Roles of insulin and growth hormone in the adaptations of fatty acid synthesis in white adipose tissue during the lactation cycle in sheep

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1. Lactation results in a substantial fall in the rate of fatty acid synthesis in sheep adipose tissue. 2. Maintenance of adipose tissue from non-lactating sheep in tissue culture for 24 or 48 h with insulin increased the rate of fatty acid synthesis. Dexamethasone, a glucocorticoid analogue, alone inhibited the rate of fatty acid synthesis, but enhanced the stimulatory effect of insulin. Growth hormone (somatotropin) antagonized the increase in the rate of fatty acid synthesis induced by insulin or insulin plus dexamethasone. 3. Maintenance of adipose tissue from lactating sheep in tissue culture resulted in a small increase in the rate of fatty acid synthesis after 24 h, and then a large increase in rate between 24 and 48 h of culture. The increase during the second 24 h period was dependent on the presence of insulin; this effect was enhanced by dexamethasone and inhibited by growth hormone. 4. The increase in the rate of fatty acid synthesis in tissue from non-lactating sheep and in tissue from lactating sheep during the major increase in rate was prevented by actinomycin D, an inhibitor of transcription. 5. Effects of insulin and growth hormone were observed with physiological concentrations of the hormones. 6. The study suggests that known changes in the serum concentrations of insulin and growth hormone are the primary causes of the changes in fatty acid synthesis in adipose tissue during the lactation cycle in sheep. 7. During lactation, adipose tissue becomes refractory to insulin in sheep; responsiveness is partly restored by tissue culture in the presence of insulin and dexamethasone.

INTRODUCTION

Lactation results in a decreased rate of fatty acid synthesis in white adipose tissue in both laboratory rodents and domestic ruminants (see Vernon & Flint, 1983). This decrease in rate is greater and quantitatively more important in ruminants than in rodents (see Vernon, 1988). The factors responsible for the decline in fatty acid synthesis in adipose tissue during lactation in either type of species have not been fully defined. Hypoinsulinaemia occurs during lactation in both ruminants and rodents (see Williamson, 1980; Vernon & Flint, 1983; Forsyth, 1986), and is thought to be at least partly responsible for the decline in rate. In the rat the high serum prolactin concentration during lactation is also implicated (Agius *et al.*, 1979; Flint *et al.*, 1981), although its action appears to depend on a functioning mammary gland and so may be indirect (Flint *et al.*, 1981). In contrast, prolactin appears not to be involved in sheep, for in this species there is a seasonal surge in serum prolactin concentration in non-lactating animals, while at the same time there is an increase in the rate of fatty acid synthesis in adipose tissue (Vernon *et al.*, 1986). Lactation results in an increase in serum growth hormone (somatotropin) in ruminants (see Forsyth, 1986), but not in rodents (Schalch & Reichlin, 1966), providing another possible explanation for the fall in the rate of fatty acid synthesis in adipose tissue in the former species. In the present study we have examined the effect of exposure to insulin and growth hormone (and several other hormones) on adipose tissue from lactating and non-lactating sheep. We present evidence for changes in the serum concentrations of insulin and growth hormone being the principal cause of changes in fatty acid synthesis in sheep adipose tissue during the lactation cycle.

EXPERIMENTAL

Sheep were 4- or 5-year-old multiparous Finn × Dorset Horn cross-bred ewes and were fed on a diet of hay and cereals (Vernon *et al.*, 1981). Lactating ewes were suckling either 2 or 3 lambs and were killed at about 18 days of lactation. On the day of killing, sheep were anaesthetized by injection of 20 ml of Sagatal (May and Baker, Dagenham, Essex, U.K.) into the jugular vein, after which they were exsanguinated and samples of subcutaneous adipose tissue were removed aseptically from the flank fat-pads immediately anterior to the hindlimbs. Adipose tissue was placed immediately in sterile iso-osmotic saline at 39–40 °C.

