The effect of starvation and refeeding on lipogenic enzymes in mammary glands and livers of lactating rats

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1. Lactating rats were starved for 48 h and refed a high-carbohydrate diet for a further 48 h. 2. Starvation stops milk secretion, which resumes shortly after refeeding. 3. Three lipogenic enzymes, fatty acid synthase, glucose 6-phosphate dehydrogenase (EC 1.1.1.49) and 'malic' enzyme (EC 1.1.1.40) all decrease in the mammary gland during starvation and are restored to the pre-starvation levels 48 h after refeeding. 4. The same enzymes in liver also decrease during starvation, but increase to values significantly higher than those for the normal fed rats after refeeding the high-carbohydrate diet. For the fatty acid synthase these values were four times the pre-starvation values. 5. Serum insulin and prolactin concentrations also increased upon refeeding the high-carbohydrate diet.

We have been studying the factors regulating the activities of three lipogenic enzymes in the mammary glands and livers of lactating rats. Recently we reported that fatty acid synthase, glucose 6-phosphate dehydrogenase and 'malic' enzyme are all suppressed in the livers of lactating rats by feeding high-fat diets. In contrast, in the mammary gland only 'malic' enzyme is reduced by fat-feeding. All three enzymes were, however, affected in the mammary gland by changes in the litter size. This treatment had no effect on the liver enzymes (Grigor *et al.*, 1982). For the fatty acid synthase and 'malic' enzyme the changes in activity correspond to changes in the amounts of immunoprecipitable enzyme protein (Grigor *et al.*, 1982; Grigor, 1983). These results, along with those of Smith *et al.* (1969) for mice, and Farid *et al.* (1978) for older lactating rats at least, would suggest that the levels of these lipogenic enzymes in the mammary gland are relatively insensitive to dietary manipulation, although the absolute rates of lipogenesis as determined by incorporation of $^3\text{H}_2\text{O}$ into lipids certainly are suppressed by fat-feeding or starvation (Robinson *et al.*, 1978; Romsos *et al.*, 1978; Grigor & Warren, 1980; Agius & Williamson, 1980).

Here we describe the effect of starving lactating rats for up to 48 h and refeeding a high-carbohydrate diet. The results show that starving for 48 h stops milk production and is accompanied by decreases in the three lipogenic enzymes in both the mammary gland and liver. However, upon refeeding, milk production resumes along with synthesis of the lipo-

genic enzymes in both the mammary glands and livers of lactating rats.

Experimental

Rats of the Wistar strain were purchased from the University of Otago Animal Breeding Station and mated as required. Litter sizes were standardized at ten within 24 h of parturition. Lactating rats were fed *ad libitum* a commercial pelleted diet (Grigor & Warren, 1980). Around day 12 *post partum* food was removed for 48 h, after which the rats were refed a high-carbohydrate diet comprising 30% sucrose, 41% starch, 20% casein, 1% vitamin mix (Goulding & Malthus, 1969) and 4% salt mix (Hubble *et al.*, 1937) by weight.

The abdominal mammary glands and livers were removed, homogenized and the 100 000 $\times$ 60 min supernatants prepared as described previously (Grigor *et al.*, 1982). Fatty acid synthase, glucose 6-phosphate dehydrogenase and 'malic' enzyme activities were determined spectrophotometrically (Grigor *et al.*, 1982) and the amounts of fatty acid synthase and 'malic' enzyme protein were determined immunochemically using single radial immunodiffusion (Mancini *et al.*, 1965; Grigor, 1983). The total soluble protein in the mammary supernatants was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as the standard.

Blood samples were also taken by cardiac puncture at death, and the serum insulin and

prolactin levels were measured by radioimmunoassay. For insulin assays, rat insulin was used as a standard (Watts *et al.*, 1976). Prolactin was assayed using rat prolactin RP-3 as standard and rabbit anti-(rat prolactin) serum-8, both supplied by the National Pituitary Agency, Baltimore, MD, U.S.A. Poly(ethylene glycol) was used to precipitate the bound prolactin.

Results and discussion

Starvation for 48 h resulted in a decrease in maternal live weights of 66 ± 8 g (mean $\pm$ s.d.). At the same time the litter weights had decreased by 17 ± 7 g, indicating that milk production had ceased. This was apparent also from the reduction of mammary-gland mass (Table 1) and in the absence of milk in the dissected glands. By 24 and 48 h after refeeding the litter weights had increased by 10 ± 5 g and 26 ± 6 g respectively. These values compare with the normal live weight gain of litters of ten pups of rats in our colony of 15.6 ± 0.5 g per day (mean for eight litters). These data show that milk production resumes shortly after refeeding and that during the second 24 h period the rate of milk production was similar to that normally found for rats when the mothers are fed *ad libitum*.

