

# Metabolism of sheep adipose tissue during pregnancy and lactation

## Adaptation and regulation

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1. Changes in the mean volume, the rate of fatty acid and acylglycerol glycerol synthesis, the activity of lipoprotein lipase and the numbers and affinities of insulin receptors of subcutaneous adipocytes are reported for sheep at different stages of pregnancy and lactation. In addition, the serum concentrations of insulin, progesterone, prolactin, choriomammotropin, somatotropin, glucose, acetate, L-lactate, glycerol and unesterified fatty acids are reported for these sheep. 2. A switch from lipid accumulation to net lipid mobilization, accompanied by a decline in the capacity for lipid synthesis, occurred at the onset of the last third of pregnancy. Net lipid mobilization continued during lactation. 3. The changes that occurred in the serum concentrations of the various hormones listed above are discussed in relation to their possible roles in the modulation of adipose tissue metabolism in sheep during pregnancy and lactation. The observations are compared with those from previous studies on the hormonal control of adipose tissue metabolism in the rat during pregnancy and lactation.

Accumulation of lipid reserves during early pregnancy for use during late pregnancy and in lactation has been observed in a variety of mammals (Spray, 1950; Vernon, 1980). The concomitant metabolic changes have been described in some detail for the rat (Fain & Scow, 1966; Otway & Robinson, 1968; Hamosh *et al.*, 1970; Knopp *et al.*, 1973; Smith, 1973; Flint *et al.*, 1979) but there is still a paucity of information for other species. In addition the factors and mechanisms responsible for the changes in adipose tissue metabolism have not been elucidated completely. For the rat, such studies are complicated by the fact that the switch from lipid accumulation to mobilization occurs close to parturition, a time when the concentration of many hormones in the blood changes markedly (see Cowie *et al.*, 1980). In sheep, on the other hand, the onset of lipid mobilization may occur several weeks before birth, especially in ewes carrying two or more lambs (Robinson *et al.*, 1978). We have therefore compared changes in adipose tissue metabolism in sheep during pregnancy and lactation with changes in the number of insulin receptors of the adipocytes and with the concentration of several hormones and metabolites in the blood. The objective of these studies was to provide further information about the mechanisms and factors that regulate adipose tissue metabolism during these physiological states.

## Experimental

### Animals

Sheep were 3-year-old Finn × Dorset Horn cross-bred ewes, all of which had lambed in the preceding spring. They were fed on hay *ad libitum* plus a cereal mix (425 g/day until day 105 of pregnancy then gradually increasing to 1400 g/day by 130 days of pregnancy and thereafter). Gestation was about 143 days in these sheep. Lambs were removed from their mothers at 50 days after birth, following which the cereal intake was returned to 425 g/day. The cereal mix was fed as two meals at approx. 07:00h and 16:30h.

### Experimental design

The ewes were divided into four groups of five or six animals. Groups 1, 2 and 3 were mated over 10-day periods in early November, late November and mid December; group 4 was mated during late February and early March. Samples of jugular venous blood and subcutaneous adipose tissue were taken from animals of groups 1, 2 and 3 at approx. 105 and 135 days of pregnancy and 18 and 50 days of lactation and, from some animals, at either 3 days or 95 or more days after removal of their lambs. Such samples were taken from ewes of group 4 either just before mating or during the first 120 days

of pregnancy. Thus during any given month of sampling, samples of blood and adipose tissue were taken from animals at several different stages of pregnancy and lactation.

#### Sampling

A cannula was inserted into a jugular vein the day before sampling, using a Medicut intravenous cannula (Sherwood Medical Industries, Crawley, West Sussex, U.K.). This cannula was filled with 40 mM-sodium citrate/123 mM-NaCl. On the day of sampling, 20 ml of jugular venous blood was withdrawn at approx. 09:15 h and the animal was anaesthetized with 18–20 ml of sodium pentobarbitone (sagatal; May and Baker, Dagenham, Essex, U.K.). An incision of about 5 cm was made in the rump region and 5–6 g of subcutaneous adipose tissue was removed and placed in 0.15 M-NaCl at approx. 37°C. The wound was closed with silk sutures and Michel clips and was dressed with an antibacterial powder (acramide; Willington Medicals, Shrewsbury, Salop, U.K.). Four samples of subcutaneous adipose tissue were removed from each animal in this way with an interval of about 30 days between biopsies; subsequent incisions were always at least 10 cm from previous incisions. For some animals a fifth biopsy sample of adipose tissue was taken after removal of the lambs; these animals were killed with a captive-bolt humane killer before they recovered from anaesthesia. All sheep were fed on the morning of biopsy and lambs were left with lactating ewes until just before sampling.

