

Evidence for a Role of Insulin in the Regulation of Lipogenesis in Lactating Rat Mammary Gland

MEASUREMENTS OF LIPOGENESIS *IN VIVO* AND PLASMA HORMONE CONCENTRATIONS IN RESPONSE TO STARVATION AND REFEEDING

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Fatty acid synthesis in the mammary gland of lactating rats *in vivo* was 5-fold higher than in the liver. Starvation decreased fatty acid synthesis in the gland 50-fold, whereas refeeding for 2h completely reversed this change. The plasma insulin concentration decreased 2-fold in starvation and was restored to the fed-rat value on refeeding. Glucagon and prolactin concentrations did not always change in parallel with lipogenesis, suggesting that insulin may be a regulator of this process in the gland.

The mammary gland makes a large demand on the total body glucose supplies (Williamson & Robinson, 1977), and a major proportion of the glucose utilized *in vitro* is accounted for by fatty acid synthesis (Baldwin & Yang, 1974; Katz *et al.*, 1974; Robinson & Williamson, 1977*a,c*). Previous studies indicate that mammary-gland metabolism responds to altered hormonal and nutritional status of the lactating rat. Arteriovenous difference measurements across the gland showed that glucose uptake is decreased, whereas lactate and pyruvate are released in starvation (Hawkins & Williamson, 1972; Robinson & Williamson, 1977*a*), and these changes may be related to the decreased activity of pyruvate dehydrogenase (Kankel & Reinauer, 1976; Baxter & Coore, 1978). Recent studies with [U - ^{14}C]glucose have shown that the rate of lactose synthesis by the gland *in vivo* is decreased on short-term withdrawal of food (Carrick & Kuhn, 1977). To obtain data for lipid synthesis we have measured the rate of lipogenesis in the gland *in vivo* with 3H_2O (Jungas, 1968; Brunengraber *et al.*, 1973; Stansbie *et al.*, 1976). In addition, the circulating concentrations of insulin, glucagon and prolactin have been measured to examine whether changes in their concentrations relate to alterations in the lipogenic rate.

Experimental

Lactating rats of the Wistar strain (250–300g) with between 6 and 14 pups were used after a lactation period of between 10 and 18 days. Virgin rats (200–300g) were used for comparison. Non-lactating rats refer to rats whose pups were removed at parturition and studied 13 to 15 days later. The rats were fed

ad libitum on Oxoid breeding diet (Oxoid Ltd., London S.E.1, U.K.). Starved rats (with pups) were deprived of food for 24h and starved-refed rats were allowed Oxoid pellets for 2h after 24h of starvation. Insulin and prolactin deficiency were induced as described by Robinson & Williamson (1977*a*). 3H_2O was obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Rates of lipogenesis were measured by intra-peritoneal injection of 3H_2O (5mCi in 0.5ml) into rats lightly anaesthetized with ether 30min before the end of the specified time of treatment. Rats were left with their litters for 30min, and litters were then removed. The rats were anaesthetized with Nembutal (50mg/kg body wt.; solution in 0.9% NaCl) at 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 the 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 was measured by radioimmunoassay by using uncoated charcoal to separate bound and free hormone (Albano *et al.*, 1972). A purified rat insulin standard (24i.u./mg; Novo Research Institute, Copenhagen, Denmark) and an anti-insulin serum reacting poorly with proinsulin were used. Plasma glucagon was determined with a pancreatic-glucagon specific antibody K47 kindly supplied by L. G. Heding (Novo Research Institute) in an assay system described by Girard *et al.* (1972). Plasma prolactin was determined with a kit from

N.I.A.M.D. by using standard rat prolactin RP1 (11 i.u./mg) and anti-rat prolactin serum 4.

The following metabolites in neutralized HClO₄ extracts of blood were determined by enzymic methods: glucose (Slein, 1963); acetoacetate and D-3-hydroxybutyrate (Williamson *et al.*, 1962).

