Time course of changes in plasma glucose and insulin concentrations and mammary-gland lipogenesis during re-feeding of starved conscious lactating rats

Stewart W. MERCER and Dermot H. WILLIAMSON

Metabolic Research Laboratory, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE, U.K.

Temporal changes in circulating insulin concentrations were measured during re-feeding of 18 h-starved lactating rats. Insulin concentrations rose rapidly over the first 20 min of re-feeding with 5 g of chow diet, and then sharply declined between 20–30 min and remained low for the rest of the 90 min experimental period. Lipogenic activity in the mammary gland also exhibited a peak during re-feeding, but there was a clear time lag between the insulin response and the lipogenic response. Blood-flow measurements failed to show any major increase to the tissue during this activation of lipogenesis. Acute suppression of insulin secretion at 30 min (after the initial surge) abolished the switch-on of lipogenesis, suggesting that the insulin-sensitivity of the gland may be acutely enhanced over this period of re-feeding.

INTRODUCTION

Recent studies on lactation in the rat have indicated the extremely high sensitivity of the mammary gland to changes in nutritional status. Short-term starvation inactivates lipogenesis in the gland (Jones *et al.*, 1984), and re-feeding with chow diet leads to a rapid restoration of lipogenic activity (Munday & Williamson, 1981; Busmann *et al.*, 1984; Jones & Williamson, 1984; Williamson *et al.*, 1985). Insulin is a key factor in the regulation of lipogenesis *in vivo* in the mammary gland (Robinson & Williamson, 1977; Robinson *et al.*, 1978; Burnol *et al.*, 1983; Jones *et al.*, 1984), and the restoration of lipogenesis during re-feeding is prevented by insulin deficiency (Robinson *et al.*, 1978). However, the temporal changes in circulating insulin concentrations during re-feeding have not been documented in the lactating rat, and thus it is not known if the restoration of lipogenesis in the mammary gland occurs in synchrony with increased plasma insulin concentrations, or whether it involves changes in the rate of insulin delivery to the gland (via increased blood flow) or in the insulin-sensitivity of the tissue itself.

The aim of the present study was to clarify these questions by measuring the time course of plasma glucose and insulin concentrations during re-feeding of starved conscious lactating rats, coupled with measurements *in vivo* of mammary-gland lipogenesis and blood flow.

METHODS

Rats

Lactating Wistar rats were kept at an ambient temperature of 22 ± 2 °C, with a 12 h-light/12 h-dark cycle (light from 07:30 h), and were fed on a low-fat/high-carbohydrate chow diet *ad libitum* (52% carbohydrate, 21% protein, 4% fat; Special Diet Services, Witham, Essex, U.K.). At 2 days before the experiments, polyethylene cannulae (internal diameter 0.63 mm, external diameter 0.96 mm) were inserted into the right

carotid artery and the left jugular vein, under general anaesthesia (Jones *et al.*, 1984). The cannulae were exteriorized between the scapulae and sealed, having been filled with 0.9% NaCl containing 10 units of heparin/ml and 3% polyvinylpyrrolidone.

The rats were used at mid-lactation (11–14 days) and were starved for 18 h before the experiments. On the morning of the experiments, the cannulae were extended to reach outside the cages, so that blood samples could be drawn during re-feeding with the minimum of disturbance to the animals. At all times the rats remained in their home cages, with their pups suckling.

Re-feeding

The 18 h-starved lactating rats were given 5 g of the chow diet immediately after the first blood sample was taken. Invariably, the rats ate the food within the first 30 min of re-feeding; in most cases the food was consumed within 15–20 min.

Blood sampling

Blood samples (0.20–0.25 ml) were drawn from the cannulated carotid artery at 0, 5, 10, 20, 30, 40, 60 and 80 min of re-feeding. Blood removed was immediately replaced with an equivalent volume of 0.9% NaCl.

