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Alterations in the Rate of Lipogenesis *in vivo* in Maternal Liver and Adipose Tissue on Premature Weaning of Lactating Rats

A POSSIBLE REGULATORY ROLE OF PROLACTIN

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Removal of pups for 24h from rats at peak lactation decreased ^3H_2O incorporation into lipid *in vivo* in mammary gland by 95%, whereas it was increased in liver (77%) and adipose tissue (330%). These increases were prevented by administration of prolactin. Plasma insulin increased 3-fold on weaning and this was partially prevented by prolactin.

Experiments *in vitro* have shown that the rate of lipogenesis is higher in livers from lactating rats than in livers from virgin rats (Smith, 1973*a*; Benito & Williamson, 1978). At peak lactation (12–16 days) the incorporation of [2-^{14}C]acetate or ^3H_2O into lipid is increased in liver slices (Smith, 1973*a*) or isolated hepatocytes (Benito & Williamson, 1978) respectively. In contrast, measurements of the rate of lipogenesis *in vivo* with ^3H_2O showed no significant difference between virgin and lactating rats (Robinson *et al.*, 1978). This apparent anomaly could be explained if the higher lipogenic capacity of the liver was suppressed *in vivo* in lactating rats. The rate of lipogenesis in adipose tissue is decreased both *in vivo* (Robinson *et al.*, 1978) and *in vitro* (Smith, 1973*b*) during lactation. A possible candidate for the regulation of lipogenesis in liver and adipose tissue of lactating rats is prolactin, especially as its circulating concentration is related to the suckling stimulus. Prolactin has been implicated in the suppression of lipoprotein lipase activity in adipose tissue during lactation (Zinder *et al.*, 1974). In the present experiments we have measured ^3H_2O incorporation into lipid *in vivo* (Jungas, 1968; Brunengraber *et al.*, 1973; Stansbie *et al.*, 1976) in mammary gland, liver and parametrial adipose tissue in situations associated with alterations in plasma prolactin concentrations: lactation, premature weaning or treatment with bromocryptine, an inhibitor of prolactin secretion (Seki *et al.*, 1974).

Experimental

Lactating rats of the Wistar strain (250–300g) with between 8 and 12 pups were used after a lactation period of between 10 and 16 days. Virgin rats (200–300g) were used for comparison. The rats were fed *ad libitum* on Oxoid breeding diet (Oxoid Ltd., London S.E.1, U.K.). Prolactin deficiency was induced with bromocryptine (3mg/kg body wt.) as described by Robinson & Williamson (1977). Where indicated ovine prolactin (2mg in 0.2ml of 154mM-NaCl, pH10) was injected subcutaneously at 10:00h and 17:00h on the day preceding the experiment. Triton WR1339 (10%, w/v; 1.0ml) was injected into a tail vein under ether anaesthesia 30min before injection of ^3H_2O. ^3H_2O was obtained from The Radiochemical Centre, Amersham, Bucks., U.K., and ovine prolactin from the Sigma London Chemical Co., Poole, Dorset, U.K. Triton WR1339 was a gift from Dr. R. B. Fears, Beecham Pharmaceutical Research Division, Tadworth, Surrey, U.K.

Rates of lipogenesis were measured by intraperitoneal injection of ^3H_2O (5mCi in 0.5ml). All experiments were carried out between 09:30 and 11:00h. Virgin rats were given an oral load of 5ml of 1M-glucose before injection (Robinson *et al.*, 1978). Lactating rats were left with their litters for 30min after injection of ^3H_2O, and the litters were then removed. The rats were anaesthetized with Nembutal (50mg/kg body wt.; solution in 0.9% NaCl) about 50min after injection of ^3H_2O, and at 60min duplicate samples of inguinal mammary gland, parametrial adipose tissue and liver were taken. Arterial blood was collected from the aorta into a heparinized tube for determination of specific radioactivity of plasma water. Weighed samples of tissue (about 1g in duplicate) and arterial blood (1ml) were added to 3ml of 30% (w/v) KOH, and lipid was saponified and extracted by the method of Stansbie *et al.* (1976).

Plasma insulin and prolactin were measured as described previously (Robinson *et al.*, 1978). Whole-blood glucose was determined in neutralized $HClO_4$ extracts (Slein, 1963).

