Effects of lactation on the signal transduction systems regulating lipolysis in sheep subcutaneous and omental adipose tissue

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The effect of lactation on the regulation of lipolysis by β - and α_2 -adrenergic agents and by adenosine has been investigated. When changes in adipocyte mean cell volume (which decreases with lactation) are allowed for, lactation increased the maximum response both to β -adrenergic agents and to the adenosine analogue *N*⁶-phenylisopropyladenosine, but had no apparent effect on the responsiveness of the α_2 -adrenergic system in both subcutaneous and omental adipocytes. For subcutaneous adipocytes, there was no significant change in the number of β -adrenergic or α_2 -adrenergic receptors, but the amount of G_s and the maximum (forskolin-stimulated) adenylate cyclase activity were increased by lactation. In contrast, in omental adipocytes, the number of β - (but not α_2 -) adrenergic receptors and the

amount of G_s were increased, whereas forskolin-stimulated adenylate cyclase activity was unchanged by lactation. In both types of adipocyte, cyclic AMP phosphodiesterase and total protein kinase A activities were unchanged. Lactation had no effect on the number of adenosine receptors but increased the amounts of the G_i isoforms expressed in both types of adipocyte. These various adaptations differ markedly in a number of respects from those described previously in the rat. Lactation, then, while having a similar overall effect on the response to β -adrenergic agonists of adipocytes, achieves this by depot-specific mechanisms. In contrast, changes in response to adenosine appear to involve the same mechanism in the two depots investigated.

INTRODUCTION

The considerable demands of the mammary gland for nutrients during lactation has major consequences for the metabolism of white adipose tissue. Lipid synthesis is suppressed in many species including rats and domestic ruminants [1] and, if the demands of the mammary gland drive the animal into negative energy balance, there is net lipolysis. The overall effect is a loss of adipose tissue lipid during early lactation which is replenished on litter removal or during the later stages of lactation when milk production declines [2–4].

Lipolysis is under complex acute and chronic endocrine control [5]. For example, it is stimulated acutely by catecholamines acting via the β -adrenergic receptor, and inhibited by catecholamines acting via the α_2 -adrenergic receptor and by adenosine acting via the adenosine A_1 receptor. Responsiveness to these acutely acting factors is modulated by steroid [6] and thyroid hormones [6,7] and by growth hormone [8].

The ability of β -agonists and adenosine to alter the lipolytic rate is changed during lactation in a species-specific manner. In rats the maximum response and sensitivity to β -agonists is unchanged, while the response to adenosine is paradoxically increased [9–11]. In cattle [4] and sheep [12,13] the response to β -agonists is increased, and, in sheep at least, the response to adenosine is again paradoxically increased [12,14].

The molecular basis of the enhanced response to β -agonists and adenosine of adipose tissue from lactating sheep has not been resolved. In the present study we have assessed the effects of lactation on the components of the β -adrenergic/adenosine signal transduction systems in sheep subcutaneous and omental adipose tissue. The study shows that several components change, that adaptations differ between depots in sheep and, furthermore,

that these adaptations differ from those recently described in lactating rats [11].

MATERIALS AND METHODS

Animals

Sheep were 3–5-year-old Finn $\times$ Dorset Horn crossbred ewes all of which had lambed previously. They were fed on hay plus a cereal mix as described previously [15]. Lactating ewes were suckling two or three lambs and were killed at about day 18 of lactation. Sheep were anaesthetized at 10.00 h by an intrajugular injection of 25 ml of Sagatal (Veterinary Drug Co., Falkirk, Scotland, U.K.). Samples of omental and subcutaneous adipose tissue (the latter from the flank fat pads immediately anterior to the hind limbs) were removed and frozen in liquid N_2 for enzyme assay. Sheep were subsequently killed by exsanguination and further samples of omental and subcutaneous adipose tissue were removed and placed in isotonic saline at about 41 °C and 37 °C respectively for transport to the laboratory (about 5 min).

Sheep were killed during the months of February through to May inclusive. Lactating and non-lactating sheep were killed on approximately alternate weeks. Some experiments were repeated over several years.

