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Regulation of lipolysis during pregnancy and lactation in sheep

Response to noradrenaline and adenosine

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1. The effects of pregnancy and lactation on lipolysis in sheep adipose tissue *in vitro* were investigated. 2. Neither pregnancy nor lactation altered the basal rate of lipolysis. 3. The rate of noradrenaline-stimulated lipolysis was directly proportional to adipocyte mean cell volume. 4. Lactation, but not pregnancy, increased the response to noradrenaline, but had no effect on the ED_{50} of noradrenaline. 5. The adenosine analogue N^6-phenylisopropyladenosine decreased the rate of lipolysis in the presence of noradrenaline; the effect was greater with adipose tissue from lactating than from control, unmated, sheep. 6. Results are discussed in relation to the need of sheep to mobilize lipid during early lactation to support milk production.

The nutrient requirements of the mammary gland for milk production are considerable and may exceed the nutrient requirements of the rest of the body (see Bauman & Elliot, 1983; Vernon & Flint, 1984). This increase in nutrient requirements is met primarily by an increase in food intake, but this is not always sufficient, and hence the animal has to mobilize reserves of fat, protein and minerals (see Bauman & Elliot, 1983; Vernon & Flint, 1984). During early lactation, lipolysis is thus the dominant metabolic activity of adipose tissue in a variety of species, including rats, mice, cows and sheep, and over 50% of adipose-tissue triacylglycerol is often expended (see Vernon & Flint, 1984).

The control of lipolysis during lactation has not been fully elucidated. In earlier studies results were expressed per g wet weight, hence conclusions are confounded by changes in cellularity (see Vernon & Flint, 1984). More-recent studies have shown that, in cattle, adipocytes show an increased response to noradrenaline during lactation (Pike & Roberts, 1980; Jaster & Wegner, 1981). In contrast, studies with rats have shown an increase in the response of adipocytes to adenosine, a local anti-lipolytic factor, during lactation (Vernon *et al.*, 1983). In the present study, we show that both phenomena occur in sheep adipose tissue.

Abbreviation used: PIA, N^6-phenylisopropyladenosine.

Experimental

Animals and experimental design

All sheep were mature 3–4-year-old Finn × Dorset Horn cross-bred ewes; all had had at least one crop of lambs before the experiment. The diet and feeding regimen have been described previously (Vernon *et al.*, 1981). For all sheep a jugular catheter was inserted 24h before biopsy samples were taken or the sheep were killed (Vernon *et al.*, 1981). Samples of subcutaneous adipose tissue were removed from the flank in the region of the hind-limb as described previously (Vernon *et al.*, 1981); when larger amounts of tissue were required, the animals were anaesthetized as for biopsy, then exsanguinated, and the tissue samples were removed immediately. Adipose-tissue samples were placed immediately in 0.15M-NaCl at 37°C.

For Expt. 1, seven sheep were sampled by biopsy in January, at 84–90 days of pregnancy (mid-pregnant), in March, at 126–133 days of pregnancy (late-pregnant), in April, at 13–16 days of lactation (peak lactation), and in May, at 43–46 days of lactation (mid-lactation). A group of five control (unmated) sheep were biopsied on each occasion. In Expt. 2, six control sheep and six sheep at 15–20 days of lactation were sampled in April or May. Pregnancy lasted for 143–145 days in these sheep.

Incubation conditions and assays

Pieces of adipose tissue were prepared as described previously (Vernon *et al.*, 1981). For

Expt. 1 (Table 1), pieces of tissue were incubated in Krebs–Ringer bicarbonate buffer (Krebs & Henseleit, 1932) (containing 1.22mM-Ca^{2+}), with 25mM-Hepes, pH7.3, 5.5mM-glucose and 30mg of bovine serum albumin/ml (0.44mM) [all albumin used was from Sigma Chemical Co. and was essentially fatty acid-free; it was dialysed before use as described by Hanson & Ballard (1968)]. Tissue was incubated in 3ml of the above medium for 15min at 37°C, after which a 0.5ml sample of medium was removed for glycerol analysis; noradrenaline (0.1mM) alone or with theophylline (2mM) was added to some flasks, and they were incubated for a further 3h at 37°C, after which samples of medium were taken for glycerol analysis. Samples of medium were deproteinized and the glycerol concentration was measured as described previously (Aitchison *et al.*, 1982). The rates of glycerol release quoted are from the difference between the amounts present in the medium after 15min and 195min of incubation. Preliminary experiments showed that release was linear with time over this period. All incubations were performed in triplicate.