#### Measurement of blood constituents

Serum was prepared from the blood as described previously (Flint *et al.*, 1980) and was stored at minus 20°C before use. Progesterone was assayed as described previously (Flint *et al.*, 1979). Serum insulin, prolactin and somatotropin ('growth hormone') were determined by double-antibody radioimmunoassays. Serum insulin concentrations were expressed in terms of a bovine insulin standard (23.4 units/mg) kindly given by Boots Pure Drug Co. (Nottingham, U.K.). Ovine prolactin (30 i.u./mg) was purchased from Sigma (London) Chemical Co. and contained less than 0.005 i.u. of somatotropin/mg. Ovine somatotropin (0.56 i.u./mg) containing less than 0.5 i.u. of prolactin/mg was kindly given by the National Institute of Arthritis, Metabolism and Digestive Diseases, Bethesda, MD, U.S.A. Antisera to bovine insulin, prolactin and somatotropin were used; these were kindly given by Dr. I. C. Hart. The antisera to prolactin and somatotropin were both highly specific (Hart, 1973; Hart *et al.*, 1975). We confirmed the findings: prolactin antiserum exhibited no cross-reaction with ovine thyrotropin, insulin or glucagon and only a slight cross reaction with ovine somatotropin, which could be explained in terms of

the stated contamination of the preparation with prolactin.

The somatotropin antiserum also showed no cross-reaction with ovine thyrotropin, insulin or glucagon and less than 0.2% cross-reaction with ovine prolactin, which could be explained in terms of the slight contamination of the prolactin preparation with somatotropin. Choriomammotropin ('placental lactogen') was assayed as described previously (Flint *et al.*, 1980) except that ovine prolactin was used instead of rat prolactin. Ovine prolactin and an ovine placental extract exhibited parallel dose-response curves, whereas ovine somatotropin did not cross-react except at very high concentrations. For this reason the placental lactogen concentrations were expressed in ovine prolactin equivalents after subtraction of the prolactin concentration of the sample (determined by radioimmunoassay).

Other samples of serum were deproteinized with HClO<sub>4</sub>, neutralized with KOH and then used for determination of glucose (Slein, 1963), L-lactate (Hohorst, 1963), glycerol (Garland & Randle, 1962) and acetate. The acetate assay was based on that of Bergmeyer & Mollering (1974) except that acetyl-CoA synthetase [Sigma (London) Chemical Co.] was used instead of acetate kinase plus phosphotransacetylase, and lactate dehydrogenase was included in the assay mixture. This latter addition eliminated a gradual creep in the absorbance with time and resulted in a clearer end-point for the reaction. The assay medium comprised 100 mM-Tris/HCl (pH 9.0), 10 mM-MgCl<sub>2</sub>, 10 mM-L-malate, 5 mM-ATP, 10 mM-reduced glutathione, 2 mM-NAD<sup>+</sup>, 0.5 mM-CoA, citrate synthetase (1.1 units/ml), malate dehydrogenase (12 units/ml), lactate dehydrogenase (5 units/ml) and acetyl-CoA synthetase (30 m-units/ml). Known amounts of acetate were added as internal standards (percentage conversion was about 80%).

The concentration of unesterified fatty acids (individual and total) of serum samples was determined as described previously (Vernon, 1975) except that serum lipids were extracted by the Folch procedure (Folch *et al.*, 1957).

#### Measurement of metabolic activities and <sup>125</sup>I-labelled-insulin-binding capacity

Pieces of adipose tissue weighing about 5 mg were cut with scissors. The rate of fatty acid synthesis in samples of these pieces was measured by the incorporation of <sup>14</sup>C from [1-<sup>14</sup>C]acetate into fatty acids as described previously (Vernon, 1976) except that albumin was not included in the incubation medium. The rate of acylglycerol glycerol synthesis of adipose-tissue pieces was measured by the incorporation of <sup>14</sup>C from [U-<sup>14</sup>C]glucose into acylglycerol glycerol as described previously (Vernon, 1976) except that acetate was not included and

1 mM-sodium palmitate was included in the incubation medium.

The methods used for the preparation of isolated adipocytes from sheep adipose tissue and measurement of their mean volume have been described previously (Vernon *et al.*, 1981) with the following modification; during digestion of the adipose tissue with collagenase, the cells were shaken gently (60 strokes/min). Sheep subcutaneous adipocytes are usually smaller and appear to be less fragile than perirenal adipocytes; when preparing the latter, we find it best not to shake the tissue during collagenase digestion (Vernon *et al.*, 1981).