Lipolysis

Pieces of adipose tissue (about 20 mg in weight) were cut with scissors, rinsed in incubation medium (below) and transferred to flasks containing 2.5 ml of Krebs–Ringer bicarbonate buffer

(containing 1.22 mM CaCl_2) with 25 mM Hepes buffer, pH 7.4, 5.5 mM glucose, 2 mM acetate and 30 mg/ml BSA (fatty acid-free and dialysed) [16]. Other agents were added as indicated in the text and Tables. Adenosine deaminase, when added, was dialysed overnight prior to use [9]. Tissue was incubated for 3 h at 37 °C after which samples of medium were removed, deproteinized [9] and stored at -20 °C until assayed for glycerol [17]. Adipocyte mean cell volume and number/g of tissue were determined as described previously [18].

Enzyme assays

Total and expressed protein kinase A (A-kinase) activities were measured as described by Clegg and Ottey [19], except that 150 mM NaCl [20] was included in the homogenizing medium and the incubation time was decreased to 2 min. AMP-stimulated kinase was assayed as described by Davies et al. [21]. For cyclic AMP phosphodiesterase (PDE) activity, tissue was homogenized and centrifuged at 14000 *g* for 1 min [22]; the enzyme activity of the supernatant was measured [23] using 2 μM cyclic AMP as substrate.

Preparation of adipocytes and adipocyte membranes

Adipocytes were prepared by collagenase digestion and washed as described previously [24], except that only 1 mg/ml collagenase was used and when preparing omental adipocytes the incubation temperature was 41 °C. Adipocytes were disrupted by suspension in 10–15 ml of 10 mM Tris/HCl, pH 7.4, containing 20 mM EDTA, 200 mM sucrose, 17.5 mg/ml benzamidine/HCl and 0.2 mM phenylmethanesulphonyl fluoride at 40 °C and vortex-mixing for 2 min. This preparation was immediately centrifuged at 2800 *g* for 5 min at 22 °C; this minimizes trapping of membranes within the lipid layer. The infranatant was removed, diluted to 50 ml with ice-cold 10 mM Tris/HCl, pH 7.4, containing 90 mM NaCl, and centrifuged at 42000 *g* for 30 min at 4 °C. The membrane pellet was resuspended in 50 mM Tris/HCl, pH 7.4, containing 10 mM MgCl_2 , snap-frozen and stored in liquid N_2 . The protein concentration of the membranes was determined by the Bradford [25] method using BSA as standard.

Receptor numbers

Numbers of β -adrenergic, α_2 -adrenergic and adenosine receptors of adipocyte membranes were determined by ligand binding. Various concentrations of [^{125}I]iodocyanopindolol ([^{125}I]ICYP) [26] and [^3H]dihydroalprenolol [11] were used to assess the number of β -adrenergic receptors, and of [^3H]rauwolscine and [^3H]phenylisopropyladenosine ([^3H]PIA) [24] to assess the numbers of α_2 -adrenergic and adenosine receptors respectively. Non-specific binding was assessed using 100 μM (-)-isoprenaline ([^{125}I]ICYP and [^3H]dihydroalprenolol), 100 μM yohimbine ([^3H]rauwolscine) or 100 μM unlabelled PIA ([^3H]PIA).

GTP-binding proteins

The amounts of GTP-binding protein α -subunits were determined by immunoblotting following separation by SDS gel electrophoresis. Anti-peptide antisera CS1 and I1C, which specifically recognized the α -subunits of the G-proteins G_s and G_i respectively, were kindly provided by Dr. G. Milligan, Department of Biochemistry, University of Glasgow [27,28]. An antiserum (SG1) which recognizes G_i 1 plus G_i 2 was generated against the C-terminal decapeptide of the α -subunit of rod transducin and characterized as described by Mitchell et al. [27].

An anti-peptide antiserum which recognizes amino acids 345–354 of the α -subunit of G_i 3 was purchased from Calbiochem (Nottingham, U.K.). These antisera are thought to be species-non-specific, and preparatory experiments showed that they recognized corresponding sheep GTP-binding proteins.

Membrane samples were resolved by SDS/PAGE on 12% gels (7.5 cm length mini-gels for detection of G_i species and 20 cm length gels for detection of G_s). Immunoblotting was performed as described previously [29]. Briefly, proteins were transferred to nitrocellulose then blocked overnight at room temperature with 2.5% (w/v) skimmed milk (Marvel). Primary antiserum, in 1% Marvel, was then added for 4 h. Following the removal of primary antiserum the blot was washed extensively then incubated with [^{125}I]labelled Protein A (Amersham) for the detection of immunoreactive proteins. Quantification of reactive proteins was achieved by γ -radiation counting of excised bands (for G_i 1 plus G_i 2 and G_s) or by densitometric scanning using a Bio-Rad 620 video densitometer (for G_i 1 and G_i 3). Control experiments established that for each antiserum the intensity of labelling was proportional to the amount of membrane protein (2–20 μg for G_i and 25–75 μg for G_s).