In Expt. 2, tissue pieces were preincubated in Krebs–Ringer bicarbonate buffer, containing 25mM-Hepes, pH7.3, 5.5mM-glucose, 1mM-acetate and 30mg of albumin/ml for 20min and were then transferred to fresh flasks containing 2.5ml of the above mixture plus other additions as indicated in the Tables and Figures: flasks were then incubated for 3h at 37°C, after which the glycerol content of the medium was assayed as described above. All incubations were performed in triplicate.

The size and number of adipocytes were determined as described previously (Robertson *et al.*, 1982), and rates of glycerol release were expressed per 10^5 cells, assuming that lipolysis was confined to the adipocyte complement of the tissue.

Statistical analysis

The results of the first experiment (Table 1) were analysed by analysis of variance. Other results were analysed by Student's *t*-test for paired or unpaired values as appropriate.

Results

Response to noradrenaline

Expt. 1. Pregnancy had no apparent effect on the adipocyte mean cell volume, basal (unstimulated) lipolysis or noradrenaline-stimulated lipolysis (Table 1). In contrast, lactation resulted in a loss of lipid from adipocytes, as evidenced by their diminished mean cell volumes. The rate of basal lipolysis was not altered significantly during lactation, but the rate of noradrenaline-stimulated lipolysis (in the absence and in the presence of theophylline) was significantly decreased (Table 1).

The mean cell volume of control (non-lactating) sheep was significantly greater ($P<0.05$) than that of the same animals when used as controls for pregnant sheep (non-pregnant) (Table 1); this is probably the result of a seasonal change which occurs in sheep adipose tissue at this time of the year (Vernon & Flint, 1984).

Expt. 2. In this second experiment, with a different group of sheep, a much wider range of adipocyte mean cell volume was found for sheep at peak lactation. The mean basal rates of lipolysis in this experiment were 15 ± 5 and 10 ± 4nmol/h per

Table 1. *Effects of pregnancy and lactation on adipocyte mean cell volume and rates of basal and noradrenaline-stimulated lipolysis in subcutaneous adipose tissue*

Pieces of adipose tissue were incubated in Krebs–Ringer bicarbonate buffer containing 25mM-Hepes, pH7.3, 5.5mM-glucose and 30mg of albumin/ml. Samples of medium were taken after 15 and 195 min and assayed for glycerol; the rates given are mean rates of release between these two times. Results for control (non-pregnant) sheep are means of values obtained with unmated sheep biopsied at the same time as the mid-pregnant and the late-pregnant sheep (see the text for further details). Similarly results for control (non-lactating) sheep are means of values obtained for unmated sheep biopsied at the same time as the peak-lactating and mid-lactating sheep. Results are means for five values (control sheep) or six values (others), and were analysed by analysis of variance; *, value significantly different ($P<0.05$) from that for unmated animals.

Variable	Control (non-pregnant)	Pregnant		Control (non-lactating)	Lactating		S.E.M.	
		Mid	Late		Peak	Mid	$n=5$	$n=6$
Adipocyte mean cell volume (pl)	519	606	525	725	374*	241*	84	77
Rate of lipolysis (nmol of glycerol released/h per 10^5 cells):								
Basal	22	15	18	8	22	26	9	8
Noradrenaline (0.1mM)	192	135	178	241	97*	102*	45	41
Noradrenaline + theophylline (2mM)	280	211	306	349	212*	159*	41	37

10^5 cells for unmated and lactating sheep respectively; results are means ± S.E.M. for six observations in each case, and do not differ significantly.

The rate of lipolysis in the presence of noradrenaline was proportional to cell size (Fig. 1): correlation coefficients were 0.88 and 0.97 respectively for tissue from control and lactating sheep, and were significant ($P<0.02$ and <0.01 respectively). When the results from Expt. 1 were pooled with those from Expt. 2 and the regression analyses repeated, the correlation coefficients were 0.95 and 0.96 respectively for control and lactating sheep; both were highly significant ($P<0.001$). Furthermore, the slope of the regression lines for tissue from lactating sheep was significantly greater than that for unmated sheep ($P<0.02$ and <0.001 respectively for results from Expt. 2 alone or Expt. 1 and 2 combined) (Fig. 1). Thus, for a given cell volume, the response to noradrenaline is greater for adipocytes from lactating than from unmated sheep; however, this difference in response diminishes as mean cell volume decreases, and the regression lines intersect at a mean cell volume of about 300 pl.