The binding and the degradation of  $^{125}\text{I}$ -labelled insulin by adipocytes were measured as described previously (Flint *et al.*, 1979), except that  $^{125}\text{I}$ -labelled insulin was prepared in our own laboratory by the chloramine-T method (Greenwood & Hunter, 1963). The specific radioactivity of the preparations was 50–100 Ci/g, of which >95% was precipitable with 10% trichloroacetic acid; the preparations retained the full biological activity of native insulin. Results for the binding of  $^{125}\text{I}$ -labelled insulin to adipocytes were corrected for non-specific binding (Flint *et al.*, 1979), which amounted on average to 19% of the total amount bound.

Lipoprotein lipase activity was measured in homogenates of adipose tissue as described previously (Flint *et al.*, 1979). The protein concentration of the homogenates was measured by the method of Wang & Smith (1975).

#### Statistical analysis

Results are expressed as means  $\pm$  S.E.M. Statistical analysis was performed by using Student's *t* test for paired or unpaired observations as appropriate. For some measurements, where a large range of individual values were found, statistical analysis was performed using either the Wilcoxon matched-pairs signed rank test (for paired observations) or the

Mann-Whitney U-test (for unpaired observations) (Siegel, 1956).

#### Results

At 1 day after parturition, the ewes of groups 1–3 weighed  $71 \pm 2$  kg; their weights decreased to  $69 \pm 2$  and  $66 \pm 2$  kg by days 18 and 50 of lactation respectively. All but two ewes had two or three lambs, the average for the flock being  $2.2 \pm 0.1$ . The mean lamb weight at birth was  $3.7 \pm 0.1$  kg and this increased to  $7.9 \pm 0.5$  and  $15.3 \pm 1.0$  kg by 18 and 50 days after birth respectively.

No statistically significant changes in the serum concentration of glucose and L-lactate were found during pregnancy and lactation (Table 1); by 95 or more days after weaning, however, the serum L-lactate concentration had risen significantly ( $P < 0.05$ ). Serum acetate concentration fell significantly ( $P < 0.05$ ) between days 1–69 and day 135 of pregnancy, and then rose significantly ( $P < 0.01$ ) by day 50 of lactation before falling significantly ( $P < 0.01$ ) by 95 or more days after weaning (Table 1). Serum glycerol concentration reached a peak at 18 days of lactation when the value was significantly greater ( $P < 0.05$ ) than that observed in animals less than 100 days pregnant, after 50 days of lactation, or post-lactating. Serum unesterified fatty acid concentration was significantly elevated at 135 days of pregnancy and 18 days of lactation compared with animals at less than 100 days of pregnancy ( $P < 0.05$ ) or post-lactating ( $P < 0.01$ ). The unesterified stearic acid/oleic acid molar ratio in the blood reached a nadir at 135 days of pregnancy, the value being significantly lower than that observed at 70–100 days of pregnancy or after lactation (Table 1).

Serum insulin concentration was the same in sheep at less than 70 days of pregnancy or after lactation (Table 2); the concentration increased

Table 1. Concentration of glucose, acetate, L-lactate, glycerol and unesterified fatty acids, and the unesterified stearic acid/oleic acid molar ratio in jugular-vein serum from sheep at different stages of pregnancy or lactation, and 95–135 days after removal of lambs

Results are means  $\pm$  S.E.M. for four to eight observations

Physiological state	[Glucose] (mM)	[Acetate] (mM)	[L-Lactate] (mM)	[Glycerol] (mM)	[Unesterified fatty acid] (mM)	Unesterified stearic acid/ oleic acid molar ratio
1–69 days pregnant	$2.9 \pm 0.1$	$2.1 \pm 0.4$	$2.2 \pm 0.4$	$0.11 \pm 0.01$	$0.27 \pm 0.05$	$1.16 \pm 0.05$
70–100 days pregnant	$3.1 \pm 0.3$	$1.6 \pm 0.2$	$1.6 \pm 0.2$	$0.10 \pm 0.01$	$0.27 \pm 0.05$	$1.41 \pm 0.17$
105 days pregnant	$3.2 \pm 0.2$	$1.1 \pm 0.2$	$2.4 \pm 0.6$	$0.14 \pm 0.04$	$0.45 \pm 0.13$	$1.28 \pm 0.18$
135 days pregnant	$3.4 \pm 0.3$	$1.0 \pm 0.2$	$1.8 \pm 0.2$	$0.14 \pm 0.02$	$0.62 \pm 0.12$	$0.84 \pm 0.15$
18 days lactating	$2.9 \pm 0.2$	$1.7 \pm 0.3$	$1.8 \pm 0.2$	$0.17 \pm 0.02$	$0.61 \pm 0.13$	$1.08 \pm 0.27$
50 days lactating	$3.5 \pm 0.3$	$1.7 \pm 0.1$	$1.6 \pm 0.2$	$0.11 \pm 0.01$	$0.34 \pm 0.06$	$1.55 \pm 0.43$
95–135 days post-lactating	$2.9 \pm 0.2$	$1.1 \pm 0.1$	$3.2 \pm 0.5$	$0.11 \pm 0.01$	$0.15 \pm 0.05$	$1.33 \pm 0.13$