Adenylate cyclase activity

The adenylate cyclase activity of adipocyte membrane fractions was determined as described previously [11]. Various additions were included in the assay as indicated in the text and Tables.

Statistical analysis

Results are expressed as means $\pm$ S.E.M. and were compared using Student's *t* test for paired and unpaired observations as appropriate. In addition, the results of Table 3 were analysed by analysis of variance using Genstat; factors comprised animals, physiological state, concentration and their interactions. Possible relationships between variables and mean cell volumes were assessed by regression analysis.

RESULTS

Some parameters of lipolysis vary with adipocyte size, thus presenting problems when trying to assess the effects of lactation as this state results in a loss of fat and hence a reduction in adipocyte volume (see Tables 1 and 2); this has been resolved in two ways. For some variables, pooling data from several experiments carried out over a number of years provided sufficient numbers of lactating and non-lactating sheep with similar mean cell volumes (600–800 pl; Table 1) to allow direct comparisons. For other variables, results were analysed by regression analysis, and where regressions were statistically significant, slopes were compared to determine if lactation altered the relationship between the variable and cell size.

For subcutaneous adipocytes the basal rate of lipolysis (i.e. measured in the absence of exogenous hormones) did not vary with cell size and was unaltered by lactation. The response to both isoprenaline (a pure β -agonist) and noradrenaline (which has both β - and α_2 -adrenergic effects) varied with cell size and was increased by lactation in subcutaneous adipocytes (Table 1). The ratio of noradrenaline/isoprenaline-stimulated lipolysis provides an indication of the α_2 -adrenergic activity of noradrenaline. This is relatively small in non-lactating sheep (about 20% of the β -adrenergic response) and did not vary with lactation (Table 1). Addition of adenosine deaminase (0.8 $\mu\text{g}/\text{ml}$) increased ($P < 0.05$, paired *t* test) the basal rate of lipolysis from 10 ± 2 to 13 ± 2 nmol/h per 10^5 cells in non-lactating sheep, and

Table 1 Effects of lactation on the rate of lipolysis of subcutaneous adipose tissue as a function of adipocyte mean cell volume

Rates of lipolysis of subcutaneous adipose tissue from non-lactating (non-lact.) and lactating (lact.) sheep were determined in the absence of exogenous agents (basal), in the presence of 10 μ M isoprenaline (Isop.) or noradrenaline (NA), and in the presence of 10 μ M isoprenaline plus 100 nM PIA, as described in the text. Rates of lipolysis are expressed as nmol of glycerol released/h per 10⁵ cells. NA/Isop. is the ratio of rates of lipolysis in the presence of 10 μ M noradrenaline and 10 μ M isoprenaline; (Isop. + PIA)/Isop. is the ratio of the rates of lipolysis in the presence of isoprenaline (10 μ M) plus PIA (100 nM) and isoprenaline (10 μ M) alone. MCV is the adipocyte mean cell volume. For each variable, values are grouped according to mean cell volume. Results are means $\pm$ S.E.M. with the numbers of observations in parentheses; * Value differs ($P < 0.05$) from corresponding value for non-lactating sheep.