Table 1 also shows the activities and amounts of the three lipogenic enzymes in the mammary homogenates. The data are expressed in four different ways: enzyme units/gland, units/g wet wt., mg of immunoprecipitable enzyme/gland and units/mg of soluble protein. Although only the abdominal glands were sampled, the activity/gland and amount of enzyme protein/gland probably represent the

most accurate indicators of enzyme turnover and synthesis. This is because of the marked changes in gland mass as a result of the complete removal of milk from the gland during the starvation period. Clearly, all three enzymes decrease during starvation and are restored after refeeding. The fatty acid synthase shows the greatest change, decreasing to some 30% on starvation.

Similar results were observed for the liver enzymes of both lactating and virgin animals (Table 2). Again there were marked changes in the liver mass during starvation and refeeding and the activities and amounts of enzyme protein/liver will represent the best basis for assessing the differences. In contrast with the mammary enzymes, the level of all three enzymes in the livers was significantly higher after refeeding than observed for the control fed animals. Results similar to these for livers of non-lactating animals have been known for some years (see Romsos & Leveille, 1974, for references).

Table 3 lists the hormone levels in sera from the rats used in our experiments. Serum insulin concentrations were significantly lower in lactating rats than in non-lactating rats and these were not significantly reduced by starvation. However, upon refeeding, serum insulin levels increased 5-fold to values similar to those found in the fed and refed virgin rats. There was also a significant increase in serum prolactin values upon refeeding. In general, the values we found for serum prolactin levels are lower than those reported by Robinson *et al.* (1978) and were more in line with those of Amenomori *et al.* (1970) and Simpson *et al.* (1973). Animals in our experiments were at days 14–16 of lactation, and this is well past the period when peak prolactin

Table 1. *Effect of starvation and refeeding on mammary lipogenic enzymes*

Values listed are means $\pm$ s.d. for *n* rats. The *t*-test was used to determine the significance of differences from the values for control fed rats (*, $P < 0.05$; **, $P < 0.01$) or rats starved for 48 h ($\dagger\dagger$, $P < 0.01$).

		Fed	Starved for 24 h	Starved for 48 h	Starved for 48 h and refed for 48 h
	<i>n</i> ...	9	4	6	6
Lipogenic enzyme	Mass (g) ...	5.6 ± 0.5	3.5 ± 0.2**	3.1 ± 0.4**	6.2 ± 1.6††
Fatty acid synthase					
(units/gland)		11.3 ± 3.6	6.3 ± 0.7*	2.1 ± 0.3**	9.7 ± 3.3††
(units/g)		1.9 ± 0.6	1.8 ± 0.3	0.7 ± 0.03**	1.6 ± 0.3††
(mg/gland)		101 ± 32	61 ± 15*	34 ± 9**	90 ± 35††
(m-units/mg of soluble protein)		25 ± 14	22 ± 2	5 ± 2**	38 ± 15††
Glucose 6-phosphate dehydrogenase					
(units/gland)		163 ± 12	123 ± 28*	105 ± 21**	189 ± 40††
(units/g)		28 ± 6	35 ± 8	34 ± 5	31 ± 3
(units/mg of soluble protein)		0.41 ± 0.12	0.50 ± 0.10	0.26 ± 0.08	0.75 ± 0.32*††
‘Malic’ enzyme					
(units/gland)		29 ± 6	23 ± 6	18 ± 1**	29 ± 5††
(units/g)		5.2 ± 0.9	6.5 ± 1.8	5.8 ± 0.6	5.0 ± 1.6
(mg/gland)		3.7 ± 1.6	2.6 ± 0.5	2.1 ± 1.1*	5.8 ± 1.0*††
(m-units/mg of soluble protein)		85 ± 15	78 ± 26	45 ± 15	116 ± 48††

Table 2. *Effect of starvation and refeeding on liver lipogenic enzymes from lactating and non-lactating rats*
Values listed and significance are as for Table 1.