Freshly collected blood was centrifuged and the plasma frozen. Plasma glucose was determined by the method of Slein (1963), and plasma insulin was measured by radioimmunoassay, with a rat insulin standard (Albano *et al.*, 1972).

Measurement of lipogenesis and blood flow

Lipogenesis was measured *in vivo* with $^3\text{H}_2\text{O}$ as previously described (Robinson *et al.*, 1978). Conscious animals were injected intraperitoneally with 2.5 mCi (0.25 ml) of $^3\text{H}_2\text{O}$. Relative blood flow was measured by the method of Herd *et al.* (1968) as previously described (Rofe & Williamson, 1983). Simultaneous with the injection of $^3\text{H}_2\text{O}$, the animals were infused (15–20 s), via the cannulated jugular vein, with 0.15 ml of [^{14}C]DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] in 0.9%

NaCl containing 2.5% (v/v) Tween 20 ($[^{14}\text{C}]\text{DDT}$ 8 $\mu\text{Ci}/\text{mg}$ per kg body wt., stock solution 80 $\mu\text{Ci}/\text{ml}$ in ethanol).

Duplicate tissue samples were removed 1 h later, the rats having been anaesthetized 3–5 min before tissue removal with pentobarbital (60 mg/kg body wt.). The samples were saponified and the lipids extracted (Stansbie *et al.*, 1976) to determine the incorporation of ^3H *in vivo* into saponified lipid and the accumulation of $[^{14}\text{C}]\text{DDT}$ *in vivo*. Measurement of $[^{14}\text{C}]\text{DDT}$ in the guts of the suckling pups indicated a negligible transfer from dam to offspring during the 1 h measurement period (less than 2% per litter of the total mammary-gland accumulation). Furthermore, the small amount transferred was the same for both starved and re-fed groups.

In the second experiment, rats received streptozotocin (50 mg/kg body wt.; 0.5% solution in 0.1 M-citrate, pH 4.9) intravenously via the jugular-vein cannula 30 min after the start of re-feeding. An additional group of rats was infused with somatostatin (100 $\mu\text{g}/\text{kg}$ body wt. per h) over the 30–90 min period.

In the third experiment, lipogenic rates were determined over 30 min periods during re-feeding at 0–30 min, 30–60 min and 60–90 min. To ensure a rapid entry of $^3\text{H}_2\text{O}$ into the circulation, these rats received the same dose of ^3H via the cannulated jugular vein. In all the experiments, the rats were conscious and undisturbed throughout the periods of measurements.

RESULTS AND DISCUSSION

Plasma insulin and glucose changes during re-feeding

The time course of the plasma insulin changes during re-feeding of the 18 h-starved lactating rats is shown in Fig. 1(a). Basal insulin concentrations were low, as previously reported (Jones *et al.*, 1984), but within only 5 min of natural re-feeding there was a significant ($P < 0.05$) 2-fold increase. By 10 min the insulin concentrations had continued to increase and the peak, which occurred at 20 min, was some 20-fold higher than the basal starved value. Surprisingly, however, the insulin concentrations decreased sharply between 20 and 30 min of re-feeding and remained low for the remainder of the 90 min period. This finding explains the previous failure to observe any appreciable increase in plasma insulin on re-feeding when measurements were made at 90 min (Williamson *et al.*, 1985). Whether this rapid fall in circulating concentrations is due to a corresponding change in the pancreatic secretion of insulin, or is related to a rapid increase in peripheral insulin removal, is an open question. It is perhaps noteworthy, however, that the 5 g of chow diet given to each animal was consumed very quickly, usually within the first 20 min. This rapid ingestion of food is reflected by the rise in plasma glucose over the initial 20 min (Fig. 1b). Between 20 and 30 min there was a slight decrease in glucose concentration, although this was not statistically significant, and the values remained significantly ($P < 0.01$) above the basal (starved) value throughout the remainder of the 90 min period.