Table 2. Concentrations of insulin, progesterone, prolactin, choriomammotropin and somatotropin in jugular-vein serum from sheep at different stages of pregnancy or lactation and 95 to 135 days after removal of lambs

Results are means  $\pm$  S.E.M. with the numbers of observations in parentheses

Physiological state	Insulin concn. (ng/ml)	Progesterone concn. (ng/ml)	Prolactin concn. (ng/ml)	Choriomammotropin concn. (ng/ml)	Somatotropin concn. (ng/ml)
1-69 days pregnant	1.53 $\pm$ 0.67 (11)	4.8 $\pm$ 1.0 (10)	60 $\pm$ 17 (10)	16 $\pm$ 4 (10)	2.0 $\pm$ 0.6 (10)
70-100 days pregnant	4.29 $\pm$ 0.70 (6)	8.8 $\pm$ 2.3 (4)	111 $\pm$ 18 (4)	127 $\pm$ 63 (4)	6.0 $\pm$ 1.5 (4)
105 days pregnant	0.87 $\pm$ 0.09 (18)	9.3 $\pm$ 1.1 (18)	105 $\pm$ 26 (18)	740 $\pm$ 135 (18)	2.8 $\pm$ 0.5 (18)
135 days pregnant	0.98 $\pm$ 0.14 (18)	24.0 $\pm$ 6.0 (18)	171 $\pm$ 28 (18)	1356 $\pm$ 154 (18)	8.5 $\pm$ 1.7 (20)
18 days lactating	0.69 $\pm$ 0.08 (15)	5.9 $\pm$ 2.5 (15)	211 $\pm$ 16 (15)	33 $\pm$ 12 (15)	17.2 $\pm$ 3.9 (17)
50 days lactating	0.93 $\pm$ 0.14 (15)	6.9 $\pm$ 2.3 (17)	156 $\pm$ 14 (16)	22 $\pm$ 23 (16)	16.5 $\pm$ 2.3 (17)
95-135 days post-lactating	1.22 $\pm$ 0.18 (7)	4.5 $\pm$ 1.6 (8)	36 $\pm$ 14 (8)	11 $\pm$ 7 (8)	3.8 $\pm$ 0.7 (8)

Table 3. The mean volume of subcutaneous adipocytes, the rate of fatty acid synthesis from acetate, the rate of acylglycerol glycerol synthesis from glucose and the activity of lipoprotein lipase of subcutaneous adipose tissue obtained by biopsy from the rump region of sheep at different stages of pregnancy or lactation, or 95-135 days after removal of lambs

Results are means  $\pm$  S.E.M. with the numbers of observations in parentheses.

Physiological state	Adipocyte mean volume (pl)	Rate of fatty acid synthesis ( $\mu$ mol of acetate incorporated/h per $10^7$ cells)	Rate of acylglycerol glycerol synthesis ( $\mu$ mol of glucose converted/h per $10^7$ cells)	Lipoprotein lipase activity (nmol of fatty acid released/min per mg of protein)
1-69 days pregnant	546 $\pm$ 26 (10)	14.2 $\pm$ 3.3 (5)	0.45 $\pm$ 0.04 (5)	6.3 $\pm$ 2.4 (5)
70-100 days pregnant	758 $\pm$ 106 (5)	8.2 $\pm$ 2.8 (4)	0.64 $\pm$ 0.20 (3)	4.4 $\pm$ 0.7 (4)
105 days pregnant	509 $\pm$ 23 (16)	1.5 $\pm$ 0.7 (10)	0.27 $\pm$ 0.03 (11)	1.7 $\pm$ 0.6 (9)
135 days pregnant	478 $\pm$ 31 (16)	1.7 $\pm$ 0.8 (9)	0.29 $\pm$ 0.06 (10)	2.4 $\pm$ 0.7 (9)
18 days lactating	307 $\pm$ 31 (16)	0.1 $\pm$ 0.0 (8)	0.21 $\pm$ 0.04 (8)	2.6 $\pm$ 0.5 (8)
50 days lactating	241 $\pm$ 31 (16)	0.3 $\pm$ 0.1 (8)	0.20 $\pm$ 0.05 (8)	2.8 $\pm$ 0.7 (8)
95-135 days post-lactating	369 $\pm$ 44 (8)	2.6 $\pm$ 0.6 (7)	0.30 $\pm$ 0.04 (7)	16.0 $\pm$ 3.5 (6)