Pieces of adipose tissue (about 10 mg) were cut with scissors and then either used immediately for assay of the rate of fatty acid synthesis or maintained in tissue culture for 24 or 48 h at 37 °C under air/CO₂ (19:1) and in Medium 199 with Earle's salts and 25 mM-Hepes, pH 7.3, plus 2 mM-acetate and antibodies in tissue-culture multi-well plates (Flow Laboratories, Paisley, Scotland, U.K.) as described previously (Robertson *et al.*, 1982). The medium was supplemented with insulin, dexamethasone (Sigma), sheep growth hormone, prolactin (gifts from NIAMDD, Bethesda, MD, U.S.A.) as described in the text and Tables. Tissue before or after tissue culture was transferred to fresh Medium 199 supplemented as described above for tissue culture, plus 17 nM-insulin and [1-¹⁴C]acetate. The rate of fatty acid synthesis was determined from the amount of incorporation of [¹⁴C]-acetate into total lipid over a 2 h incubation (Vernon *et al.*, 1981) (over 90 % of the carbon used for fatty acid synthesis is derived from acetate in sheep, whereas negligible amounts of ¹⁴C are incorporated into acylglycerol).

The amount of glucose removed from the culture media and the amounts of lactate and glycerol released into the culture media were determined as described previously (Vernon *et al.*, 1981). The number of adipocytes/g of tissue was determined (Robertson *et al.*, 1982). Results are expressed per 10^7 cell units; this is the rate/g of tissue $\div 10^{-7} \times$ number of adipocytes/g of tissue (see Vernon *et al.*, 1987). Results are expressed as means $\pm$ S.E.M.; each individual value contributing to a given mean value was obtained from a separate sheep. Results were analysed by using Student's *t* test for paired observations. As the rate of fatty acid synthesis can vary considerably between sheep in the same physiological state, statistical analysis was performed with both untransformed and also log-transformed data.

RESULTS AND DISCUSSION

Effects of insulin and dexamethasone

Maintaining adipose tissue from non-lactating sheep in tissue culture in the absence of added hormones resulted in no change in the rate of fatty acid synthesis over the first 24 h, but then the rate declined ($P < 0.05$) (Fig. 1a). Addition of insulin (17 nM) resulted in an increase in rate by 24 h ($P < 0.05$), but then again the rate decreased ($P < 0.05$) during the subsequent 24 h period (Fig. 1a), as found previously (Vernon, 1979). Addition of dexamethasone (10 nM) along with the insulin, however, prevented the decrease in rate over the second 24 h period (Fig. 1a). In the absence of insulin, dexamethasone is inhibitory (Table 1), and its effect remains inhibitory until insulin concentration in the culture medium exceeds about 0.25 nM. Above this concentration the effect of dexamethasone is synergistic

(Fig. 2; Table 1); the overall effect of dexamethasone is thus to accentuate the insulin dose-response curve. Dexamethasone also inhibited ($P < 0.001$) glucose utilization (Table 1; Plested *et al.*, 1987a; Vernon & Taylor, 1988); the degree of inhibition is partly alleviated by the presence of insulin, but even with 17 nM-insulin dexamethasone was still inhibitory ($P < 0.001$) (Table 1). Thus in sheep, at least, dexamethasone acts both synergistically and antagonistically with respect to insulin. As found previously, dexamethasone also inhibits glycerol release from sheep tissue (Table 1; Plested *et al.*, 1987a); this effect is not ameliorated by insulin. Glucocorticoids act synergistically with insulin to stimulate fatty acid synthesis in pig (Walton *et al.*, 1986) and bovine adipose tissue (Etherton *et al.*, 1987). In contrast, for the rat there are conflicting reports, claiming antagonism (Philips *et al.*, 1965), synergism (Minshall & Strong, 1985; Plested *et al.*, 1987b) and no effect (Hallowes *et al.*, 1973)! Some differences may be due to use of different insulin concentrations, but in addition glucose is the major precursor for fatty acid synthesis in rat adipocytes; hence inhibitory effects of glucocorticoids on glucose uptake (see Fain, 1979) could counter-balance enhancing effects on fatty acid synthesis. In ruminants, acetate rather than glucose is the major precursor, hence any inhibitory effects of glucocorticoids on the conversion of glucose into acetyl-CoA for fatty acid synthesis are avoided.