Results and Discussion

Lipogenesis in rat mammary gland was 5-fold greater than the rate in liver of lactating or virgin rats, and 25-fold greater than the rate in the gland of virgin rats (Table 1). Lipogenic rates were similar in the liver of lactating rats and virgin rats fed glucose intragastrically immediately before injection of ³H₂O (Table 1), which is not consistent with the increased lipogenesis observed in liver *in vitro* during lactation (Smith, 1973a; Benito & Williamson, 1978). Although lipogenesis was 36% lower in the liver of fed virgin rats not given glucose (results not shown), comparison with the virgin rats given glucose seems more valid in view of the high food consumption and continuous feeding of lactating rats. These lipogenic rates are similar to those reported in liver of male rats (Brunengraber *et al.*, 1973; Stansbie *et al.*, 1976; Cook *et al.*, 1977).

In starvation lipid synthesis in the mammary gland decreased 50-fold, and this dramatic change was completely reversed on refeeding for 2h (Table 1). Lipogenesis in the liver of lactating rats decreased 10-fold in starvation and remained 50% less than the rate in fed rats on refeeding. The increased lipogenesis found in the gland on refeeding was prevented by administration of streptozotocin immediately before refeeding, but not by bromocryptine injection 1h before refeeding. Further evidence that the mammary gland may be particularly sensitive to short-term regulation is the finding that streptozotocin treatment of fed rats decreased lipogenesis in the gland 7-fold, whereas the liver was not affected.

In parametrial adipose tissue of lactating and virgin rats lipogenesis was 3.4 ± 3.0 ($n = 6$) and 6.2 ± 3.8 ($n = 6$) μmol of ³H₂O/h per g respectively.

Lipogenesis in adipose tissue of lactating rats was not changed in starvation, on refeeding or after streptozotocin treatment (results not shown). The lower lipogenic rate in adipose tissue during lactation is consistent with the finding that fatty acid synthesis *in vitro* from glucose is decreased in parametrial adipose tissue from lactating rats (Smith, 1973b).

It is possible that a portion of the radioactivity in the gland may represent lipid synthesized in the liver and subsequently released into the circulation and taken up by the gland. However, this is unlikely to account for a large proportion of the ³H-labelled lipid found in the gland, since incorporation of ³H₂O into blood lipid in fed lactating rats was not increased compared with virgin rats and paralleled changes in the lipogenic rate in the liver rather than the gland (Table 1).

By using a gland weight and blood flow from the data of Chatwin *et al.* (1969), lipid synthesis in the gland is 70mmol of ³H₂O/day compared with glucose utilization by the gland of 30mmol/day (Williamson & Robinson, 1977). Thus it seems likely that glucose utilization by the gland *in vivo* is sufficient to account for the large demand made by lipogenesis in fed lactating rats. In starvation the decreased lipogenesis (Table 1), in addition to the suppression of lactose synthesis (Carrick & Kuhn, 1977), may decrease the glucose requirement of the gland, thus sparing body supplies.

In fed lactating rats plasma insulin concentrations were 60% lower than in virgin rats or non-lactating rats (Table 2). After starvation (24h) insulin concentrations decreased by 50% in both groups and the 2.5-fold difference in concentration between lactating and virgin rats was maintained (Table 2). Refeeding starved rats for 2h restored insulin concentrations to the value found for fed lactating rats. Plasma insulin concentrations were decreased to the starved value by streptozotocin treatment for 2h, but were not affected by bromocryptine treatment for 24h.