Variable	State	Adipocyte MCV range ...	Rate (nmol/h per 10 ⁵ cells)			
			< 400 pl	400–600 pl	600–800 pl	> 800 pl
Basal	Non-lact.	—	—	15 $\pm$ 6 (4)	10 $\pm$ 1 (8)	10 $\pm$ 3 (10)
	Lact.	9 $\pm$ 3 (8)	—	10 $\pm$ 2 (11)	13 $\pm$ 2 (7)	—
Isop.	Non-lact.	—	—	166 $\pm$ 19 (3)	230 $\pm$ 20 (9)	360 $\pm$ 49 (10)
	Lact.	134 $\pm$ 27 (8)	—	207 $\pm$ 25 (10)	353 $\pm$ 29 (7)*	—
NA	Non-lact.	—	—	112 $\pm$ 4 (4)	217 $\pm$ 24 (8)	296 $\pm$ 43 (11)
	Lact.	90 $\pm$ 23 (8)	—	148 $\pm$ 26 (9)	307 $\pm$ 21 (7)*	—
100 $\times$ NA/Isop.	Non-lact.	—	—	77 $\pm$ 9 (4)	88 $\pm$ 6 (8)	80 $\pm$ 5 (9)
	Lact.	66 $\pm$ 10 (8)	—	70 $\pm$ 5 (10)	89 $\pm$ 6 (7)	—
100 $\times$ (Isop. + PIA)/isop	Non-lact.	—	—	36 $\pm$ 14 (3)	55 $\pm$ 9 (7)	46 $\pm$ 7 (8)
	Lact.	11 $\pm$ 2 (5)	—	22 $\pm$ 5 (10)	22 $\pm$ 8 (6)*	—
MCV (pl)	Non-lact.	—	—	549 $\pm$ 24 (4)	727 $\pm$ 13 (9)	932 $\pm$ 25 (13)
	Lact.	282 $\pm$ 28 (8)	—	505 $\pm$ 18 (14)	691 $\pm$ 11 (12)	973 $\pm$ 68 (3)

from 9 $\pm$ 2 to 14 $\pm$ 2 nmol/h per 10⁵ cells in lactating sheep (values are means $\pm$ S.E.M. for subcutaneous adipose tissue from 12 non-lactating and 11 lactating sheep). Adenosine deaminase increased isoprenaline-stimulated lipolysis by 25 $\pm$ 7% and by 19 $\pm$ 8% in subcutaneous adipose tissue from non-lactating and lactating sheep respectively; the effects of adenosine deaminase did not vary with cell size. Sensitivity to isoprenaline was also measured in the presence of adenosine deaminase: ED₅₀ values did not vary with cell size and were 21.0 $\pm$ 7.8 nM and 2.6 $\pm$ 1.0 nM for subcutaneous adipose tissue from non-lactating and lactating sheep respectively (results are means $\pm$ S.E.M. of seven and six values and differ significantly; $P < 0.05$).

Isoprenaline (10 μ M)-stimulated lipolysis was decreased by 100 nM PIA (an adenosine analogue) in subcutaneous adipose tissue; the degree of inhibition did not vary significantly with cell size (Table 1), and was significantly increased by lactation in cells of 600–800 pl (Table 1) and also for pooled values for cells of 400–800 pl ($P < 0.05$). Addition of adenosine deaminase (0.8 μ g/ml) had no effect on the response to PIA in subcutaneous adipose tissue from either non-lactating or lactating sheep.

Omental adipocytes are significantly larger ($P < 0.001$) than subcutaneous adipocytes (Tables 1 and 2). Analysis of paired observations for seven non-lactating and four lactating sheep showed that omental adipocytes had higher rates of basal lipolysis and of isoprenaline- and also noradrenaline-stimulated lipolysis ($P < 0.01$) than subcutaneous adipocytes. The ratio of noradrenaline/isoprenaline-stimulated lipolysis was the same for omental and subcutaneous adipocytes, but subcutaneous adipocytes showed a significantly ($P < 0.01$) greater response to PIA.

Lactation decreased the size of omental adipocytes (Table 2). Rates of isoprenaline-stimulated lipolysis varied significantly ($P < 0.01$) with mean cell volume (correlation coefficients were 0.86 and 0.88 for non-lactating and lactating sheep respectively). The slope of the regression was significantly greater ($P < 0.05$) for lactating than non-lactating sheep, suggesting that, for a given cell volume, lactating sheep would have a higher rate of isoprenaline-stimulated lipolysis. Basal lipolysis was not altered by lactation (Table 2). Addition of adenosine deaminase

Table 2 Effect of lactation on adipocyte mean cell volume and rates of lipolysis in omental adipose tissue

Rates of lipolysis were measured in adipose tissue from non-lactating and lactating sheep in the absence of exogenous agents (basal) and in the presence of various agents as described in the legend to Table 1; in addition, adenosine deaminase (AD; 0.8 μ g/ml) was included in some incubations. Rates of lipolysis are expressed as nmol of glycerol release/h per 10⁵ cells. MCV is adipocyte mean cell volume. Results are means $\pm$ S.E.M. of seven observations. See Table 1 for definition of abbreviations.