Adipose tissue from lactating sheep had a much lower ($P < 0.001$) rate of fatty acid synthesis ($0.18 \pm 0.04 \mu\text{mol}$ of acetate incorporated/h per 10^7 cell units) than that from non-lactating sheep (8.3 ± 0.14) (results are means $\pm$ S.E.M. of 13 and 12 observations respectively), as found previously (Vernon *et al.*, 1981). When adipose tissue from lactating sheep was maintained in tissue culture in the presence of insulin and dexamethasone,

Fig. 1. Effects of preincubation with various agents on the rate of fatty acid synthesis in adipose-tissue pieces from (a) non-lactating and (b) lactating sheep

Adipose-tissue pieces were preincubated for 0 h (□), or for either 24 or 48 h in the absence of exogenous hormones (■), or in the presence of insulin (○), insulin and dexamethasone (●), insulin, dexamethasone and growth hormone (▲), or insulin, dexamethasone and actinomycin D (△), after which the rate of fatty acid synthesis was measured as described in the text. Concentrations of agents used were: insulin, 17 nM; dexamethasone, 10 nM; growth hormone, 4.5 nM; actinomycin D, 80 nM. Results are means of five and six observations for tissue from lactating and non-lactating sheep respectively, except for values for insulin plus dexamethasone and actinomycin D, which are means of four observations.

Fig. 2. Effects of insulin concentration during tissue culture for 48 h in the presence and the absence of dexamethasone and growth hormone on the rate of fatty acid synthesis in adipose tissue from lactating and non-lactating sheep

Adipose-tissue pieces from non-lactating ($\square$, $\circ$, $\triangle$) and lactating ($\blacksquare$, $\bullet$, $\blacktriangle$) sheep were preincubated for 48 h in the presence of various concentrations of insulin, either alone ($\square$, $\blacksquare$) or with 10 nM-dexamethasone ($\circ$, $\bullet$) or with 10 nM dexamethasone plus 4.5 nM-growth hormone ($\triangle$). The rate of fatty acid synthesis was subsequently measured in the presence of 17 nM-insulin as described in the text. Results are means of two values for non-lactating sheep and of three values for lactating sheep.

there was a lag phase of about 24 h, when the rate of fatty acid synthesis increased ($P < 0.01$) by 2–3-fold, and then during the second 24 h period there was a massive surge in the rate ($P < 0.001$) (Fig. 1b), achieving a rate which was about 50% of that of non-lactating sheep (before culture). In the absence of added hormones, the rate of fatty acid synthesis still increased over the first 24 h in culture ($P < 0.01$), but there was no further change during the subsequent 24 h. Dexamethasone alone was again inhibitory (Table 1): the rate achieved after 48 h with insulin was lower ($P < 0.05$) than that with insulin plus dexamethasone (Table 1). The effect of dexamethasone is again to accentuate the insulin dose-response curve (Fig. 2), but the interaction between the two agents is only partly restored during 48 h of culture (dexamethasone enhanced the rate in the presence of 17 nM-insulin by 38 and 62% on average in tissue from lactating and non-lactating sheep respectively).

The above adaptations are reminiscent of another study in which we found that the ability of insulin to stimulate the conversion of glucose into products other than lactate was negligible in adipose tissue from lactating sheep over the first 24 h period of incubation, but then the ability to respond to insulin was partly restored during the second 24 h period (i.e. between 24 and 48 h of culture) (Vernon & Taylor, 1988).

It thus appears that lactation results in an impaired ability of insulin to stimulate at least two aspects of adipose-tissue metabolism in sheep, and that responsiveness to the hormone is partly restored on prolonged incubation of adipose tissue in culture in the presence of insulin.

Effects of actinomycin D

A lag phase of about 24 h suggested a requirement for protein synthesis for restoration of insulin-responsiveness in tissue from lactating sheep. Addition of actinomycin D, an inhibitor of transcription, had no significant effect on the small increase in the rate of fatty acid synthesis over the first 24 h of incubation (Fig. 1b), but it prevented completely the subsequent major increase in rate between 24 and 48 h of culture ($P < 0.001$), clearly indicating that restoration of insulin-responsiveness was dependent on transcription. However, addition of actinomycin D after 24 h (i.e. at the end of the lag phase) also prevented the increase ($P < 0.05$) in the rate of fatty acid synthesis over the next 24 h (Table 1). This shows that transcription is required during the period of rapid increase in rate. During the lag phase there may be accumulation of some essential component required for stimulation of transcription during the second 24 h period. An alternative possibility is that some inhibitory component is lost during the initial 24 h period; once gone, the rate of fatty acid synthesis can then increase. This increase is clearly dependent on transcription, and may reflect synthesis of enzymes (acetyl-CoA carboxylase activity falls to a very low value in sheep adipose tissue during lactation; Vernon *et al.*, 1987) and/or some component of the signal-transduction system.