	<i>n</i>	Mass (g)	Lactating rats			Non-lactating rats		
			Fed	Starved for 24 h	Starved for 48 h	Fed	Starved for 48 h	Starved for 48 h and refed for 48 h
Lipogenic enzyme	...	...	9	4	6	4	3	5
Fatty acid synthase								
(units/liver)			15.1 ± 2.8	10.0 ± 1.0**	8.3 ± 0.3**	15.0 ± 1.2††	6.7 ± 0.3	10.6 ± 1.0**††
(units/g)			7.3 ± 2.6	4.1 ± 1.0*	1.8 ± 0.3**	29 ± 8**††	2.9 ± 0.2	16.8 ± 2**††
(mg/liver)			0.46 ± 0.17	0.41 ± 0.08	0.22 ± 0.04**	2.0 ± 0.5**††	0.44 ± 0.04	1.63 ± 0.28**††
(m-units/mg of soluble protein)			80 ± 36	29 ± 4*	18 ± 2**	115 ± 40††	28 ± 5	204 ± 40**††
Glucose 6-phosphate dehydrogenase			9.3 ± 5.6	8.8 ± 3.3	2.2 ± 0.5	45.5 ± 13.2	7.3 ± 0.6	34.5 ± 4.7**††
(units/liver)			134 ± 41	98 ± 15	60 ± 10**	189 ± 44**††	26 ± 2	97 ± 10**††
(units/g)			8.8 ± 2.0	9.7 ± 0.7	7.3 ± 1.1	12.7 ± 2.3**††	3.9 ± 0.4	9.2 ± 1.3**††
(units/mg of soluble protein)			0.15 ± 0.03	0.19 ± 0.03	0.08 ± 0.02**	0.30 ± 0.9**††	0.065 ± 0.007	0.198 ± 0.030**††
'Malic' enzyme								
(units/liver)			26 ± 10	11 ± 4*	11 ± 4*	39 ± 7**††		
(units/g)			1.7 ± 0.5	1.1 ± 0.3	1.5 ± 0.6	2.6 ± 0.4**††		
(mg/liver)			4.3 ± 1.5	3.2 ± 0.6	1.7 ± 0.2**	7.5 ± 1.1**††	6.9 ± 1.1	5.5 ± 0.7††
(m-units/mg of soluble protein)			19 ± 9	21 ± 8	4 ± 1	59 ± 16	2.8 ± 1.1**	

Table 3. *Effect of starvation and refeeding on serum hormone levels in lactating and non-lactating rats*
Values are means $\pm$ s.d. for the numbers of rats given in parentheses. Symbols for significance of differences are as for Table 1.

	Control fed	Starved for 24 h	Starved for 48 h	Starved for 48 h and refed for 48 h
Lactating-rat serum insulin (pM)	206 ± 139 (9)	198 ± 46 (3)	158 ± 43 (6)	705 ± 303 (6) ^{***††}
Serum prolactin (ng/ml)	37 ± 19 (9)	38 ± 39 (4)	30 ± 15 (6)	65 ± 20 (6) ^{†*}
Non-lactating-rat serum insulin (pM)	583 ± 207 (4)	—	102 ± 12 (3) [*]	616 ± 171 (4) ^{††}

concentrations are observed (Amenomori *et al.*, 1970; Simpson *et al.*, 1973).

From our experiments it is not possible to claim any causal relationship between the changes in concentration of either hormone and the rates of synthesis of the lipogenic enzymes at mid-lactation. In each case the change in hormone levels on starvation was very small considering that there appears to be a complete cessation of the synthesis of the enzymes. Certainly, both insulin and prolactin are required for the induction of these enzymes at the initiation of lactation (Oka & Perry, 1974; Speake *et al.*, 1976), although clearance of milk from the gland appears to be important for the induction of both acetyl-CoA carboxylase and ATP citrate-lyase (Martyn & Hansen, 1981).

At this stage it is not clear what is regulating the synthesis of these enzymes in the mammary gland either at the cellular level or at the molecular level. Experiments done with liver suggest that two forms of molecular control may be involved in the response to nutritional stimuli. These are changes in the concentrations of the mRNA species for the lipogenic enzymes (Nakanishi *et al.*, 1976; Flick *et al.*, 1977; Hutchison & Holten, 1978; Miksicek & Towle, 1982) and possibly changes in the translational efficiency of the mRNA species (Sun & Holten, 1978; Towle *et al.*, 1980; Schwartz & Abraham, 1983). The question as to which of these is important in the mammary gland has yet to be addressed and, as for the liver, will be best answered by hybridization studies with cloned complementary-DNA species of the genes for each enzyme. This approach has already been used in studies on the synthesis of fatty acid synthase and 'malic' enzyme in neonatal avian livers (Morris *et al.*, 1982; Winberry *et al.*, 1983). In both cases the increases in enzyme content correlated with the increases in the concentration of the appropriate mRNA species.

A further mechanism that may be important in regulating the changes in the activities of the lipogenic enzymes in response to starvation and refeeding involves changes in the rates of degradation of these enzymes. Wilde *et al.* (1980) have used mammary explants from mid-pregnant rabbits to show that the degradation of fatty acid synthase is regulated by the presence of lactogenic hormones such that it is suppressed during periods of rapid enzyme accumulation and activated when the hormones are removed.

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