significantly by 70-100 days of pregnancy ( $P < 0.05$ ) but fell significantly by 105 days of pregnancy ( $P < 0.001$ ). A minimum mean value was found at 18 days of lactation, which was significantly lower ( $P < 0.01$ ) than that found in post-lactating animals (Table 2). The serum concentrations of both progesterone and choriomammotropin increased during the second half of pregnancy but had fallen by day 18 of lactation (Table 2), whereas serum prolactin was elevated throughout the latter half of pregnancy and through all of lactation (Table 2). Serum somatotropin concentration rose significantly ( $P < 0.05$ ) between days 105 and 135 of pregnancy and there was a further significant increase ( $P < 0.01$ ) by day 18 of lactation. Serum somatotropin remained elevated throughout lactation (Table 2).

The mean volume of subcutaneous adipocytes increased significantly ( $P < 0.05$ ) by 70-100 days of pregnancy and then fell significantly ( $P < 0.05$ ) by 105 days of pregnancy; there was also a statistically significant decline in the mean cell volume during lactation ( $P < 0.05$ ) (Table 3). The mean cell volume of the post-lactating animals was greater ( $P < 0.01$ )

than that of animals at 50 days of lactation but was lower ( $P < 0.01$ ) than that of animals at 1-69 days of pregnancy (Table 3).

There was no statistically significant change in the rate of fatty acid or acylglycerol glycerol synthesis per cell or in the activity of lipoprotein lipase over the first 100 days of pregnancy, but all three activities fell significantly ( $P < 0.05$  or better) between 70-100 days and 105 days of pregnancy (Table 3). There was no further statistically significant change in the rate of acylglycerol glycerol synthesis per cell or the activity of lipoprotein lipase until at least 50 days of lactation. In contrast, there was a further decline ( $P < 0.05$ ) in the rate of fatty acid synthesis per cell between 135 days of pregnancy and 18 days of lactation (Table 3). The rate of fatty acid synthesis per cell and the lipoprotein lipase activity were both significantly ( $P < 0.01$ ) higher in post-lactating than in 50-day lactating sheep. The rates of fatty acid and acylglycerol glycerol synthesis per cell of post-lactating sheep were lower ( $P < 0.01$  and  $P < 0.05$  respectively) than those of sheep 1-69 days pregnant.

Scatchard analysis (Scatchard, 1949) of the

results of the binding of  $^{125}\text{I}$ -labelled insulin to adipocytes gave non-linear plots (Fig. 1) as found previously for adipocytes and other tissues from other species (see Bradshaw & Frazier, 1977; Ginsburg, 1977). Such plots could be due to a heterogeneous population of receptors, negative co-operativity between receptors or a combination

of both, but at present it is not clear which, if any, of these explanations is correct. We have therefore reported the numbers of receptors with a high affinity for insulin ( $K_d$  about 1 nM) and also the total number of insulin receptors (Table 4). When the number of receptors for insulin was expressed on a per cell basis, a significant increase in the number with a high affinity for insulin (but not the total number) was found between 1–69 and 70–100 days of pregnancy. There was a subsequent fall in the number of high-affinity receptors ( $P < 0.01$ ) and the total number of receptors ( $P < 0.05$ ) by day 105 of pregnancy. There was no further change in the number of high-affinity receptors per cell until after weaning, whereas the total number of insulin receptors per cell fell between days 18 and 50 of lactation ( $P < 0.05$ ). There was a significant fall ( $P < 0.05$ ) in the number of both high-affinity and total receptors per cell between 50 days of lactation and 95 or more days after weaning when the number of both were significantly lower ( $P < 0.05$ ) than at 1–69 days of pregnancy.