Addition of actinomycin D also inhibited ($P < 0.05$) the rise in rate of fatty acid synthesis induced by insulin plus dexamethasone in adipose tissue from non-lactating sheep, but in this state an effect was apparent over 48 h (Table 1) and over the initial 24 h period ($P < 0.05$). The rate after 24 h was 10.8 ± 3.3 μmol of acetate incorporated/h per 10^7 cell units in the absence of exogenous hormone, 16.7 ± 4.0 in the presence of insulin plus dexamethasone, and 12.2 ± 3.3 in the presence of insulin, dexamethasone plus actinomycin D (results are means $\pm$ S.E.M. for four observations; concentrations as in Fig. 1 and Table 1). Addition of actinomycin D after 24 h prevented ($P < 0.01$) a further rise in the rate of fatty acid synthesis in the presence of insulin plus dexamethasone, but did not result in a decrease in the increased rate achieved over the first 24 h period (see above results for 24 h, and Table 1). In contrast, if actinomycin D was added at zero time, then there was a decrease ($P < 0.05$) in the rate of fatty acid synthesis between 24 and 48 h (above results, and Table 1). These observations suggest that the postulated inhibitory component present in lactating sheep is (effectively) absent in non-lactating sheep, hence effects of the hormone are seen during the initial 24 h period. Again, the increase in fatty acid synthesis induced by insulin plus dexamethasone is dependent on transcription, but an increased rate, once achieved, can be sustained for at least 24 h in the presence of actinomycin D.

Effects of growth hormone

Addition of growth hormone to the culture medium prevented the increase in fatty acid synthesis induced by the addition of insulin alone ($P < 0.05$) or insulin plus dexamethasone ($P < 0.001$) in adipose tissue from both non-lactating and lactating sheep (Figs. 1 and 2; Table 1). A similar inhibitory effect of growth hormone has been found in non-lactating pigs (Walton *et al.*, 1986) and cattle (Etherton *et al.*, 1987). Growth hormone alone had an inhibitory effect on fatty acid synthesis in adipose tissue from non-lactating sheep, and exacerbated

Table 1. Effects of tissue culture for 48 h in the presence of hormones and other agents on the rate of fatty acid synthesis, glucose uptake and glycerol release by adipose-tissue pieces from non-lactating and lactating sheep

Pieces of adipose tissue from non-lactating and lactating (16–24 days *post partum*) sheep were incubated in Medium 199 supplemented as described in the text and with various combinations of insulin (17 nM), dexamethasone (10 nM), growth hormone (4.5 nM), actinomycin D (80 nM) and 3-methylisobutylxanthine (0.5 mM) for 48 h, after which the tissue was transferred to fresh Medium 199 containing insulin (17 nM) and the rate of fatty acid synthesis was measured as described in the text. Samples of culture medium were analysed for glucose and glycerol: *growth hormone or actinomycin D added after 24 h of culture (i.e. for 24–48 h of culture). Results are expressed as means $\pm$ s.e.m., with the numbers of observations in parentheses. Results were analysed by Student's *t* test for paired observations: †, ††, †††, value significantly different ($P < 0.05$, < 0.01 , < 0.001 respectively) from that obtained after culture in the absence of added hormones; ‡, ‡‡, ‡‡‡, value significantly different ($P < 0.05$, < 0.01 , < 0.001 respectively) from that obtained after tissue culture with insulin plus dexamethasone. Other details are given in the text. Rates of fatty acid synthesis of tissue before tissue culture were 8.3 ± 0.14 (12) and 0.18 ± 0.04 (13) μ mol of acetate incorporated/h per 10^7 cells for adipose tissue from non-lactating and lactating sheep respectively.