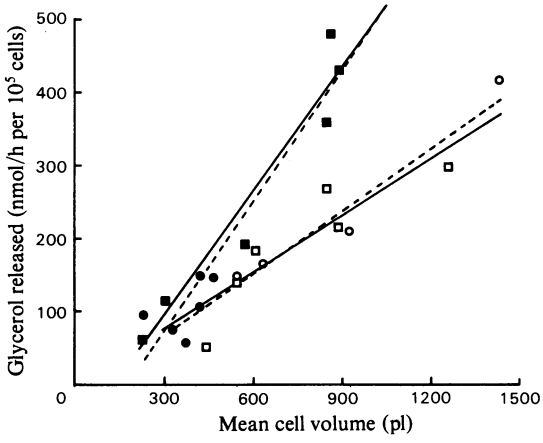

Fig. 1. *Effect of lactation on the relationship between the rate of noradrenaline-stimulated lipolysis and adipocyte mean cell volume*

Pieces of adipose tissue from control (unmated) sheep (○ and □) and sheep at peak lactation (● and ■) were incubated in Krebs–Ringer bicarbonate buffer, supplemented as described in the text for either Expt. 1 or Expt. 2, in the presence of 100 μM-noradrenaline (Expt. 1) (○ and ●) or 10 μM-noradrenaline (Expt. 2) (□ and ■). Least-squares linear-regression analysis was performed on the results: continuous regression lines are for results from Expt. 2 alone, broken lines are for results from Expts. 1 and 2 combined. Each point represents a value from a different sheep.

As the response to noradrenaline varies with cell size, further comparisons of results from Expt. 2 were made with results for unmated and lactating sheep with similar cell volumes (range 500–900 pl). As shown in Table 2, the response to noradrenaline was significantly greater at all concentrations examined for adipose tissue from lactating sheep. When results were expressed as a fraction of that obtained with 10 μM-noradrenaline, the dose–response curves were essentially the same for both groups of sheep (results not shown) and indicate that there was no apparent change in the concentration of noradrenaline required to elicit half-maximum response (ED_{50}) (about 0.5 μM) with lactation.

Response to adenosine

Preparatory experiments with adipose tissue from unmated sheep showed that inclusion of 1 μM-PIA, an analogue of adenosine, in the incubation medium decreased the sensitivity of adipose tissue to noradrenaline, the ED_{50} for noradrenaline increasing from 0.5 μM to 1.8 μM (Fig. 2). The anti-lipolytic effect of PIA was most marked when 1 μM-noradrenaline was used (Fig. 2). When adipose tissue from lactating and unmated sheep was incubated with 1 μM-noradrenaline plus various concentrations of PIA, the rate of lipolysis was significantly decreased ($P<0.05$) (Table 3; results analysed by a *t* test for paired observations). PIA at all concentrations tested caused a significantly greater decrease in the rate of noradrenaline-

Table 2. *Increase in the rate of glycerol release from adipose pieces obtained from control (non-lactating) sheep and sheep at about 15 days of lactation in response to various concentrations of noradrenaline*

Pieces of adipose tissue were incubated in Krebs–Ringer bicarbonate buffer supplemented as described in the text (Expt. 2). Basal rates of lipolysis have been subtracted. Results are for animals with adipocyte mean cell volumes in the range 500–900 pl, and are means ± S.E.M. for four observations in each case: * and **, results significantly different from that for control sheep, $P<0.05$ and <0.01 respectively.

State	Concn. of noradrenaline ...	Glycerol released (nmol/h per 10^5 cells) 0.01 μM	0.1 μM	1.0 μM	10 μM	Adipocyte mean cell volume (pl)
Non-lactating		0.7 ± 0.7	6.3 ± 1.0	152 ± 24	189 ± 17	722 ± 86
Lactating		7.0 ± 4.3*	19.7 ± 3.3**	267 ± 34*	361 ± 66*	796 ± 76

stimulated lipolysis with adipose tissue from lactating sheep than with that from non-lactating animals (Table 3). The effect of PIA was to decrease the different rates of noradrenaline-stimulated lipolysis of unmated and lactating sheep to the same value (Table 3).

