult animals, leptin is known to have an important role in the control of appetite and energy expenditure, and in the maintenance of normal immune and reproductive function (1, 3, 4). Furthermore, leptin release is regulated by nutrient availability, energy expenditure, the sympathetic nervous system, and by other hormones, such as insulin and glucocorticoids (1, 3). However, in the fetus, the functions and control of leptin are poorly understood.

Leptin is present in the circulation of human and porcine fetuses from mid-gestation (5, 6), and in a number of species, mRNA for leptin and leptin receptors has been detected in various fetal tissues, including adipose tissue and the placenta (2, 7–10). In human infants, cordocentesis and umbilical blood sampling at delivery have shown that the circulating leptin concentration increases close to term (5, 11–13). Furthermore, in fetal sheep, leptin mRNA abundance in perirenal adipose tissue increases with gestational age toward term (2). These ontogenic changes in plasma leptin and tissue mRNA levels coincide with the rise in plasma cortisol concentration seen in the fetus near term (14). Glucocorticoids have been shown to stimulate leptin gene expression and secretion from adult adipocytes both in vivo and in vitro (15–17), but to date, the role of glucocorticoids in the developmental control of leptin before birth is unknown.

Therefore, the present study investigated the ontogeny of plasma leptin concentration in chronically catheterized sheep fetuses close to term, and in neonatal and adult sheep. The effect of glucocorticoids on plasma leptin concentration in utero was also examined by fetal adrenalectomy and exogenous glucocorticoid infusion.

Materials and Methods

Animals

Seven nonpregnant adult Welsh Mountain ewes, 8 lambs and 30 ewes carrying fetuses of known gestational age were used in this study. Of the total of 33 fetuses studied, 21 were twins and the remainder were singletons; 17 of the fetuses were male and 16 were female. All pregnant and nonpregnant adult animals were maintained on 200 g d⁻¹ concentrates with free access to hay, water, and a salt-lick block. The lambs were suckled naturally by their mothers. Food, but not water, was withheld for 18–24 h before surgery, which occurred at 120 ± 1 d of gestation (n = 33, term 145 ± 2 d). All surgical and experimental procedures were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986.

Surgical procedures

Under halothane anesthesia (1.5% in O₂-N₂O) with positive pressure ventilation, catheters were inserted into the femoral artery and a branch of the femoral vein of all fetuses using surgical techniques described previously (18). All catheters were exteriorized through a small incision in the flank of the ewe and secured in a plastic bag sutured to the skin. From the day after surgery, the catheters were flushed daily with heparinized saline solution (100 IU ml⁻¹ heparin in 0.9% saline). Antibiotic (procaine penicillin: Depocillin, Mycofarm, Cambridge, UK) was administered im to the ewes on the day of surgery and for 3 d thereafter. The fetuses were given 100 mg ampicillin (Penbritin, Beecham Animal Health, Brentford, UK) iv at surgery. In a similar procedure, a catheter was inserted into the femoral artery of 4 of the 7 nonpregnant adult ewes.
In a separate surgery carried out 9–11 d before vascular catheterization, 5 of the fetuses were adrenalectomized (ADX) using surgical techniques described previously (19). All animals were studied at least 3 d after surgery.

**Experimental procedures**

Wherever possible, daily blood samples (2 ml) were taken from 10 of the intact fetuses and from the 5 ADX fetuses from 130 d to between 137–142 d of gestation. The remaining 18 fetuses were infused iv from between 123 and 127 d of gestation with either saline (0.9% NaCl, n = 6) or cortisol (3–5 mg kg⁻¹ d⁻¹ in 0.9% saline, n = 6; Efcortelan, Glaxo-Wellcome Ltd., Ware, UK) for 5 d, or with dexamethasone (45–60 μg kg⁻¹ d⁻¹ dexamethasone sodium phosphate in 0.9% saline, n = 6; Merck, Sharp, Dohme Ltd., Harlow, UK) for 2 d. The dose of cortisol infused was calculated to increase the plasma cortisol concentration to that normally seen close to term (14), and the dexamethasone dose was calculated to produce a plasma dexamethasone concentration approximately one-fifth of that measured in human infants after antenatal maternal glucocorticoid treatment (20). Arterial blood samples (2 ml) were taken daily from 2 d before and throughout the period of infusion. Nine of the intact, one of the ADX, three of the saline-infused, two of the cortisol-infused and six of the dexamethasone-infused fetuses were twins.