Additions to culture medium	State...	Rate of fatty acid synthesis (μ mol acetate incorporated/h per 10^7 cell units)		Glucose utilization (μ mol/48 h per 10^7 cell units)		Glycerol release (μ mol/48 h per 10^7 cell units)	
		Non-lactating	Lactating	Non-lactating	Lactating	Non-lactating	Lactating
None		5.8 ± 0.9 (12)†††	0.4 ± 0.1 (13)†††	155 ± 19 (11)†††	115 ± 12 (13)†††	50 ± 7 (11)†††	21 ± 2 (13)†††
Insulin		11.6 ± 1.9 (11)††	2.9 ± 0.8 (9)††	364 ± 32 (11)†††††	223 ± 20 (9)††††	55 ± 10 (10)††	25 ± 3 (9)††
Dexamethasone		2.2 ± 0.5 (10)†††††	0.2 ± 0.0 (6)††††	25 ± 8 (9)†††††	23 ± 4 (6)†††††	21 ± 4 (9)††	8 ± 1 (6)††
Growth hormone		2.4 ± 0.4 (7)††††	0.1 ± 0.0 (3)††	134 ± 14 (7)†††	88 ± 19 (3)†	40 ± 8 (7)††	30 ± 6 (3)
Insulin + dexamethasone		18.8 ± 1.6 (12)†††	4.0 ± 0.6 (12)†††	273 ± 32 (11)†††	174 ± 9 (12)†††	21 ± 4 (11)†††	11 ± 1 (13)†††
Insulin + growth hormone		5.5 ± 0.6 (6)††	0.3 ± 0.1 (3)†	254 ± 31 (6)††	166 ± 13 (3)†	43 ± 8 (5)†††	36 ± 9 (3)
Dexamethasone + growth hormone		1.0 ± 0.2 (7)†††††	—	42 ± 9 (5)†††	—	32 ± 5 (6)††	—
Insulin + dexamethasone + growth hormone		3.4 ± 0.6 (12)†††††	0.3 ± 0.1 (9)†††	214 ± 24 (11)††	89 ± 8 (9)†††	47 ± 10 (11)††	16 ± 3 (9)†††
Insulin + dexamethasone + growth hormone*		4.1 ± 1.3 (5)†††	0.3 ± 0.0 (3)†	223 ± 32 (5)††	142 ± 8 (3)	37 ± 7 (5)†††	15 ± 2 (3)
Insulin + dexamethasone + actinomycin D		7.6 ± 2.1 (4)††	0.1 ± 0.0 (4)†††	161 ± 19 (4)†	75 ± 15 (4)††	45 ± 12 (4)†	11 ± 2 (4)††
Insulin + dexamethasone + actinomycin D*		14.2 ± 4.2 (4)††	0.6 ± 0.0 (4)††	322 ± 12 (4)†	128 ± 17 (4)††	34 ± 7 (4)†	8 ± 2 (4)
Insulin + dexamethasone + 3-methylisobutylxanthine		2.1 ± 0.9 (4)††	0.4 ± 0.0 (2)	333 ± 52 (4)††	289 ± 36 (2)	85 ± 22 (4)††	50 ± 7 (2)

($P < 0.05$) the inhibitory effects of dexamethasone (Table 1). By 48 h of culture, growth hormone in the presence of insulin and dexamethasone not only prevented the increase induced by the last two agents, but decreased the rate ($P < 0.01$) below that found in the absence of hormones in adipose tissue from non-lactating sheep. This effect was also seen if growth hormone was added after 24 h of culture in the presence of insulin plus dexamethasone ($P < 0.05$) (Table 1). Similarly, addition of growth hormone after 24 h blocked the increase in the rate of fatty acid synthesis induced in adipose tissue from lactating sheep ($P < 0.05$).

Dose-response curves for growth hormone in the presence of insulin (17 nM) plus dexamethasone (10 nM) were essentially identical for tissue from both lactating and non-lactating sheep (results not shown), and gave an ED_{50} of 170 pM. In our non-lactating sheep, serum growth-hormone concentration is about 130 pM (Vernon *et al.*, 1981, 1986), but during lactation this increased to over 700 pM. Thus the increase in serum growth hormone during lactation is probably a major cause of the decline in fatty acid synthesis in adipose tissue in sheep. Furthermore, the serum growth-hormone concentration in non-lactating sheep is still sufficient to have a small inhibitory effect on fatty acid synthesis; the increase in rate seen in tissue from such animals during tissue culture may thus be at least partly due to relief from inhibition by growth hormone.