In other studies of lactating rats (Sutter-Dub *et al.*, 1974; Kuhn, 1977) plasma insulin concentrations

Table 1. Lipogenesis *in vivo* in mammary gland and liver of lactating and virgin rats

For experimental details see the text. The results are mean values \pm s.d. with the numbers of rats shown in parentheses. Rates of lipogenesis are expressed as μmol of ³H₂O 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	Blood
Lactating fed	(6) 121 \pm 57	22.8 \pm 9.4	0.94 \pm 0.11
Lactating starved (24h)	(6) 2.1 \pm 1.1**	2.1 \pm 0.9**	0.26 \pm 0.14**
Lactating starved (24h)+refed (2h)	(6) 148 \pm 28	9.0 \pm 1.9**	0.44 \pm 0.14**
Lactating starved (24h), bromocryptine-treated (3h)+refed (2h)	(6) 110 \pm 48	8.8 \pm 3.1*	0.31 \pm 0.09**
Lactating starved (24h), streptozotocin-treated (2h)+refed (2h)	(3) 3.2 \pm 1.6*	5.6 \pm 2.0*	0.18 \pm 0.01**
Lactating fed, streptozotocin-treated (2h)	(4) 17 \pm 11**	22.5 \pm 9.0	1.00 \pm 0.29
Virgin fed, glucose intubated	(6) 4.7 \pm 1.2**	19.1 \pm 10.6	1.13 \pm 0.10*

Table 2. Concentrations of glucose, ketone bodies, insulin, glucagon and prolactin in blood of lactating and virgin rats

Blood was collected from the aorta and metabolites and hormones were determined as described in the Experimental section. The results are mean values \pm s.d. with the numbers of rats shown in parentheses. Values that are significantly different from those for fed lactating rats are shown: * $P < 0.05$; ** $P < 0.005$.

	[Glucose] (μ mol/ml of whole blood)	[Ketone bodies] (μ mol/ml of whole blood)	[Insulin] (μ units/ml of plasma)	[Glucagon] (pg/ml of plasma)	[Prolactin] (ng/ml of plasma)
Lactating fed	4.74 \pm 0.48 (8)	0.14 \pm 0.08 (8)	19.7 \pm 4.9 (9)	198 \pm 60 (9)	381 \pm 175 (9)
Lactating starved (24h)	4.80 \pm 0.40 (7)	0.54 \pm 0.22 (7)**	10.6 \pm 4.9 (10)**	147 \pm 45 (10)*	352 \pm 168 (7)
Lactating starved (24h)+refed (2h)	5.15 \pm 0.66 (8)	0.16 \pm 0.03 (8)	20.5 \pm 8.8 (8)	322 \pm 123 (8)*	—
Lactating fed, streptozotocin-treated (2h)	10.6 \pm 3.4 (7)**	0.15 \pm 0.04 (7)	10.4 \pm 8.7 (8)*	294 \pm 98 (8)*	53 \pm 46 (6)**
Lactating fed, bromocryptine-treated (24h)	5.33 \pm 0.53 (6)	0.14 \pm 0.06 (6)	24.3 \pm 8.1 (3)	160 \pm 18 (3)	19 \pm 9 (3)**
Virgin fed	6.79 \pm 0.51 (5)**	0.26 \pm 0.01 (5)**	49.3 \pm 23.0 (7)**	251 \pm 36 (7)*	—
Virgin starved (24h)	5.14 \pm 0.35 (7)*	1.14 \pm 0.18 (7)**	27.9 \pm 15.0 (10)	167 \pm 46 (10)	138 \pm 140 (10)**
Non-lactating fed	6.51 \pm 0.12 (3)**	0.28 \pm 0.02 (3)*	44.0 \pm 21.4 (5)**	201 \pm 21 (5)	191 \pm 189 (5)*