Variable	Non-lactating	Lactating
MCV (pl)	1719 $\pm$ 230	825 $\pm$ 91
Lipolysis (nmol/h per 10 ⁵ cells)		
Basal	28 $\pm$ 9	23 $\pm$ 10
AD	43 $\pm$ 11	34 $\pm$ 8
Isop.	246 $\pm$ 65	149 $\pm$ 27
Isop. + AD	241 $\pm$ 61	122 $\pm$ 18
Isop. + PIA	150 $\pm$ 50	58 $\pm$ 10
Isop. + AD + PIA	151 $\pm$ 51	53 $\pm$ 10
NA	211 $\pm$ 56	115 $\pm$ 24
NA + AD	225 $\pm$ 58	118 $\pm$ 27
Lipolysis ratios		
NA/Isop.	0.92 $\pm$ 0.08	0.76 $\pm$ 0.06
(NA + AD)/(Isop. + AD)	0.91 $\pm$ 0.05	0.77 $\pm$ 0.06
(PIA + Isop.)/Isop.	0.61 $\pm$ 0.09	0.42 $\pm$ 0.09
(PIA + AD + Isop.)/(Isop. + AD)	0.61 $\pm$ 0.09	0.45 $\pm$ 0.08

increased basal lipolysis but had no effect on the rate of isoprenaline-stimulated lipolysis (Table 2).

The ratio of noradrenaline/isoprenaline-stimulated lipolysis was unchanged by lactation in omental adipocytes, but the response to PIA was again increased by lactation ($P < 0.05$); in the initial experiment with seven sheep in each group, significance was marginal (Table 2), so measurements were repeated on further sheep in the following year; analysis of pooled results showed a significant effect of lactation.

The number of β -receptors on adipocyte membranes was assessed using a range of concentrations of both [³H]dihydro-

Table 3 Effect of lactation on ligand binding to β -adrenergic, α_2 -adrenergic and adenosine receptors of membranes from subcutaneous and omental adipocytes

Subcutaneous and omental adipocytes were isolated from non-lactating and lactating sheep and used for the preparation of membranes as described in the text. Binding of various concentrations of [3 H]dihydroalprenolol to β -adrenergic receptors, [3 H]rauwolscine to α_2 -adrenergic receptors and [3 H]PIA to adenosine receptors was determined as described in the text. Results are means $\pm$ S.E.M. of six or seven observations; *Value differs ($P < 0.05$) from corresponding value for non-lactating sheep.

Ligand	Concn. (nM)	Tissue ... State ...	Ligand bound (fmol/mg of protein)			
			Subcutaneous		Omental	
			Non-lactating	Lactating	Non-lactating	Lactating
[3 H]Dihydroalprenolol	5		134 $\pm$ 13	189 $\pm$ 51	120 $\pm$ 26	202 $\pm$ 23*
	10		175 $\pm$ 17	249 $\pm$ 73	132 $\pm$ 21	218 $\pm$ 20*
	20		179 $\pm$ 17	256 $\pm$ 59	137 $\pm$ 19	238 $\pm$ 34*
[3 H]Rauwolscine	5		32 $\pm$ 6	34 $\pm$ 9	24 $\pm$ 4	18 $\pm$ 6
	10		58 $\pm$ 16	75 $\pm$ 18	40 $\pm$ 7	40 $\pm$ 6
	20		125 $\pm$ 31	130 $\pm$ 28	52 $\pm$ 10	67 $\pm$ 10
[3 H]PIA	5		108 $\pm$ 31	133 $\pm$ 33	116 $\pm$ 22	173 $\pm$ 28
	10		153 $\pm$ 35	189 $\pm$ 42	142 $\pm$ 27	198 $\pm$ 20
	20		187 $\pm$ 29	192 $\pm$ 26	161 $\pm$ 31	224 $\pm$ 25

alprenolol (Table 3) and [125 I]ICYP (results not shown). Essentially maximum binding was achieved with 20 nM dihydroalprenolol (Table 3). Correlation analysis of results obtained with 20 nM dihydroalprenolol and 150 mM ICYP for both non-lactating and lactating sheep showed a highly significant ($P < 0.001$) correlation. Lactation did not increase the amount of either dihydroalprenolol (Table 3) or ICYP bound to membranes from subcutaneous adipocytes at any concentration used; this was confirmed for both ligands by analysis of variance, which showed a significant effect of ligand concentration ($P < 0.001$) for both [3 H]dihydroalprenolol and [125 I]-ICYP but no effect of physiological state and no significant physiological state-concentration interaction with either ligand. Scatchard analysis of data for ICYP binding to subcutaneous adipocyte membranes showed that lactation had no effect on the affinity of the receptor for the ligand (K_d 104 $\pm$ 18 and 109 $\pm$ 18 pM for control and lactating sheep respectively; means $\pm$ S.E.M. of seven observations). In contrast to subcutaneous adipocytes, lactation increased the binding of [3 H]dihydroalprenolol to membranes from omental adipocytes (Table 3); this was confirmed by analysis of variance ($P < 0.05$). Lactation had no effect on ligand binding to either the α_2 -adrenergic or adenosine receptors of subcutaneous or omental adipocyte membranes (Table 3).