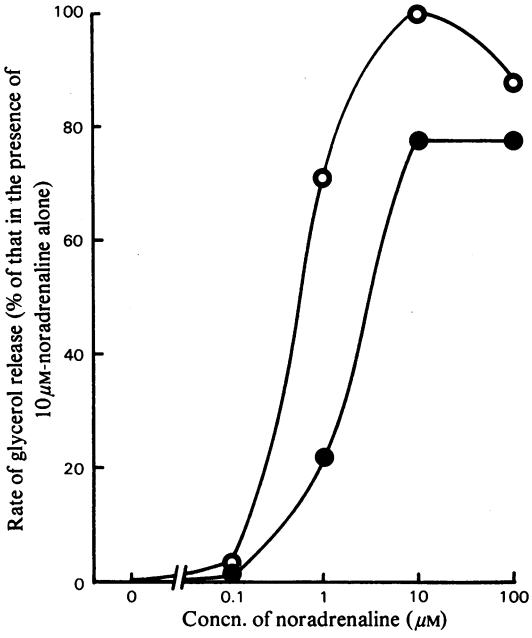

Fig. 2. *Effects of PIA on the rate of noradrenaline-stimulated lipolysis in sheep adipose tissue*

Pieces of adipose tissue were incubated in Krebs–Ringer bicarbonate buffer supplemented as described in the text for Expt. 2, in the presence of various concentrations of noradrenaline without (○) or with (●) 1 μM-PIA. Results are mean values from two separate experiments with tissue from control sheep: basal rates of lipolysis have been subtracted, and results are expressed as a percentage of the rate found with 10 μM-noradrenaline in the absence of PIA.

Other studies with adipose tissue from non-lactating sheep showed that PIA caused much greater inhibition of noradrenaline-stimulated lipolysis than did either adenosine itself or its precursor, AMP; neither of the latter two agents had a significant effect at concentrations lower than 10 nM, although significant inhibition ($P<0.05$) was apparent with 100 nM-adenosine and 50 nM- and 500 nM-AMP (results not shown).

Discussion

Several points arise from this study: the rate of noradrenaline-stimulated lipolysis varied directly with cell size; the response to noradrenaline increased during lactation; the response to PIA increased during lactation; pregnancy had no apparent effect on lipolysis.

A correlation between the rate of lipolysis and cell volume has not been reported previously for sheep. Also most studies with other species on the relationship between cell size and the rate of lipolysis have used animals of different ages to achieve a range of adipocyte sizes, and so conclusions are confounded by an age effect. For rats (Zinder & Shapiro, 1971) and man (Jacobsson & Smith, 1972; Arner & Östman, 1978), however, a correlation between fat-cell volume and the rate of lipolysis has been found that was independent of age. In contrast, studies with adipocytes from pregnant and from lactating rats suggested that the rate of noradrenaline-stimulated lipolysis did not vary with cell volume over the volume range of 200–600 pl (Vernon *et al.*, 1983); however, the rates of lipolysis were modulated by inhibition by adenosine (the incubation medium, Medium 199, contained 0.5 μM-AMP, from which adenosine is synthesized). In the presence of theophylline (an

Table 3. *Effects of PIA on the rate of noradrenaline-stimulated lipolysis in adipose tissue from controls (non-lactating) and sheep at about 15 days of lactation*

Pieces of adipose tissue were incubated in Krebs–Ringer bicarbonate buffer supplemented as described in the text (Expt. 2) containing 1 μM-noradrenaline with and without PIA. Results are for the same animals as described in Table 2, and are means ± S.E.M. for four observations in each case; *, value significantly different from that for control sheep, $P<0.05$.

		Rate of lipolysis (nmol/h per 10^5 cells)		Decrease in rate of lipolysis on addition of PIA (nmol/h per 10^5 cells)	
Concn. of PIA (nM)	State ...	Control	Lactating	Control	Lactating
0		170 ± 31	275 ± 28*	—	—
2		117 ± 39	122 ± 12	52 ± 16	154 ± 28*
20		103 ± 39	114 ± 12	66 ± 17	162 ± 24*
1000		110 ± 39	118 ± 12	60 ± 17	157 ± 31*

adenosine antagonist), the rate of noradrenaline-stimulated lipolysis was again positively correlated with cell volume for adipocytes from lactating rats, but not pregnant rats (Vernon *et al.*, 1983). Thus a marked dependence of the rate of noradrenaline-stimulated lipolysis on cell volume during lactation appears to be common to at least two species. In accordance with this conclusion, the rate of fat loss from the body of lactating sheep, on a given energy intake, is proportional to the initial fat content of the body (Cowan *et al.*, 1982; Vernon & Flint, 1984).

In cattle and sheep, fat loss is greatest during early lactation when fat reserves are largest; about half the fat reserves of adipose tissue are normally used during this period, after which increasing food intake coupled with a falling milk yield result in a gradual cessation of net lipolysis to support lactation (Bauman & Currie, 1980; Vernon & Flint, 1984). Thus an increased response to noradrenaline and a marked dependence of the rate of lipolysis on cell size is well suited to the needs of the animal during early lactation. The system also appears to provide a brake, minimizing the possibility of the complete exhaustion of lipid reserves.