The effects of growth hormone are very similar to those of actinomycin D, suggesting that growth hormone impairs the synthesis of key proteins required for the increase in fatty acid synthesis induced by insulin plus dexamethasone. In non-lactating sheep, however, exposure to actinomycin D for 48 h prevented the effects of insulin plus dexamethasone, whereas addition of growth hormone decreased the rate below that of control tissue (incubated in the absence of hormones). This difference in effect was also found when actinomycin D or growth hormone was added after 24 h in the presence of insulin plus dexamethasone. Thus, not only may growth hormone antagonize the synthesis of key proteins required to increase the rate of fatty acid synthesis, but it may also promote the production of the putative inhibitory component, the disappearance of which is a necessary prelude to the induction of fatty acid synthesis in tissue from lactating sheep.

Effects of 3-methylisobutylxanthine

Addition of 3-methylisobutylxanthine (0.5 mM) mimicked the effect of growth hormone in inhibiting the effects of insulin plus dexamethasone on fatty acid synthesis in adipose tissue from both lactating and non-lactating sheep (Table 1). 3-Methylisobutylxanthine is an inhibitor of cyclic AMP phosphodiesterase and an antagonist of adenosine, and so is likely to increase cyclic AMP and hence protein kinase A activity in the tissue. This was reflected by increased ($P < 0.05$) glycerol release (Table 1) in adipose tissue from both lactating and non-lactating sheep (analysis of pooled results for both states). Growth hormone also increased glycerol release ($P < 0.01$) in the presence of insulin and dexamethasone (Table 1). In the absence of dexamethasone, growth hormone had no effect on glycerol release, as observed previously during prolonged incubation with sheep adipose tissue (Vernon, 1982) and with adipose tissue from several other species, including rats (Maloff *et al.*,

1980), man (Nyberg & Smith, 1977), pigs (Walton *et al.*, 1986) and cattle (Etherton *et al.*, 1987). A requirement for a glucocorticoid for a lipolytic effect of growth hormone *in vitro* has been reported for rat adipocytes (Fain *et al.*, 1965). Growth hormone in the presence or absence of dexamethasone had no lipolytic effect on sheep adipose tissue during a 4 h incubation (Duquette *et al.*, 1984), showing that prolonged incubation is required for the effect to be manifest. However, the stimulatory effect of growth hormone on glycerol release in sheep adipose tissue was small and was significantly less ($P < 0.001$) than that induced by 3-methylisobutylxanthine (Table 1). The effects of this latter agent also differed from those of growth hormone in that it induced an increase in glucose utilization in adipose tissue from both lactating and non-lactating sheep ($P < 0.05$, analysis of pooled results for both states) in the presence of insulin plus dexamethasone; this additional glucose utilization was almost all due to increased lactate production (results not shown). In contrast, growth hormone in the presence of insulin and dexamethasone either had no significant effect on glucose utilization (in non-lactating sheep) or caused inhibition ($P < 0.001$) (in lactating sheep). These observations suggest that growth hormone and 3-methylisobutylxanthine exert their inhibitory effects on fatty acid synthesis by different mechanisms.

Effects of other hormones

The effect of insulin on fatty acid synthesis in adipose tissue from lactating sheep was not mimicked by insulin-like growth-factor-1; this was found in two experiments firstly using 0.65 nM- and secondly 6.5 nM-insulin-like growth factor-1 in the presence or absence of dexamethasone (10 nM). This is in accord with the absence of insulin-like growth factor-1 receptors on adipocytes, at least in rats (Massague & Czech, 1982), and the conclusion that effects of high concentrations of insulin-like growth factor-1 on fatty acid synthesis in bovine adipose tissue were due to it acting via the insulin receptor (Etherton & Evoke, 1986). Prolactin at 4.2 nM did not mimic the action of growth hormone on the stimulation of fatty acid synthesis by insulin plus dexamethasone in adipose tissue from lactating or non-lactating sheep. A further experiment using 20 nM-prolactin with adipose tissue from lactating sheep produced a 10% inhibition of insulin + dexamethasone-induced fatty acid synthesis, compared with a 96% inhibition achieved with 4.5 nM-growth hormone. This is in agreement with our previous conclusions, derived from circumstantial evidence (see the Introduction), that increased serum prolactin is not responsible for the fall in fatty acid synthesis in adipose tissue during lactation or sheep. Etherton *et al.* (1987) found no effects of prolactin on adipose tissue from non-lactating cattle, either. Progesterone (10 nM) did not mimic the effect of dexamethasone on insulin stimulation of fatty acid synthesis.