On the last day of the study, the fetuses were weighed after delivery of the ewes either by jugular venepuncture (n = 3) or via indwelling arterial catheters (n = 4). Arterial blood samples (2 ml) were taken by jugular venepuncture from the lambs within 0–1 d of birth and at 1, 2, and 5 wk of age. Blood samples (2 ml) were taken from the nonpregnant adult ewes immediately after delivery. Perirenal fat was weighed from the intact untreated and ADX fetuses and weighed.

**Biochemical analyses**

Arterial blood samples obtained from the fetuses were analyzed immediately for the partial pressure of oxygen (p \(\text{O}_2\)) by an ABL330 Radiometer analyzer corrected for maternal and fetal body temperature, and for hemoglobin content and \(\text{O}_2\) saturation by an OSM2 Hemoximeter (Radiometer, Copenhagen, Denmark). Arterial blood \(\text{O}_2\) content was calculated from these values, assuming the amount of \(\text{O}_2\) dissolved in plasma as insignificant:

\[
\text{Blood } \text{O}_2 \text{ content (mol ml}^{-1}) = 0.00062 \times \text{O}_2 \text{ saturation (\%)} \times \text{hemoglobin content (g dl}^{-1})
\]

All arterial and venous blood samples were placed into EDTA-containing tubes which were centrifuged at 1,000 × g and 4 C for 5 min. The plasma was removed and stored at −20 C until analysis.

Plasma cortisol concentration was determined by RIA as described previously (21). The intraassay and interassay coefficients of variation were 8.5 and 11.8%, respectively, and the lower limit of detection was 1.0–1.5 ng ml⁻¹.

Plasma leptin concentration was measured by an ovine-specific sand-
Results

Ontogeny of plasma leptin concentration

In the intact, untreated fetuses, a significant increase in plasma leptin concentration was observed toward term (P < 0.001, Fig. 1A). Plasma leptin increased from 217 ± 16 pg ml⁻¹ at 130 d to a maximum of 405 ± 89 pg ml⁻¹ at 139 d of gestation (P < 0.05, Fig. 1A). When values from all of the fetuses were combined, there was a significant positive relationship between plasma leptin concentration and gestational age (r = 0.51, n = 70, P < 0.001).

The ontogenic rise in plasma leptin coincided with the significant increase in plasma cortisol concentration seen in the intact fetuses near term (P < 0.001, Fig. 1). Overall, a significant positive correlation was observed between plasma concentrations of leptin and cortisol (r = 0.43, n = 69, P < 0.001, Fig. 2). In addition, plasma leptin showed a significant inverse relationship with arterial pO₂ (r = -0.41, n = 59, P < 0.005, Fig. 3), but not with O₂ content (r = 0.01, n = 59, P > 0.05) or pH (r = 0.03, n = 60, P > 0.05) in utero. A weak but significant correlation was observed between plasma leptin and arterial pCO₂ (r = 0.26, n = 60, P < 0.05).

There were no effects of gender on the plasma leptin concentration measured in the fetuses and, on the day of delivery, no relationships were observed between plasma leptin and either fetal body weight (2.19–4.30 kg; r = 0.31, n = 10, P > 0.05) or perirenal fat weight (P > 0.05 for both absolute and percentage weights).

Plasma leptin concentration in the fetuses near term increased to within the range of values seen in the neonatal lambs over the first few weeks of life (Table 1). When analyzed by unpaired t test, plasma leptin in the lambs at 0–1 d of age (211 ± 36 pg ml⁻¹, n = 4) was significantly lower than that seen in the fetuses between 136 and 140 d of gestation (350 ± 26 pg ml⁻¹, n = 10, P < 0.01). Plasma leptin
in the newborn lambs was also significantly lower compared with values observed at 1, 2, and 5 wk of age ($P < 0.05$, Table 1). By 1 wk of age, plasma leptin increased significantly to $635 \pm 149$ pg ml$^{-1}$ and remained within this range for up to 5 wk after birth (Table 1). However, the plasma leptin level observed in the fetuses and lambs was significantly lower than the concentration measured in the adult ewes ($P < 0.05$, Table 1).

A significant difference in plasma cortisol concentration was observed between the groups of neonatal lambs and adult ewes ($P < 0.005$, Table 1). However, after birth, no significant relationships between plasma leptin and cortisol concentrations were seen in the neonatal lambs ($r = 0.29$, $n = 27$, $P > 0.05$) or adult ewes ($r = 0.08$, $n = 7$, $P > 0.05$).