Certain differences were found when the numbers of insulin receptors were expressed per unit area of cell surface instead of per cell (Table 4). No increase in the number of receptors with a high affinity for insulin per unit surface area was found over the first 100 days of pregnancy and although there was a significant ( $P < 0.05$ ) fall in the number of this 'class' of receptor between 70–100 and 105 days of pregnancy, there was no significant change in the total number of receptors over this period. However, there was a significant increase ( $P < 0.001$ ) in the numbers of both groups of receptors between day 135 of pregnancy and 18 day of lactation; the numbers of both groups of receptors did not change significantly during lactation, but they fell significantly ( $P < 0.05$ ) by 95 or more days after the end of lactation.

The dissociation constant for insulin of the

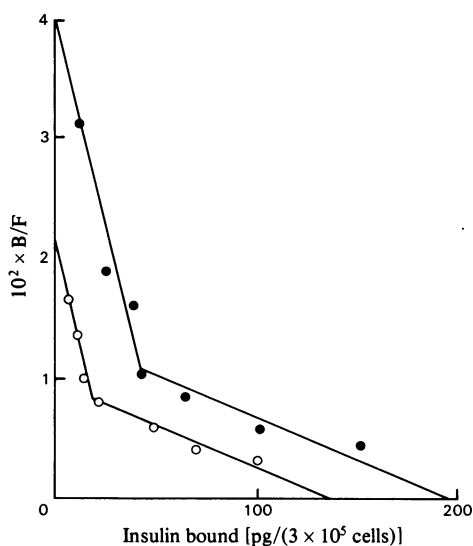


Fig. 1. Scatchard plots of the insulin binding to subcutaneous adipocytes from sheep either 70–100 days (●) or 105 days pregnant (○)

$^{125}\text{I}$ -labelled insulin binding was measured and corrected for non-specific binding as described in the text. Each point is the mean value obtained from separate determinations with adipocytes from four sheep (70–100 days pregnant) or 11 sheep (105 days pregnant). B/F is the ratio of insulin bound to adipocytes/free (unbound) insulin.

Table 4. The number of insulin receptors with a high affinity for insulin ( $K_d$  about 1 nM) and the total number of insulin receptors of subcutaneous adipocytes isolated from samples of adipose tissue taken by biopsy from the rump of sheep at different stages of pregnancy or lactation or at 95–135 days after removal of lambs

Results are means  $\pm$  S.E.M.

Physiological state	No. of observations	Insulin receptors with a $K_d$ of about 1 nM		Total insulin receptors	
		( $10^{-3} \times$ number/cell)	(Number/ $\mu\text{m}^2$ of cell surface)	( $10^{-3} \times$ number/cell)	(Number/ $\mu\text{m}^2$ of cell surface)
1–69 days pregnant	9	$9.4 \pm 1.2$	$0.29 \pm 0.04$	$67.6 \pm 10.7$	$2.12 \pm 0.38$
70–100 days pregnant	4	$16.8 \pm 4.1$	$0.41 \pm 0.09$	$87.0 \pm 25.3$	$2.11 \pm 0.57$
105 days pregnant	11	$8.0 \pm 0.9$	$0.26 \pm 0.02$	$40.0 \pm 4.9$	$1.31 \pm 0.14$
135 days pregnant	15	$8.0 \pm 0.9$	$0.29 \pm 0.04$	$41.7 \pm 6.7$	$1.51 \pm 0.23$
18 days lactating	16	$12.0 \pm 1.6$	$0.59 \pm 0.08$	$51.6 \pm 3.9$	$2.52 \pm 0.22$
50 days lactating	16	$9.2 \pm 1.1$	$0.50 \pm 0.04$	$34.0 \pm 4.4$	$1.92 \pm 0.21$
95–135 days post-lactating	4	$4.0 \pm 0.9$	$0.21 \pm 0.05$	$19.0 \pm 3.8$	$0.91 \pm 0.19$

high-affinity receptor ( $0.6 \pm 0.1$  nM) did not change significantly during the experimental period and there was no significant change in the amount of insulin degraded by the adipocytes ( $13 \pm 1\%$ ).

Samples of blood and subcutaneous adipose tissue were taken from five sheep 3 days after removal of their lambs (at 50 days of lactation). Removal of the lambs for 3 days had no significant effects on the concentration of hormones, metabolic activities or numbers of insulin receptors reported in Tables 2–4 (results not shown).