General discussion

A peculiar and unexplained difference between adipose tissue from ruminants and rodents is that the former does not respond reproducibly to insulin during short-term incubations (1–3 h), and the response when seen is usually small (< 50% increase) (see Vernon, 1980). In contrast, incubation of adipose tissue from sheep and cattle with insulin for 24–48 h results in a reproducible response (> 50% increase), and with physiological con-

centrations of hormone (e.g. Vernon, 1982; Vernon *et al.*, 1985; Etherton *et al.*, 1987; Plested *et al.*, 1987a). Curiously, both stimulation of glucose utilization (Plested *et al.*, 1987a) and fatty acid synthesis (the present work) by insulin during prolonged incubations are inhibited by actinomycin D. This suggests there may be a labile protein required for insulin action in ruminant tissue which is lost during preparation of tissue for studies *in vitro*. An alternative view, that insulin stimulation of adipose-tissue metabolism is normally suppressed *in vivo* and is expressed only *in vitro*, seems unlikely (see Vernon, 1980). The time course of changes in fatty acid synthesis in adipose tissue of sheep and other ruminants during the lactation cycle has not been documented in great detail, but it is clear that changes take place gradually over a number of weeks (Chilliard *et al.*, 1978; Vernon *et al.*, 1981; Smith & Walsh, 1984; McNamara & Hillers, 1986). A decrease in serum insulin from about 0.2 nM to 0.1 nM and an increase in serum growth hormone from 0.1 nM to 0.7 nM, as found in our sheep with lactation (Vernon *et al.*, 1981, 1986), are together sufficient to decrease the rate of fatty acid synthesis from about 7 to 2.5 $\mu\text{mol/h}$ per 10^7 cell units (Fig. 2) within 48 h; at such a rate of decrease, the rate found during a peak lactation could be achieved in less than 1 week. Thus changes in serum insulin and growth-hormone concentrations (which also occur gradually during the lactation cycle in ruminants) can well explain the change in the rate of fatty acid synthesis in adipose tissue seen during the lactation cycle in these species.

It also appears that the adipose tissue loses its ability to respond to insulin temporarily during lactation in sheep. Hence transient surges of insulin (e.g. after a meal) should not alter the rate of fatty acid synthesis during lactation, and a prolonged increase in serum insulin would appear to be necessary (along with a fall in serum growth hormone concentration) to induce a restoration of fatty acid synthesis. A diminished ability of insulin to stimulate fatty acid synthesis has also been observed in adipose tissue from lactating rats (Burnol *et al.*, 1983, 1986), but in rats the impairment is less severe than in sheep. The nature of the mechanism responsible is unknown in either species, but in the rat there is evidence for a failure to release a putative insulin second messenger from the adipocyte plasma membrane during lactation (Kilgour & Vernon, 1988). For sheep we postulate the presence of an inhibitory factor during lactation which disappears during the first 24 h of tissue culture. Alternatively there may be synthesis of components during the first 24 h of culture which are necessary for the synthesis of the actinomycin D-sensitive component (and hence increase in fatty acid synthesis) during the second 24 h. Identification of these factors should not only clarify the mechanisms operating to modulate insulin action during lactation, but should also provide further insight into the mechanisms of action of insulin itself.