### Effect of adrenalectomy on plasma leptin concentration in utero

The rise in plasma leptin concentration seen in the intact fetuses close to term was abolished when the prepartum cortisol surge was prevented by adrenalectomy (Fig. 4). Between 133 and 140 d of gestation, no significant changes in plasma leptin or cortisol concentrations were observed in the ADX fetuses; overall, the mean plasma leptin and cortisol concentrations were $244 \pm 31$ pg ml$^{-1}$ and $6.8 \pm 0.6$ ng ml$^{-1}$, respectively ($n = 5$). When values were averaged over 136–140 d of gestation, plasma concentrations of leptin ($244 \pm 37$ pg ml$^{-1}$, $n = 5$) and cortisol ($6.9 \pm 0.7$ ng ml$^{-1}$, $n = 5$) in the ADX fetuses were both significantly lower than those measured in the intact fetuses ($350 \pm 26$ pg ml$^{-1}$ and $34.7 \pm 3.6$ ng ml$^{-1}$, respectively, $P < 0.05$ in both cases, $n = 10$). On the day of delivery, there were no significant differences in body weight or perirenal fat weights, expressed either as an absolute value or as a percentage of body weight, between the intact and ADX fetuses (Table 2).

### Effect of glucocorticoid infusion on plasma leptin concentration in utero

Before the glucocorticoid or saline infusions began, the plasma leptin concentration in the infused fetuses at 123–127 d of gestation was within the range of values seen in the intact untreated fetuses studied at 130 d (Figs. 1A and 5A). Both cortisol and dexamethasone infusions caused a significant increase in plasma leptin concentration ($P < 0.05$ in both cases, Fig. 5A). Within 1 d of the start of the glucocorticoid treatment, plasma leptin concentration was increased by $127 \pm 21\%$ in the cortisol-infused fetuses, and by $268 \pm 61\%$ in the fetuses infused with dexamethasone ($P < 0.05$ in both cases, Fig. 5A). On the first day of infusion, plasma leptin concentration in both groups of glucocorticoid-treated fetuses was significantly increased to above the respective basal values and was significantly greater than that seen in

### Table 1. Mean (±SEM) plasma leptin concentration in intact sheep fetuses near term, lambs over the first 5 wk of life and adult nonpregnant sheep

<table>
<thead>
<tr>
<th>Animals</th>
<th>Number</th>
<th>Gestational or postnatal age (days)</th>
<th>Plasma leptin (pg ml$^{-1}$)</th>
<th>Plasma cortisol (ng ml$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact untreated fetuses</td>
<td>10</td>
<td>136–140</td>
<td>$350 \pm 26^a,b$</td>
<td>$34.7 \pm 3.6^a,b$</td>
</tr>
<tr>
<td>Neonatal lambs</td>
<td>4, 8, 7</td>
<td>0–1, 7</td>
<td>$211 \pm 36^a$</td>
<td>$95.3 \pm 40.0^a$</td>
</tr>
<tr>
<td>Adult nonpregnant sheep</td>
<td>7</td>
<td>Adult</td>
<td>$635 \pm 149^a$</td>
<td>$34.6 \pm 9.4^{b,d}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$670 \pm 143^a$</td>
<td>$24.9 \pm 9.7^{b,d}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$612 \pm 126^a$</td>
<td>$13.7 \pm 4.3^{d}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$1318 \pm 254^a$</td>
<td>$51.6 \pm 10.0^{a,c}$</td>
</tr>
</tbody>
</table>

Within each column, values with different letters are significantly different from each other ($P < 0.05$).
the saline-infused fetuses \((P < 0.05\) in all cases, Fig. 5A). However, the rise in plasma leptin was not maintained throughout the period of cortisol infusion; by the fifth day of infusion, plasma leptin concentration was restored to within the basal range and was similar to that seen in the saline-infused fetuses (Fig. 5A). In the dexamethasone-infused fetuses, the mean plasma leptin concentration observed on the second day of infusion remained significantly above the basal values and significantly greater than that seen in the saline-infused fetuses \((P < 0.05\) in both cases, Fig. 5A). Saline infusion had no effect on plasma leptin concentration: values remained within the basal range throughout the period of the infusion (Fig. 5A).