were about 2-fold higher than those in Table 2, which may be due to differences in the radioimmunoassay techniques and site of blood sampling. Plasma insulin concentrations were higher in lactating rats than in non-lactating rats, whereas plasma glucose concentrations were about 6.0mm for both groups of rats in blood from the tail vein after starvation for 12h (Sutter-Dub *et al.*, 1974). This contrasts with the present studies where the lower plasma insulin concentrations in lactating rats compared with virgin rats are consistent with the lower blood glucose concentrations found in lactating rats (Table 2). In view of the increased food intake during lactation (Cole & Hart, 1938; Fell *et al.*, 1963) it might be expected that plasma insulin concentrations would increase during lactation as in pregnancy (Saudek *et al.*, 1975), where the increased synthesis of insulin and enhanced secretory response to glucose by the pancreatic β -cells has been related to the increased food intake (Green & Taylor, 1974; Bone & Howell, 1977). A possible explanation is that removal and degradation of insulin by liver (Terris & Steiner, 1975) and mammary gland (Osborne *et al.*, 1978) may be increased.

Plasma glucagon concentrations in fed lactating rats were similar to those of non-lactating rats, but were 20% lower than those of virgin rats (Table 2). Since insulin concentrations were decreased in lactating rats compared with virgin rats, the ratio of [insulin]/[glucagon] was decreased 2-fold in lactating rats, suggesting that a catabolic state may prevail (Unger, 1971). Glucagon concentrations tended to decrease in starvation and increase on refeeding or following streptozotocin treatment (Table 2). The concentrations of glucagon found in these studies are similar to those reported previously (Saudek *et al.*, 1975; De Jong *et al.*, 1977).

Prolactin concentrations were 2-fold higher in lactating rats compared with non-lactating rats and were not affected by starvation (Table 2). In previous studies (Amenomori *et al.*, 1970; Simpson *et al.*, 1973) somewhat lower prolactin concentrations were found. As was expected prolactin concentrations were decreased by bromocryptine treatment, but surprisingly streptozotocin also caused a decrease, although not to the same extent as bromocryptine (Table 2).

Insulin is the only one of the three hormones whose concentrations altered in parallel with lipogenesis in all situations studied, which suggests that insulin may control this process *in vivo*. Some evidence that the decreased lipogenesis after streptozotocin treatment may be related to decreased insulin concentrations rather than the accompanying prolactin deficiency is the finding that bromocryptine treatment (3h) did not abolish the effect of refeeding on lipogenesis in the gland (Table 1). Previously it was proposed that ketone bodies as well as insulin

may control mammary-gland glucose metabolism in starvation (Williamson *et al.*, 1975). Ketone-body concentrations increase in starvation (Table 2) and there is evidence both *in vivo* (Robinson & Williamson, 1977b) and *in vitro* (Williamson *et al.*, 1975; Robinson & Williamson, 1977c) that acetoacetate decreases glucose utilization in the gland. Insulin *in vitro* relieves the acetoacetate inhibition of glucose utilization and lipogenesis (Williamson *et al.*, 1975; Robinson & Williamson, 1977c), and the decreased insulin concentrations in starvation may potentiate inhibition of glucose utilization and lipogenesis by the increased blood acetoacetate concentrations.

Decreased lipogenesis in the gland in starvation may involve long-term changes in enzyme activities, as is the case in other lipogenic tissues (Volpe & Vagelos, 1976), but the complete restoration of lipogenesis in the gland after 2h of refeeding suggests that the regulation is short term. Pyruvate dehydrogenase and acetyl-CoA carboxylase are possible regulatory steps in lipogenesis in the gland (Baldwin & Yang, 1974; Williamson & Robinson, 1977). Pyruvate dehydrogenase activity shows parallel changes to lipogenesis in starvation (Kankel & Reinauer, 1976; Baxter & Coore, 1978) and after streptozotocin treatment (Field & Coore, 1976). It is not known whether acetyl-CoA carboxylase in the gland undergoes changes in activity in starvation and on refeeding, and whether insulin has direct effects on its activity.

In conclusion, these studies have demonstrated the high lipogenic rate in the mammary gland and its extreme sensitivity to the nutritional state. Such tight regulation would be expected in view of the large demand normally made by the gland on body glucose supplies. Insulin may be of prime importance in regulation of lipogenesis and glucose utilization, but its site of action remains a challenging question.

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