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REFERENCES

- Agius, L., Robinson, A. M., Girard, J. R. & Williamson, D. H. (1979) *Biochem. J.* **180**, 689–692
- Burnol, A.-F., Leturque, A., Ferré, P. & Girard, J. R. (1983) *Am. J. Physiol.* **245**, E351–E358
- Burnol, A.-F., Guerre-Millo, M., Lavau, M. & Girard, J. R. (1986) *FEBS Lett.* **194**, 292–296
- Chilliard, Y., Durand, G., Sauvant, D. & Morand-Fehr, P. (1978) *C. R. Seances Acad. Sci. Ser. D* **287**, 1131–1134
- Duquette, P. F., Scanes, C. G. & Muir, L. A. (1984) *J. Anim. Sci.* **58**, 1191–1197
- Etherton, T. D. & Evock, C. M. (1986) *J. Anim. Sci.* **62**, 357–362
- Etherton, T. D., Evock, C. M. & Kensinger, R. S. (1987) *Endocrinology (Baltimore)* **121**, 699–703
- Fain, J. N. (1979) in *Glucocorticoid Hormone Action* (Baxter, J. D. & Rousseau, G. G., eds.), pp. 547–560, Springer-Verlag, Berlin
- Fain, J. N., Kovacev, V. P. & Scow, R. O. (1965) *J. Biol. Chem.* **240**, 3522–3529
- Flint, D. J., Clegg, R. A. & Vernon, R. G. (1981) *Mol. Cell. Endocrinol.* **22**, 265–275
- Forsyth, I. A. (1986) *J. Dairy Sci.* **69**, 886–903
- Hallowes, R. C., Wang, D. Y., Lewis, D. J., Strong, C. R. & Dils, R. (1973) *J. Endocrinol.* **57**, 265–276
- Kilgour, E. & Vernon, R. G. (1988) *Biochem. J.* **252**, 667–672
- Massague, T. & Czech, M. P. (1982) *J. Biol. Chem.* **257**, 5038–5045
- Maloff, B. L., Levine, J. H. & Lockwood, D. H. (1980) *Endocrinology (Baltimore)* **107**, 538–554
- McNamara, J. P. & Hillers, J. K. (1986) *J. Lipid Res.* **27**, 150–157
- Minshall, M. & Strong, C. R. (1985) *Int. J. Biochem.* **17**, 529–532
- Nyberg, G. & Smith, U. (1977) *Horm. Metab. Res.* **9**, 22–27
- Philips, G. R., Herrera, M. G. & Renold, A. E. (1965) *Biochim. Biophys. Acta* **106**, 234–240
- Plested, C. P., Taylor, E., Brindley, D. N. & Vernon, R. G. (1987a) *Biochem. J.* **247**, 459–465
- Plested, C. P., Finley, E., Brindley, D. N. & Vernon, R. G. (1987b) *Biochem. Soc. Trans.* **15**, 948
- Robertson, J. P., Faulkner, A. & Vernon, R. G. (1982) *Biochem. J.* **206**, 577–586
- Schalch, D. S. & Reichlin, S. (1966) *Endocrinology (Baltimore)* **79**, 275–280
- Smith, R. W. & Walsh, A. (1984) *Res. Vet. Sci.* **37**, 320–323
- Vernon, R. G. (1979) *Int. J. Biochem.* **10**, 57–60
- Vernon, R. G. (1980) *Prog. Lipid Res.* **19**, 23–106
- Vernon, R. G. (1982) *Int. J. Biochem.* **14**, 255–258
- Vernon, R. G. (1988) in *Nutrition and Lactation in the Dairy Cow* (Garnsworthy, P. C. ed.), pp. 32–52, Butterworths, London
- Vernon, R. G. & Flint, D. J. (1983) *Proc. Nutr. Soc.* **42**, 315–331
- Vernon, R. G. & Taylor, E. (1988) *Biochem. J.* **256**, 509–514
- Vernon, R. G., Clegg, R. A. & Flint, D. J. (1981) *Biochem. J.* **200**, 307–314
- Vernon, R. G., Finley, E., Taylor, E. & Flint, D. J. (1985) *Endocrinology (Baltimore)* **116**, 1195–1199
- Vernon, R. G., Clegg, R. A. & Flint, D. J. (1986) *Horm. Metab. Res.* **18**, 308–312
- Vernon, R. G., Faulkner, A., Finley, E., Pollock, H. & Taylor, E. (1987) *J. Anim. Sci.* **64**, 1395–1411
- Walton, P. E., Etherton, T. D. & Evock, C. M. (1986) *Endocrinology (Baltimore)* **118**, 2577–2581
- Williamson, D. H. (1980) *FEBS Lett.* **118**, K93–K105