Lactation increased ($P < 0.05$) the amounts of both the 42 kDa and 45 kDa forms of the α -subunit of the stimulatory GTP-binding protein G_s in both subcutaneous and omental adipocyte membranes (Table 4). Immunoblotting with antisera CS1 and SG1 showed significant similar increases in the amounts of α -subunit of G_{i1} and G_{i1} plus G_{i2} respectively (Table 4). These results suggested that G_{i2} was also increased in membranes from both depots during lactation. Confirmation of this was obtained by resolving the α -subunits of G_{i1} and G_{i2} on urea gels (10% acrylamide/0.2% bisacrylamide gels containing 6 M urea, run at 30 mA for about 9 h) [30] followed by immunoblotting with antiserum SG1. This procedure produced two bands of similar intensity corresponding to G_{i1} and G_{i2} α -subunits; the amounts of both were increased by lactation (results not shown). In contrast, the amount of G_{i3} changed only in omental adipocyte membranes in lactation (Table 4). Further 20 cm gels were run as for G_s analysis, and stained for protein with Coomassie Blue. The pattern of protein bands was unchanged by lactation and there were no obvious differences in the staining intensity of individual bands (with the exception of one component with a molecular mass of about 100 kDa) with lactation, indicating that the relative amount of plasma membranes in the membrane fractions from either depot was unchanged by lactation.

Table 4 Effect of lactation on the amounts of GTP-binding protein α -subunits of sheep adipocyte membranes

Subcutaneous and omental adipocytes were isolated from non-lactating and lactating sheep and used for preparation of membranes. Amounts of G-protein α -subunits were determined by immunoblotting and either γ -radiation counting of excised bands (both the 45 and 42 kDa forms of G_s and $G_{i1} + G_{i2}$) or densitometric scanning (G_{i1} and G_{i3}) as described in the text. Results are means $\pm$ S.E.M. with the numbers of observations in parentheses. *, ** Value differs ($P < 0.05$ and < 0.01 respectively) from corresponding values for non-lactating sheep.

G-protein α -subunit	Tissue ... State ...	Amount of G-protein (arbitrary units)			
		Subcutaneous		Omental	
		Non-lactating	Lactating	Non-lactating	Lactating
G_s (45 kDa)		744 $\pm$ 58 (7)	1124 $\pm$ 110 (5)**	718 $\pm$ 167 (7)	1071 $\pm$ 134 (5)
G_s (42 kDa)		1476 $\pm$ 286 (7)	3348 $\pm$ 785 (5)*	1814 $\pm$ 270 (7)	3120 $\pm$ 363 (5)*
G_{i1}		1.37 $\pm$ 0.21 (6)	3.88 $\pm$ 0.54 (6)**	1.81 $\pm$ 0.46 (7)	4.15 $\pm$ 0.58 (6)**
$G_{i1} + G_{i2}$		240 $\pm$ 52 (7)	817 $\pm$ 242 (5)*	524 $\pm$ 155 (7)	905 $\pm$ 144 (6)*
G_{i3}		1.56 $\pm$ 0.27 (6)	2.25 $\pm$ 0.52 (5)	1.33 $\pm$ 0.25 (7)	2.66 $\pm$ 0.48 (6)*

Table 5 Effect of lactation on enzyme activity of subcutaneous and omental adipose tissue and adipocyte membranes

Adipocytes were isolated from subcutaneous and omental adipose tissue of non-lactating and lactating sheep and used for preparation of membranes which were subsequently assayed for adenylate cyclase in the absence of exogenous agents (basal) in the presence of 100 μ M forskolin or 1 μ M isoprenaline alone or with 1 μ M GTP or GTP + 100 nM PIA, as described in the text. PDE activity, AMP-stimulated protein kinase activity (AMP-PK) and A-kinase (total and expressed) were assayed in tissue removed from sheep prior to slaughter as described in the text. Adenylate cyclase activities are given as pmol/min per mg of protein; PDE and AMP-PK activities as pmol/min per 10^6 cells and A-kinase activity as nmol/min per 10^6 cells. Results are means $\pm$ S.E.M. with the numbers of observations in parentheses. *, **Value differs ($P < 0.05$ and < 0.01 respectively) from corresponding values for non-lactating sheep.