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Results and Discussion

Removal of the pups for 24h at peak lactation decreased (95%) the rate of ^3H_2O incorporation *in vivo* into lipid in mammary gland, whereas the rates in liver and adipose tissue increased by 77 and 330% respectively (Table 1). The amount of ^3H_2O incorporated into blood lipid was also increased by approx. 200%. The ^3H_2O incorporation into mammary gland and adipose tissue of weaned rats was similar to that in virgin rats, whereas the incorporation into liver and blood was higher than that in virgin rats (Table 1). Increases in hepatic and adipose-tissue lipogenesis from [6-^{14}C]glucose or [2-^{14}C]acetate have been observed *in vitro* when lactating rats were weaned at 21 days and then killed 3 days later (Smith, 1973*a*,*b*). The increase in the rate of hepatic lipogenesis *in vitro* occurred without significant changes in the concentrations of acetyl-CoA carboxylase or ATP citrate lyase (Smith, 1973*a*), suggesting that short-term regulation may be responsible.

Plasma prolactin decreases on removal of the pups for 24h (Table 2; Amenomori *et al.*, 1970). Administration of ovine prolactin to weaned rats during this period completely suppressed the increase in ^3H_2O incorporation into liver, adipose tissue and blood lipids (Table 1). Conversely, inhibition of prolactin secretion by administration of bromocryptine (Table 2; Seki *et al.*, 1974) while allowing the pups to suckle increased hepatic lipogenesis by 200%; this increase was higher ($P<0.05$) than that observed on removal of the pups for 24h (Table 1). Incorporation of ^3H_2O into adipose-tissue lipid also increased, but not significantly. Prolactin deficiency depressed the incorporation of ^3H_2O into mammary-gland lipid (Table 1), and this decrease is presumably in part due to lower uptake of triacylglycerol by the gland (Zinder *et al.*, 1974). Administration of bromocryptine plus prolactin to lactating and suckled rats produced a pattern of ^3H_2O incorporation in mammary gland and liver similar to that of untreated lactating rats, but the incorporation into adipose tissue and blood was lower (Table 1).

Table 1. *Effects of premature weaning on ^3H_2O incorporation in vivo in mammary gland, liver and adipose tissue of lactating rats*
For experimental details see the text. The results are mean values ± S.D. with the numbers of rats shown in parentheses. Rates of lipogenesis are expressed as μmol of ^3H_2O incorporated into lipid/h per g wet wt. of tissue or per ml of whole blood. Values that are significantly different from the corresponding values for fed lactating rats are shown: *$P<0.05$; **$P<0.005$.

State of rats	Mammary gland	Liver	Adipose tissue	Blood
Lactating (7)	118±32	21.5±10.2	2.2±1.0	1.4±0.51
Lactating, weaned (24h) (13)	5.2±2.5**	38.0±18.8*	9.5±4.6**	4.0±3.3*
Lactating, weaned (24h) + prolactin injected (9)	3.2±1.3**	17.5± 9.2	2.8±1.4	1.7±0.9
Lactating, bromocryptine-treated (24h) (5)	63.2±40.8*	61.6±14.2**	6.4±7.7	4.4±1.8**
Lactating, bromocryptine-treated (24h) + prolactin injected (3)	79.5±56.1	14.2±10.3	0.59±0.25**	0.57±0.16**
Lactating, Triton-treated (1.5h) (4)	57.8±23.4*	15.9±4.3	2.4±0.76	2.3±1.3
Lactating, weaned (24h), Triton-treated (1.5h) (5)	5.2±3.0**	60.9±6.6**	5.8±1.0**	18.0±3.8**
Virgin, glucose intubated (20)	7.8±4.7**	17.7±9.4	9.6±7.8*	1.1±0.68

Table 2. *Concentrations of glucose, insulin and prolactin in blood or plasma of lactating rats*
Blood was collected from the aorta, and glucose and the hormones were determined as described in the Experimental section. The results are mean values ± S.D. with the numbers of rats shown in parentheses. The values for lactating rats are taken from Robinson *et al.* (1978). Values that are significantly different from those for lactating rats are shown (*P<0.05; **P<0.005) and values that are significantly different from untreated weaned rats are shown (†P<0.005).