As only two ewes carried single lambs, a search for correlations between number of lambs and the various concentrations and activities measured was limited. However, at day 105 of pregnancy a positive correlation ( $r$  0.55;  $P < 0.05$ ) between the concentration of choriomammotropin and the number of lambs, and a negative correlation ( $r$  0.63;  $P < 0.01$ ) between the serum insulin concentration and the number of lambs were found, but no relationship between the number of lambs and either adipocyte mean volume or the serum progesterone concentration was apparent. No statistically significant correlations between the number of lambs and the various properties denoted above were found at 135 days of pregnancy or during lactation. However, by 50 days of lactation, the mothers suckling single lambs had adipocyte mean volumes of 442 and 510 pl compared with  $215 \pm 28$  and  $199 \pm 53$  for those suckling two and three lambs respectively.

## Discussion

The amount of maternal fat mobilized during pregnancy and lactation in sheep depends on the number of lambs and the plane of nutrition (Robinson *et al.*, 1978). The type of sheep used in the present study normally carries two or three lambs and so were expected to mobilize fat during late-pregnancy and when lactating. Net mobilization of lipid from subcutaneous adipocytes began around 100 days of gestation as shown by the fall in adipocyte mean volume. Our normal husbandry practice of increasing energy intake of the ewes during the last month of pregnancy and throughout lactation retarded fat mobilization during late-pregnancy, at least in subcutaneous adipose tissue as evinced by the lack of a significant change in mean adipocyte volume between days 105 and 135 of pregnancy. Some mobilization was probably occurring at the end of this period, however, as indicated by the rise in the concentration of unesterified fatty acids in the blood; this is usually observed in sheep at this stage of pregnancy (see Vernon, 1980). In addition, the fall in the unesterified stearic acid/oleic acid molar ratio in the blood is also thought to be an indication of increased lipid mobilization (see Vernon, 1980). Net lipid mobilization occurred between

days 135 of pregnancy and 18 of lactation as indicated by the fall in adipocyte mean volume and the high concentration of unesterified fatty acids in the blood. A further fall in adipocyte mean volume during lactation indicated that net lipid mobilization continued during this period.

The switch from net lipid accumulation to net mobilization in these sheep occurred at a relatively earlier stage of gestation than we previously found in rats (Flint *et al.*, 1979). In both species the decrease in adipocyte mean volume is accompanied by a fall in the rate of fatty acid and acylglycerol glycerol synthesis and the activity of lipoprotein lipase and hence the capacity to synthesize lipid (Flint *et al.*, 1979). Furthermore, in both species there is an apparently concomitant decrease in both the serum insulin concentration and the number of insulin receptors of adipocytes (Flint *et al.*, 1979) and it would seem probable that it is these changes that are at least partly responsible for the switch to net lipid mobilization and the decreased capacity for lipid synthesis during late-pregnancy. A fall in serum insulin during late-pregnancy in sheep was also found by Blom *et al.* (1976).

The factors responsible for the fall in the number of insulin receptors of adipocytes and the serum insulin concentration during late-pregnancy are less certain. Choriomammotropin may be involved for, as also shown previously (see Cowie *et al.*, 1980), there is a massive rise in the serum concentration of this hormone at this time and choriomammotropin may be responsible for the development of insulin resistance during pregnancy (Tyson & Felig, 1971). On the other hand, placental lactogen begins to rise around mid-pregnancy in the rat (see Cowie *et al.*, 1980) but this is not associated with a fall in the number of insulin receptors of adipocytes or the serum insulin concentration (Flint *et al.*, 1979; D. J. Flint, R. A. Clegg & R. G. Vernon, unpublished work). The rat and the sheep, however, differ markedly in their serum progesterone concentrations during pregnancy (see Cowie *et al.*, 1980). In the rat, serum progesterone concentrations of about 50 ng/ml are reached during early pregnancy and are maintained until almost the end of pregnancy. In contrast, in the sheep, serum progesterone concentration is much lower than in the rat for most of pregnancy and begins to rise to high values only during the last month of pregnancy. Thus in the rat there is a major rise in serum progesterone concentration that precedes that of choriomammotropin, whereas in the sheep this sequence is reversed. We have suggested that in the rat progesterone protects adipose tissue metabolism from insulin antagonistic effects of choriomammotropin (Flint *et al.*, 1979). In addition, we have shown that the fall in the number of insulin receptors of rat adipocytes that occurs around parturition is probably due to the fall in serum

progesterone at this time (Flint *et al.*, 1980). There is also evidence that progesterone promotes insulin secretion in the rat (Costrini & Kalkhoff, 1971; Ashby *et al.*, 1981) and so may be at least partly responsible for the hyperinsulinaemia observed during pregnancy (Knopp *et al.*, 1973; Flint *et al.*, 1979). Thus the relatively early switch to lipid mobilization in sheep adipose tissue during pregnancy may be the result of a large rise in serum choriomammotropin concentration preceding a rise in serum progesterone concentration. The possibility of other factors being involved, however, cannot be excluded; the rises in serum prolactin and serum somatotropin concentrations would not appear to be responsible for the initial decline in the capacity for lipid synthesis, for they did not occur until after day 105 of gestation. A rise in serum somatotropin concentration during late-pregnancy in sheep has also been shown by Bassett *et al.* (1970) and Blom *et al.* (1976).