Enzyme	Tissue ...	Enzyme activity			
		Subcutaneous		Omental	
	State ...	Non-lactating	Lactating	Non-lactating	Lactating
Adenylate cyclase					
Basal		173 $\pm$ 25 (12)	125 $\pm$ 19 (10)	75 $\pm$ 11 (6)	91 $\pm$ 7 (7)
+ Forskolin		855 $\pm$ 113 (13)	1695 $\pm$ 358 (10)*	2386 $\pm$ 484 (6)	2787 $\pm$ 328 (7)
+ Isop.		185 $\pm$ 23 (12)	272 $\pm$ 97 (10)	99 $\pm$ 12 (6)	159 $\pm$ 13 (7)**
+ Isop. + GTP		240 $\pm$ 19 (7)	254 $\pm$ 60 (7)	184 $\pm$ 20 (6)	345 $\pm$ 41 (7)**
+ Isop. + GTP + PIA		216 $\pm$ 22 (7)	186 $\pm$ 26 (7)	189 $\pm$ 29 (6)	354 $\pm$ 50 (7)**
PDE		275 $\pm$ 49 (7)	272 $\pm$ 35 (7)	589 $\pm$ 64 (7)	626 $\pm$ 73 (7)
AMP-PK		110 $\pm$ 22 (7)	114 $\pm$ 26 (7)	—	—
A-kinase					
Total		16.8 $\pm$ 1.3 (14)	11.4 $\pm$ 1.6 (14)*	39.5 $\pm$ 10.3 (7)	18.9 $\pm$ 1.1 (7)
Expressed		3.45 $\pm$ 0.5 (7)	2.5 $\pm$ 0.8 (7)	9.5 $\pm$ 2.3 (7)	3.1 $\pm$ 0.3 (7)*
Expressed/total		0.19 $\pm$ 0.02 (7)	0.18 $\pm$ 0.03 (7)	0.25 $\pm$ 0.02 (7)	0.17 $\pm$ 0.02 (7)*

Lactation had no effect on the basal or isoprenaline-stimulated adenylate cyclase activity of subcutaneous adipocyte membranes, but it did increase forskolin-stimulated activity ($P < 0.05$) (Table 5). Isoprenaline in the presence and absence of GTP had no effect on the adenylate cyclase activity of subcutaneous adipocyte membranes from non-lactating sheep, but it did increase ($P < 0.05$) activity in such membranes from lactating sheep (Table 5). In contrast, lactation had no effect on either the basal or forskolin-stimulated adenylate cyclase activity of omental adipocyte membranes (Table 5). Isoprenaline had no effect by itself with omental adipocyte membranes from non-lactating sheep, but did increase ($P < 0.01$) the adenylate cyclase activity of membranes from lactating sheep (Table 5). Addition of GTP increased ($P < 0.01$) the activity of adenylate cyclase in the presence of isoprenaline in omental adipocyte membranes from sheep in both states; in the presence of isoprenaline and GTP, adenylate cyclase activity was significantly ($P < 0.01$) higher in membranes from lactating sheep. Addition of PIA (100 nM) had no effect on the adenylate cyclase activity in the presence of isoprenaline and GTP in adipocyte membranes from either depot (Table 5).

PDE activity did not change with lactation (Table 5) and did not vary with cell volume (i.e. correlation coefficient non-significant) in either depot. Omental adipocytes had greater PDE ($P < 0.01$) and total A-kinase ($P < 0.05$) activities than subcutaneous adipocytes (Table 5). Total A-kinase activity was decreased by lactation in subcutaneous adipocytes (Table 5) and tended to decrease in omental adipocytes (Table 5). However, these changes are probably due to the decrease in cell size with lactation, as there was a significant correlation ($P < 0.05$ and $P < 0.002$ for subcutaneous and omental membranes respectively) between cell size and total A-kinase activity. These relationships were not altered by lactation. A significant correlation ($P < 0.001$) was also found for total A-kinase from both subcutaneous and omental adipocytes and mean cell volume, suggesting that the higher ($P < 0.05$) A-kinase activity of omental adipocytes is just due to them being larger cells than subcutaneous adipocytes. Expressed A-kinase activity and the ratio of expressed/total

activity were both significantly decreased in omental but not subcutaneous adipocytes by lactation (Table 5).