A significant increase in plasma cortisol concentration was observed in the cortisol-infused fetuses throughout the period of infusion \((P < 0.05\) at all time points, Fig. 5B). From within 1 d of the start of the infusion, plasma cortisol concentration in the cortisol-infused fetuses was significantly greater than that seen in the saline and dexamethasone-infused fetuses \((P < 0.05\) Fig. 5B). A small but significant decrease in plasma cortisol concentration was observed in the dexamethasone-treated fetuses on the first day of the infusion \((P < 0.05\) Fig. 5B); however, throughout the infusion, there were no significant differences in plasma cortisol concentration between the dexamethasone and saline-treated fetuses (Fig. 5B). No significant change in plasma cortisol concentration was observed in the fetuses infused with saline (Fig. 5B).

**Discussion**

This study is the first to report the circulating concentration of leptin in fetal sheep and the first to describe sequential changes in plasma leptin with gestational age and glucocorticoid treatment in any species before birth. The plasma leptin concentration measured in the adult sheep is within the range of values previously reported (23). The present study showed, in the sheep fetus, that the circulating concentration of leptin increases close to term in association with the prepartum rise in plasma cortisol concentration. Fetal adrenalectomy prevented the ontogenic rise in plasma leptin and glucocorticoid infusions elevated the plasma leptin level in utero. Furthermore, in intact untreated fetuses, a significant relationship was observed between plasma concentrations of leptin and cortisol. Overall, these findings suggest that the prepartum cortisol surge may be responsible, at least in part, for the rise in plasma leptin seen in the sheep fetus near term, and that antenatal glucocorticoid therapy may lead to increased leptin concentration in utero.

The leptin present in the circulation of the sheep fetus may originate primarily from the placenta and fetal adipose tissue, both of which express the leptin gene (2, 10). The human placenta perfused in vitro has been shown to deliver over 95% of the leptin produced into the maternal circulation and less than 2% into the fetal circulation (24). Furthermore, the present and previous studies have shown a fall in plasma leptin over the immediate postnatal period that may be indicative of a placental source of leptin before birth (25).
Leptin does not appear to cross the placental barrier to any significant extent, as no relationship has been found between plasma leptin concentrations in blood samples taken from human infants and mothers at delivery (26), and administration of human recombinant leptin to pregnant mice does not alter the fetal leptin concentration within 15 min of treatment (27).

Circulating concentrations of leptin are likely to reflect the amount of adipose tissue present both in fetal and postnatal animals, and, hence, the rise in plasma leptin with gestational age and adulthood may be associated with the growth and development of the adipose tissue. In adult sheep, plasma leptin concentration correlates with body fat and with the body condition score (23, 28). Furthermore, although no correlation was observed between plasma leptin and body weight in the small number of intact fetuses in the present study, positive relationships have been shown between umbilical blood leptin concentration and both birth weight and adiposity in human infants at term (5, 26). In contrast to human infants, lambs are born with a relatively small percentage of their body weight as fat: only 2% in newborn lambs compared with 15% in human infants (29, 30). Correspondingly, the plasma leptin concentration measured in fetal sheep near term in the present study is approximately ten times lower than that seen in human infants (9). In fetal sheep, perirenal adipose tissue grows rapidly from 70–120 d of gestation with a slower increase in mass from 120 d until term (31). In the last month of gestation, adipocyte mean volume increases by 30–40%, although the number of adipocytes per gram of tissue decreases (32). Therefore, in the present study, the increase in plasma leptin seen in the sheep fetus near term may be due, in part, to increases in adipocyte size and leptin secretion per adipocyte, but not to an increase in adipocyte number. Larger adipocytes are known to contain more leptin mRNA than smaller ones (33), and a concomitant increase in leptin mRNA abundance is observed in fetal perirenal adipose tissue toward term in this species (2). In the present study, the ontogenic rise in fetal plasma leptin concentration seen near term may also be due, in part, to the prepartum rise in plasma cortisol concentration. Fetal adrenalectomy suppressed the plasma cortisol concentration and abolished the rise in plasma leptin near term, without affecting perirenal fat weight. Furthermore, an exogenous infusion of cortisol or dexamethasone increased the plasma leptin concentration to the level normally seen near term. Previous studies have shown that administration of glu-