The study shows that it is the response and not the sensitivity to noradrenaline that increased during lactation. In the studies with bovine adipocytes, only a single, maximum, concentration of noradrenaline was used (Pike & Roberts, 1980; Jaster & Wegner, 1981). An increased response to noradrenaline was clearly apparent even with low concentrations (10nM), which is important, for it is unlikely that the concentratons of noradrenaline required to achieve the maximum response occur naturally *in vivo*. From changes in adipocyte volume over the period of study, the average net rate of lipolysis was in the range 25–50nmol/h per 10^5 cells during the first few weeks of lactation, which is much less than the maximum rates observed *in vitro*.

The mechanism responsible for the increased response to noradrenaline is uncertain. Jaster & Wegner (1981) found an increased number of β-adrenergic receptors per adipocyte in lactating cattle, and a decrease in phosphodiesterase activity has been found in adipocytes from lactating rats (Aitchison *et al.*, 1982), so more than one mechanism is probably involved.

Apart from an increased response to noradrenaline, there was also an increased response to PIA of sheep adipocytes during lactation. PIA has been used widely for studying the response of adipocytes to adenosine, as it is not subject to degradation by adenosine deaminase. Sheep adipose tissue has an adenosine deaminase activity of 50–100nmol/min per mg of protein (assayed as described by Vernon *et al.*, 1983), which is higher than that of rat adipose tissue (Vernon *et al.*, 1983), and probably accounts for the small effect of adenosine itself or its precursor AMP on sheep adipose tissue. The dose–response curve for PIA for adipose tissue from unmated sheep is similar to that reported for man (Ohisalo, 1981) and rats (Ohisalo & Stouffer, 1979; Saggerson, 1980), and the primary effect of PIA appears to be to decrease the sensitivity of the tissue to noradrenaline, which is again in keeping with studies on the effects of adenosine on rat adipocytes (Fernandez & Saggerson, 1978; Shechter, 1982).

In the present study it was assumed that endogenous adenosine is minimal in the extracellular fluid of adipose-tissue pieces, owing to their adenosine deaminase activity. Studies with rat adipose tissue have shown that, unlike adipocytes, there appeared to be no accumulation of adenosine (sufficient to inhibit the effects of isoprenaline) during incubation of pieces of adipose tissue (Shechter, 1982). This conclusion is supported by the dose–response curve for PIA, which is similar to that found with rat adipocytes in the absence of endogenous adenosine (see above), and the poor response to exogenous adenosine. Furthermore, the 5′-nucleotidase activity of the tissue, the enzyme responsible for most of the adenosine production in adipose tissue (see Arch & Newsholme, 1978), in this study was the same in tissue from both unmated and lactating sheep (1.8 ± 0.3 and 2.4 ± 0.7nmol/min per mg of protein respectively; results are means $\pm$ S.E.M. for six observations, assayed as described by Vernon *et al.*, 1983), whereas the sensitivity to noradrenaline was the same for adipose tissue from both control and lactating sheep. These observations suggest that the differential effects of PIA were not due to differences in the amounts of adenosine accumulating in adipose tissue from control and lactating sheep.

Adipocytes from lactating rats also show an increased response to adenosine compared with adipocytes from pregnant rats (Vernon *et al.*, 1983) and virgin rats (R. G. Vernon & E. Finley, unpublished work). However, the physiological significance of this adaptation during a state of net lipid mobilization is still not clear. Lactation thus differs from starvation, for which there is a decrease in the response to PIA (Chohan *et al.*, 1984).

The lack of effect of pregnancy on the rate of lipolysis is in accordance with studies on human adipose tissue (Elliott, 1975) and rat adipocytes (Aitchison *et al.*, 1982). However, the observation was surprising, as fat mobilization often begins relatively early during pregnancy in sheep (Robinson *et al.*, 1978).

This study shows that the response to both noradrenaline and PIA is increased during lactation in sheep adipose tissue. An increased response to adenosine is thus apparent in two distinct species, whereas an increased response to noradrenaline during lactation appears to be a feature of ruminants. Furthermore, the response to noradrenaline is dependent on cell size, and the study emphasizes the importance, when comparing the effect of physiological state on lipolysis in adipose tissue and its response to hormones, to take differences in cell size into account; the results of Table 1 alone would have given a totally misleading picture of the effect of lactation on the response of sheep adipose tissue to noradrenaline.

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