State of rats	[Glucose] (μmol/ml of whole blood)	[Insulin] (μunits/ml of plasma)	[Prolactin] (ng/ml of plasma)
Lactating	4.74±0.48 (8)	19.7±4.9 (9)	381±175 (9)
Lactating, weaned (24h)	5.35±0.51 (6)*	59.5±11.9 (11)**	83±72 (15)**
Lactating, weaned (24h) + prolactin injected	—	33.0±13.4 (5)*†	—
Lactating, bromocryptine-treated (24h)	—	52.8±11.0 (5)**	19±3 (5)**
Lactating, bromocryptine-treated (24h) + prolactin injected	—	37.0±8.5 (3)**†	—

The activity of adipose-tissue lipoprotein lipase is suppressed during lactation but increases on removal of the pups (Hamosh *et al.*, 1970), and therefore increased uptake of newly synthesized lipid from the plasma might contribute to the increase in ^3H_2O incorporation into maternal adipose tissue on weaning. To test this, the effects of Triton WR1339, an inhibitor of triacylglycerol uptake (Scanu, 1965), were examined. Injection of Triton WR1339 into lactating rats caused a 50% decrease in ^3H_2O accumulation in lipid in mammary gland, but no significant changes in liver and adipose tissue (Table 1). In contrast, Triton WR1339 decreased the radioactivity in adipose tissue in weaned rats ($P<0.05$; Table 1) and this was accompanied by a 4-fold increase in ^3H_2O incorporation into blood lipid. Thus it would appear that a proportion of the ^3H_2O incorporated into adipose-tissue lipid in the weaned rat is transferred from the liver. Nevertheless, if the ^3H_2O incorporation into adipose-tissue lipid of lactating and weaned rats treated with Triton WR1339 is compared, there is a 140% increase ($P<0.005$) in weaned rats, indicating that weaning increases lipogenesis in adipose tissue.

Insulin is involved in the regulation of adipose-tissue and liver lipogenesis *in vivo* (Stansbie *et al.*, 1976). We have reported that plasma insulin is decreased in lactating rats compared with virgin and non-lactating rats (Robinson *et al.*, 1978) and therefore insulin was measured in the present experiments. Removal of the pups for 24h increased plasma insulin by 200%, and bromocryptine treatment of lactating rats produced a similar rise (Table 2). The increase in plasma insulin was accompanied by a small, but significant, increase in blood glucose both in weaned rats (Table 2) and bromocryptine-treated rats (Robinson & Williamson, 1977). Administration of prolactin to weaned rats decreased the insulin concentration towards that found in lactating rats. We have suggested that the lower plasma insulin in lactating rats may be due to removal of the hormone by the gland (Robinson *et al.*, 1978), and therefore the lower mammary-gland flow in weaned rats (Hanwell & Linzell, 1973) might decrease extraction of insulin and increase the circulating concentration. However, this explanation does not hold for weaned rats injected with prolactin, where the decreased insulin concentration (Table 2) is not likely to be due to an increased blood flow to the gland.

Experiments on lactating mice similar to those reported in the present paper showed no increase in hepatic lipogenesis on removal of the pups, but a 2-fold increase in ^3H_2O incorporation into adipose-tissue lipid (Romsos *et al.*, 1978). Apart from the species difference, the mice pups were removed for only 12h and mammary-gland lipogenesis decreased by 50% only.

Although experiments with exogenous prolactin must be treated with some caution because of the presence of other hormones as contaminants in prolactin preparations (Hwang *et al.*, 1972; Vorherr, 1978), the present results suggest that alteration of the plasma prolactin concentration causes a reciprocal change in the rate of lipogenesis in both liver and adipose tissue. We therefore postulate that the elevation of prolactin in response to the suckling stimulus results in a depression of lipogenesis in liver and adipose tissue so that glucose and lactate, the two main lipogenic precursors (Katz, *et al.*, 1974), are made available for the lactating gland. On weaning, the decrease in prolactin allows an increase in lipogenesis in these tissues, so that lipid stores depleted during lactation can be replenished. The increase in lipoprotein lipase activity in adipose tissue (Hamosh *et al.*, 1970) and the decreased uptake of glucose and increased output of lactate by the mammary gland on weaning (L. Agius, A. M. Robinson & D. H. Williamson, unpublished results) also play a role in the redirection of circulating substrates for this process. The present experiments give no information on the site or type of regulation involved or whether prolactin is a primary regulator.

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