![Image](https://academic.oup.com/endo/article-abstract/143/4/1166/2989062/1171)
corticoids can increase the circulating concentration of leptin in adult rodents and human subjects in vivo (17, 34, 35). In rats, the glucocorticoid-induced rise in plasma leptin is associated with an increase in the expression of the leptin gene in adipose tissue (34). High plasma concentrations of cortisol and leptin are also observed in patients with Cushing’s syndrome where levels of both hormones decrease after surgical removal of the tumor responsible (17). In addition, there is some evidence that glucocorticoids may increase plasma cortisol and leptin are also observed in patients with Cushing’s syndrome where levels of both hormones decrease after surgical removal of the tumor responsible (17). In addition, there is some evidence that glucocorticoids may increase plasma cortisol and leptin are also observed in patients with Cushing’s syndrome where levels of both hormones decrease after surgical removal of the tumor responsible (17).

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In rats, the glucocorticoid-induced rise in plasma leptin is observed in newborn human infants that is independent of gender, birth weight, and gestational age (37). Glucocorticoids may influence the circulating leptin concentration in utero by a number of different mechanisms. First, they may have direct actions on the leptin gene. Previous studies have demonstrated that glucocorticoids increase leptin gene expression in adult adipocytes in vitro (16, 17), and preliminary work has shown that cortisol can up-regulate leptin mRNA abundance in perirenal adipose tissue of fetal sheep (38). A glucocorticoid-response element has been identified in the sequence for the human leptin gene (39), although glucocorticoids may also regulate leptin gene expression via a mechanism independent of glucocorticoid-response element binding (34). Second, glucocorticoids may increase the plasma leptin concentration in utero indirectly via actions on other regulatory systems. Leptin production by adipose tissue is stimulated by insulin and oestrogen in rats and human subjects (16, 40, 41), and the sensitivity of adipose or placental tissue to these hormones may be influenced by gestational age and glucocorticoid treatment. In sheep, cortisol and dexamethasone are known to promote oestrogen synthesis within the placenta (42). However, the effects of insulin and oestrogen on leptin secretion from fetal and placental tissues, and the extent to which their actions are modulated by glucocorticoids, are unknown in any species.

The effect of exogenous glucocorticoids on plasma leptin in utero appears to be transitory. In the present study, the increase in plasma leptin induced by cortisol treatment was maximal within 1 d of the start of the infusion, and, on the fifth day of infusion, the concentration was restored to the baseline value and was no different from that measured in the fetuses infused with saline. The mechanism responsible for the transient effect of the glucocorticoid infusion in utero remains to be established, but similar findings have been observed in studies where the effects of glucocorticoids have been investigated over a longer period of time. In human subjects treated with physiological doses of cortisol for 4 d, the rise in plasma leptin was short-lived and the pretreatment level was restored by the last day of the study (35). In addition, dexamethasone causes a transient increase in leptin gene expression and secretion from adipocytes taken from obese human subjects (43); a rise in leptin mRNA abundance and secretion was observed within 1 d of incubation with dexamethasone but basal levels were restored after 3 and 7 d (43).

The physiological importance of the gestational and glucocorticoid-induced increases in fetal plasma leptin is unknown, although leptin receptor mRNA and protein have been detected in a number of fetal tissues including the hypothalamus, lung, and kidney, and in the placenta (7, 44, 45). In the fetus near term, leptin may be involved in the onset of parturition and/or in the preparation of the fetus for extrauterine life. Pregnant ob/ob mice, mated with ob/ob male mice and treated with leptin until d 0.5 after conception, establish and maintain a normal pregnancy, but the duration of gestation is prolonged by up to 2 d (46). In addition, the ontogenic rise in plasma leptin may contribute to activation of the thyroid axis near term (47) and, hence, promote processes such as thermogenesis and glucogenesis in the neonate (48, 49). Leptin treatment has also been shown to correct the reduction in brain weight and proteins, and impaired locomotor activity seen in young ob/ob mice (50).

Finally, in the present study, the correlations observed between fetal plasma leptin and both plasma cortisol and arterial oxygen tension suggest that adverse intrauterine conditions may cause a rise in the circulating leptin concentration in utero. The effects of experimental intrauterine stressors, such as hypoxemia, cord compression, and placental insufficiency, on plasma leptin concentration in the fetus remain to be established. However, a high plasma leptin level, per kilogram of body weight, is seen in human infants with intrauterine growth retardation and evidence of fetal distress such as lactacidemia and abnormal Doppler imaging of the umbilical cord (12). Further studies are required to determine the role of leptin as a physiological signal both during normal development and in a stressful intrauterine environment.

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