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Lipolysis in rat adipocytes during recovery from lactation

Response to noradrenaline and adenosine

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The replenishment of lipid reserves of adipocytes following the removal of litters from lactating rats is associated with a 3-fold decrease in both the response of lipolysis to noradrenaline and the maximum rate of lipolysis (measured in the presence of noradrenaline plus adenosine deaminase); these adaptations do not appear to result from the changes in serum prolactin and insulin concentrations that occur on litter removal.

INTRODUCTION

Lactation in rats and in a variety of other mammals is usually associated with the mobilization of adipose-tissue lipid owing to the demand for nutrients for milk production exceeding the ability of the animal to increase its food intake (see Bauman & Elliot, 1983; Vernon & Flint, 1984). There is some lipid accumulation during pregnancy in rats, but this does not normally meet the demands during lactation, when the animal may lose more than half of its reserves of adipose-tissue lipid (see Vernon & Flint, 1984; Moore & Brasel, 1984). Lipid lost during lactation is replaced on removal of the young, so reproductively active female animals undergo cycles of lipid loss and accumulation (Johnson, 1973).

Removal of the young results in a rise in the activity of lipoprotein lipase (Hamosh *et al.*, 1970; Scow *et al.*, 1977; Flint *et al.*, 1981) and the rate of fatty acid synthesis (see Vernon & Flint, 1983) in adipose tissue of rats. In addition, there is a rise in the number of insulin receptors (Flint *et al.*, 1981) and the response of adipocytes to insulin (R. G. Vernon & E. Taylor, unpublished work). The factors responsible for these adaptations have not been fully resolved, but an increase in serum insulin (Agius *et al.*, 1979; Burnol *et al.*, 1983) and a decrease in serum prolactin (Amenomori *et al.*, 1970; Agius *et al.*, 1979; Flint *et al.*, 1981), which accompany litter removal, are probably involved.

In the present study we show that the increased ability of adipocytes to synthesize lipid on litter removal is augmented by a transient decrease in their lipolytic capacity.

EXPERIMENTAL

Wistar rats (A. Tuck and Son, Rayleigh, Essex, U.K.) were fed on Labsure irradiated CRM diet (Labsure, Poole, Dorset, U.K.) and water *ad libitum*. They were mated at 2–3 months of age, and the number of pups per mother was adjusted to eight within 24 h after birth. Rats were killed at 12–16 days of pregnancy or 12–16 days of lactation; litters were removed at 12–16 days of lactation also. Rats injected with bromocriptine (a gift from Sandoz Ltd.) received 500 μg of the drug twice daily by subcutaneous injection for 2 days (Flint *et al.*, 1981) and were then killed on day 3; injections were begun at 12–16 days of lactation. Virgin, pregnant and lactating rats were all at a similar age at slaughter. All experiments were performed from September through to December.

Rats were killed at about 10:00 h by cervical dislocation. The parametrial fat-pads were removed, and adipocytes were prepared and their size and number determined as described previously (Aitchison *et al.*, 1982). The rate of lipolysis (glycerol release) of isolated adipocytes was determined as described previously in Medium 199 containing Earle's salts, L-glutamine and 25 mM-Hepes, pH 7.3, and 4% (w/v) essentially fatty-acid-free bovine serum albumin as incubation medium (Aitchison *et al.*, 1982). Albumin and adenosine deaminase were dialysed before use as described previously (Vernon *et al.*, 1983).

RESULTS

Maximum rates of lipolysis were determined by measuring the rate of glycerol release in the presence of 100 μM-noradrenaline plus 0.8 μg of adenosine deaminase/ml (to prevent the accumulation of adenosine in the medium); addition of 2 mM-theophylline to this combination resulted in no further increase in the rate of lipolysis in any of the states investigated (results not shown). As indicated in Table 1, pregnancy and lactation did not alter the maximum rate of lipolysis. Removal of the litter for 2 days from lactating rats, however, resulted in a significant ($P < 0.01$) fall in the maximum rate of lipolysis, which returned to the value found in lactating and also virgin control rats by 7 days after litter removal. Administration of bromocriptine for 2 days to lactating rats had no effect on the maximum rate of lipolysis.