The activity of AMP-stimulated kinase was not changed by lactation (Table 5).

DISCUSSION

Previous studies have shown that, for omental adipocytes, lactation in sheep results in an increased maximum response to and sensitivity to the pure β -agonist isoprenaline [13,14]. In those studies mean cell volume did not change during lactation, indicating that changes in response and sensitivity were not due to a change in cell size. The results for omental adipose tissue in the present study are consistent with these previous findings. For subcutaneous adipose tissue, we showed previously that lactation increased the response to noradrenaline, which has both β - and α_2 -adrenergic effects; the present study confirms this previous finding and, by using isoprenaline as well as noradrenaline, shows that the enhanced response is due to an increased β -adrenergic response with no apparent change in the α_2 -adrenergic response. No change in the response to α_2 -adrenergic agents with lactation has also been noted in cattle [31]. In our earlier study we found no change in the sensitivity to noradrenaline [12], but this was assessed without the addition of adenosine deaminase to remove endogenous adenosine and so an increase in sensitivity to catecholamines could have been masked by an increased response to adenosine. Thus from these various studies it is apparent that lactation increases both the maximum response and the sensitivity to β -agonists in sheep omental and subcutaneous adipose tissue and also, paradoxically, increases the response to adenosine.

As fat cell size decreases during lactation due to a loss of lipid, an increased response to catecholamines could be due to the plasma membrane not being lost at a commensurate rate so that, for cells of the same size, there is more plasma membrane in those from lactating animals. However, while this may contribute to the changes, the present study shows that there are changes in the relative amounts of membrane constituents. Curiously, some of these changes are depot-specific. Thus for omental adipose tissue

there is an increase in the number of β -receptors of adipocyte membranes. Such an effect was found also in a preliminary study of ours [32]. In contrast, it would appear that there is little or no change in the number of β -receptors of subcutaneous adipocytes with lactation. Numbers tended to increase in the present study but the difference was not significant; no change was found in one preliminary study [32] and a marginal increase in another [33]; it is possible that a small change does occur but is difficult to demonstrate due to inter-animal variability.

In contrast to the number of β -adrenergic receptors, the maximum (forskolin-stimulated) activity of adenylate cyclase was increased in subcutaneous adipocyte membranes by lactation but remained unchanged in omental adipocyte membranes, while for both tissues the amounts of G_s α -subunits were increased by lactation. As activities of PDE and A-kinase (when changes in cell size are taken into account) are unchanged by lactation, it would appear that differences in signal are due to adaptations at the level of the plasma membrane, with an increased amount of G_s and adenylate cyclase in subcutaneous adipocyte membranes, and an increased number of β -receptors and amount of G_s in omental adipocyte membranes. The reason for such depot-specific adaptations with lactation is not clear, but this finding is not confined to the β -adrenergic system, since lactation results in a much greater degree of insulin-resistance in subcutaneous than omental adipocytes in sheep [34].

While there appear to be depot-specific differences responsible for the increased response to β -agonists, the reason for the increased response to adenosine would seem to be the same in both depots, with the key factor being an increase in the amounts of G_i α subunits. In contrast to the present study, an apparent increase in the number of adenosine receptors of subcutaneous adipocytes with lactation was found in a preliminary study [35]. However, that study was conducted during June and July and a marked seasonal change in both response to PIA and receptor number was occurring during the course of the experiment, probably confounding the results.

Not only are the effects of lactation on the β -adrenergic system depot-specific, they are also species-specific. In rats, lactation does not increase the response or sensitivity of parametrial adipocytes to catecholamines [9,11], although it does increase the response to adenosine [10]. Despite this, the number of β -receptors in adipocyte membranes is increased, but in rats the maximum adenylate cyclase activity fell as did the PDE activity, the overall effect being no change in the strength of the signal reaching A-kinase [11]. In rats most adipose tissue lipid is lost around parturition when there is a transient fall in food intake, so the lack of an increased response to adrenergic stimuli may reflect a less important role for lipolysis during lactation itself in this species than in ruminants, where it is of considerable quantitative importance [3].

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