The continuing net lipid mobilization during lactation is probably due in part to the low serum insulin concentration (relative to that of mid-pregnant or post-weaning animals). Hypoinsulinaemia during lactation has been reported for cows (see Vernon, 1980) and rats (see Williamson, 1980). It appears to be at least partly due to a decreased response of the pancreas to insulinotropic agents (Lomax *et al.*, 1979); in addition insulin removal by the mammary gland (Beck & Tucker, 1978) may also be involved (Williamson, 1980). In sheep, although serum insulin concentration falls during lactation, the number of insulin receptors per  $\mu\text{m}^2$  of adipocyte surface rises, as a result of a decline in cell volume without a concomitant fall in the number of receptors per cell. No such increase in the number of insulin receptors per adipocyte or per unit area of cell surface was found in the rat during lactation (Flint *et al.*, 1979).

A high serum prolactin concentration is thought to be responsible for the low capacity for lipid synthesis in adipose tissue of lactating rats (see Bauman & Currie, 1980; Williamson, 1980), although the hormone may not act directly on adipose tissue itself (Flint *et al.*, 1981). Prolactin could have a similar role in the ruminant. In addition, the high serum somatotropin concentration found in sheep during lactation in the present study and elsewhere (J. M. Bassett, personal communication) is likely to influence adipose tissue metabolism. Somatotropin promotes lipid mobilization and inhibits fatty acid synthesis in non-ruminants (see Goodman & Schwarz, 1974). Somatotropin is thought to promote lipolysis in ruminants (see Vernon, 1980). Preliminary studies suggest that somatotropin antagonizes the ability of insulin to maintain fatty acid synthesis in sheep adipose tissue maintained in tissue culture (Vernon,

1979). Serum somatotropin is elevated in cows during lactation and it is possible that the somatotropin/insulin ratio in the blood may have an important role in regulating the partitioning of nutrients between the mammary gland and adipose tissue in lactating ruminants (see Cowie *et al.*, 1980; Vernon, 1980). The role of growth hormone in the lactating rat is not clear and available information suggests that serum concentration of the hormone may not increase in lactation (Schalch & Reichlin, 1966).

For rats, weaning results in a rapid increase in the rate of fatty acid synthesis (Smith, 1973; Agius *et al.*, 1979), lipoprotein lipase activity (Hamosh *et al.*, 1970) and the number of insulin receptors of adipocytes (Flint *et al.*, 1981) and a rise in the serum insulin concentration (Agius *et al.*, 1979). No rapid (i.e. within 3 days) effects of weaning were observed on any of these features in sheep, but this may be due to a slower response to weaning, perhaps as a result of the elevated serum somatotropin concentration, for the capacity to synthesize lipids was increased by 3 months after weaning.

The rate of fatty acid synthesis, the activity of lipoprotein lipase and the number of insulin receptors of adipose tissue of the post-weaning ewes differed from those of ewes during early pregnancy. The reason for this is not clear but it probably represents a seasonal change, for the post-weaning animals were biopsied in the autumn, whereas the early-pregnant animals were biopsied in the spring. Seasonal changes in the concentration of plasma unesterified fatty acids have been reported for sheep (Leat, 1974) and preliminary experiments suggest that seasonal changes in fatty acid synthesis occur (R. G. Vernon, R. A. Clegg & D. J. Flint, unpublished work). One purpose of staggering the mating times of the groups of ewes was to minimize seasonal effects, for it meant that in most months when biopsies were taken there were animals at several stages of pregnancy and lactation. The post-weaning animals, however, were an isolated group.

Changes in adipose tissue metabolism in sheep during pregnancy and lactation are similar to those of rats, the major differences being the timing of the onset of lipid mobilization. Similar metabolic changes during pregnancy and lactation have also been indicated for cows and goats (see Vernon, 1980). Thus although there are differences between species in the time of onset of lipid mobilization and perhaps in the regulatory mechanisms, a pattern of lipid accumulation and then mobilization during pregnancy and lactation may well be ubiquitous amongst mammals.

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