The rate of lipolysis in the presence of noradrenaline alone was significantly ($P < 0.01$) lower in adipocytes from lactating than from virgin or pregnant rats (Table 1). Litter removal for 2 days resulted in a further significant ($P < 0.02$) fall in the rate of lipolysis in the presence of noradrenaline alone, which had returned to that observed in lactating rats by 7 days after litter removal. However, litter removal for 2 days had no effect

Abbreviation used: PIA, N^6-phenylisopropyladenosine.

Table 1. Effects of lactation and of litter removal or bromocriptine treatment of lactating rats on the rates of lipolysis of parametrial adipocytes

Parametrial adipocytes were prepared from virgin rats, from rats at 12–16 days of pregnancy or lactation, or from lactating rats after their litters had been removed for 2 or 7 days, or which had retained their litters but had been treated with bromocriptine. The amount of glycerol release over 1 h in the presence of various additions was determined as described in the text. Flasks contained about 10^5 cells/ml, and, when added, 100 μM-noradrenaline, 0.8 μg of adenosine deaminase/ml or 100 nM-PIA. Results are means ± S.E.M. for four to six observations; values in parentheses are results expressed as a percentage of those obtained in the presence of noradrenaline plus adenosine deaminase.

State	Additions to medium...	Glycerol released (nmol/h per 10^5 cells)			
		None	Noradrenaline	Noradrenaline, adenosine deaminase	Noradrenaline, adenosine deaminase, PIA
Virgin		19 ± 4 (2.6 ± 0.2)	601 ± 122 (78.1 ± 3.6)	771 ± 159 (100)	610 ± 112 (79.5 ± 3.6)
Pregnant		12 ± 4 (1.4 ± 0.5)	532 ± 124 (73.4 ± 6.9)	704 ± 124 (100)	538 ± 122 (74.1 ± 5.8)
Lactating		20 ± 4 (2.4 ± 0.6	193 ± 38 (25.8 ± 4.3)	783 ± 120 (100)	208 ± 43 (27.2 ± 4.4)
Lactating, litter removed 2 days		14 ± 4 (7.4 ± 4.0)	66 ± 8 (24.7 ± 2.4)	283 ± 47 (100)	89 ± 12 (33.8 ± 4.0)
Lactating, litter removed 7 days		17 ± 4 (2.6 ± 0.4)	244 ± 40 (37.0 ± 3.3)	676 ± 124 (100)	254 ± 49 (38 ± 3.1)
Lactating, given bromocriptine for 2 days		17 ± 2 (2.9 ± 0.4)	237 ± 26 (33.2 ± 0.4)	626 ± 70 (100)	280 ± 41 (40.1 ± 4.8)

on the dose–response curve for noradrenaline (results not shown), which was identical with that found previously for both pregnant and lactating rats (Aitchison *et al.*, 1982): in all three states a concentration of noradrenaline of about 0.5 μM was required to elicit half-maximum stimulation of glycerol release. Treatment of lactating rats with bromocriptine for 2 days had no significant effect on the rate of noradrenaline-stimulated lipolysis as such, but, when results were expressed as a percentage of the maximum rate, then bromocriptine treatment did result in a small, but significant ($P < 0.05$), increase (Table 1).

Differences between the rate of lipolysis measured in the presence of noradrenaline alone and in the presence of noradrenaline plus adenosine deaminase in virgin, pregnant and lactating rats appear to be due to a greater responsiveness of adipocytes from lactating rats to the local anti-lipolytic agent, adenosine (Vernon *et al.*, 1983). The response to adenosine can be assessed by removing endogenous adenosine from the medium with adenosine deaminase and adding an analogue, PIA, which is not degraded by the deaminase. Addition of 100 nM-PIA has a maximum effect in the system used (Vernon *et al.*, 1983); it decreased the maximum rate of lipolysis by 161 ± 48, 166 ± 32 and 571 ± 105 nmol/h per 10^5 cells for adipocytes from virgin, pregnant and lactating rats respectively: the value for lactating rats is significantly greater ($P < 0.01$) than those for virgin and pregnant rats. Litter removal for 2 days decreased the response to PIA to 193 ± 39 nmol/h per 10^5 cells, a value significantly ($P < 0.01$) lower than that for lactating rats. However, when the decrease caused by addition of PIA is expressed as a percentage of maximal lipolytic activity, the percentage response to PIA was the same in lactating rats and rats 2 days after litter removal. By 7 days after litter removal or after 2 days of bromocriptine treatment of lactating rats, the decrease in lipolytic rate caused by PIA was 422 ± 78 and 345 ± 63 nmol/h per 10^5 cells respectively, values intermediate between those of lactating rats and virgin rats: the response, as a percentage of maximum lipolytic rate, was also intermediate between values for lactating rats and those for virgin or pregnant rats (see Table 1).

DISCUSSION

As described above, removal of the litter from lactating rats results in a rapid (within 24 h) increase in the capacity of adipocytes for lipid synthesis. The present study shows that this is augmented by a transient 3-fold fall in the lipolytic capacity of the tissue.

The mean cell volume of adipocytes of rats used in the present experiment was 276 ± 51 pl for the lactating rats, and increased significantly ($P < 0.05$) by about 70% to 458 ± 60 pl by 7 days after litter removal, whereas 2 days after litter removal the mean cell volume was intermediate (376 ± 45 pl). In a previous study in which changes in lipogenic capacity were measured on litter removal, no evidence was found for any change in adipocyte mean cell volume by 24 h after litter removal. However, as in the present study, there was some evidence for an increase in cell volume and hence net lipid deposition by 2 days after litter removal (Flint *et al.*, 1981), i.e. when there is both an increased capacity for lipid synthesis and a decreased capacity for lipolysis. The female rat thus has a very effective mechanism for replenishing fat reserves lost during lactation; this is likely to be of considerable importance in the wild, for the female rat would probably be pregnant again within a few days after loss of a litter. In addition, the first lactation may have lasting effects,

which benefit subsequent pregnancies, for, although the lipolytic capacity had returned to normal control values by 7 days after litter removal, the increased response to the anti-lipolytic effects of adenosine had still not returned to control values. Similarly, in mice, although the suppression of the capacity for non-shivering thermogenesis observed during lactation returned to control values by 7 days after litter removal (Trayhurn, 1983), the amount of the GDP-binding protein of brown-adipocyte mitochondria was still low at 3 weeks after litter removal (Trayhurn & Richard, 1985). These various observations suggest that lipid accumulation should be facilitated during a second and subsequent pregnancies, as has been observed in mice (Johnson, 1973).

The factors responsible for the fall in lipolytic capacity on litter removal are uncertain. The main lactogenic hormone in the rat is prolactin: the bromocriptine treatment used in the present study was shown previously to decrease serum prolactin concentrations to the low values found on litter removal, and also mimicked the effect of litter removal on fatty acid synthesis and lipoprotein lipase activity of adipocytes (Flint *et al.*, 1981): similar effects were also observed by Agius *et al.* (1979). The bromocriptine treatment used in the present experiment again greatly decreased serum prolactin concentration and retarded litter growth (results not shown), but clearly failed to elicit the fall in lipolytic capacity. Litter removal and bromocriptine treatment both result in a similar rise in serum insulin concentration (Agius *et al.*, 1979; Flint *et al.*, 1981). Thus changes in serum prolactin and insulin by themselves are insufficient to account for the change in lipolytic capacity. Furthermore, it would seem unlikely that the changes in the serum concentrations of these hormones are responsible for the increased response of adipose tissue to adenosine during lactation (Vernon *et al.*, 1983).

Lactation followed by litter removal resembles starvation followed by re-feeding in that both involve a loss of lipid reserves followed by replenishment, and both involve similar changes in lipogenic capacity of adipose tissue. In contrast, there appear to be distinct differences in the modifications to lipolysis. Most studies suggest that there is no change in the lipolytic response to catecholamines of adipose tissue on starvation; rather there is an increase in sensitivity to such agents (Zapf *et al.*, 1977, 1981; Dax *et al.*, 1981; Chohan *et al.*, 1984), probably owing to a decrease in response to adenosine (Chohan *et al.*, 1984). However, another study has reported a decreased response to catecholamines on starvation (Giudicelli *et al.*, 1982), but the reason for this discrepancy is not clear. Re-feeding of starved rats has no apparent effect on the lipolytic response to catecholamines of adipose tissue, but it did restore the normal sensitivity to noradrenaline (Zapf *et al.*, 1977, 1981). The reason why the two situations evoke such different responses in the lipolytic system is not obvious, but the lactating rat, once rid of her litter, appears to have a much more potent system for replenishing her lipid reserves than does the starved rat when allowed access to food.

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