Mammary-gland lipogenesis and blood flow in starved and re-fed lactating rats

The rates of lipogenesis *in vivo* in mammary glands of starved and re-fed lactating rats are shown in Table 1;

Fig. 1. Time course of plasma insulin (a) and glucose (b) changes in conscious lactating rats during re-feeding, after starvation for 18 h

Arterial blood samples were drawn from conscious lactating rats during re-feeding, as described in the text. The animals received 5 g of chow diet, which they rapidly consumed within approx. 20 min. Results are expressed as means $\pm$ S.E.M. for four to eight samples at each time point. For further details see the text. $\circ$, Starved rats; $\bullet$, re-fed rats.

lipogenesis in the latter group was measured from 30 to 90 min after the onset of re-feeding. In confirmation of previous findings (Williamson *et al.*, 1985), there was a large increase (23-fold) in the rate of lipogenesis in the re-fed animals compared with the low rate found in the starved group. This large switch-on of lipogenesis in the gland thus occurred over a period when the circulating insulin concentrations were relatively low, i.e. after the transitory hyperinsulinaemia (Fig. 1a). Previous work has shown that blockade of pancreatic insulin release by streptozotocin immediately before re-feeding *ad libitum* prevents this re-activation of lipogenesis in the gland (Robinson *et al.*, 1978). This raises three possible explanations of the present data: (a) the rapid transient hyperinsulinaemia over the first 30 min of re-feeding is followed by a large increase in blood flow to the gland (and hence in the rate of hormone and substrate delivery); (b) the initial surge in insulin concentration serves to stimulate a system(s) in the gland, which remains active thereafter for a certain period of time, irrespective of the ensuing circulating insulin concentrations; (c) after the insulin surge there is a rapid increase in the insulin-sensitivity of the mammary gland, such that insulin-sensitive processes involved in the lipogenic switch-on are stimulated even by the relatively low insulin concentrations existing over the 30–90 min period. The effects of insulin may either be direct (e.g. activation of key glycolytic/lipogenic enzymes by phosphorylation/dephosphorylation; Munday &

Table 1. Mammary-gland rates of lipogenesis and relative blood flow measured *in vitro* in conscious lactating rats during re-feeding

For details see the text. The results are means $\pm$ S.E.M., with the numbers of observations shown in parentheses. Rates of lipogenesis are expressed as μmol of $^3\text{H}_2\text{O}$ incorporated into saponified lipid/h per g fresh wt. of tissue, or per total dissectable tissue. Relative blood flow is given as the [^{14}C]DDT accumulated into lipid as a percentage of the injected dose. A significant difference by Student's *t* test between the starved and re-fed values is indicated by: *P < 0.05; **P < 0.01.

	Starved (18 h)	Re-fed (90 min)
Lipogenesis per g of tissue (μmol of $^3\text{H}_2\text{O}$ incorporated into saponified lipid/h per g wet wt.)	2.5 ± 0.2 (7)	58.5 ± 10.1 (6)**
Lipogenesis per total gland (μmol of $^3\text{H}_2\text{O}$ incorporated into saponified lipid/h per total dissectable tissue)	36.3 ± 4.2 (7)	714 ± 125 (6)**
Blood flow per g of tissue ([^{14}C]DDT accumulated/h per g wet wt.)	1.01 ± 0.13 (7)	1.51 ± 0.18 (7)*
Blood flow per total gland ([^{14}C]DDT accumulated/h per total tissue)	14.3 ± 2.0 (7)	18.6 ± 2.2 (7)
Mammary-gland fat content (%)	8.8 ± 1.4 (7)	8.1 ± 0.6 (7)

Hardie, 1986) or indirect (e.g. decrease of putative inhibitors, ketone bodies, citrate; Williamson *et al.*, 1975; Zammit, 1979).

To test the first possibility, we have measured the relative change in blood flow to the mammary gland during re-feeding, using [^{14}C]DDT (Herd *et al.*, 1968; Rofe & Williamson, 1983). Although the preferred technique for assessing blood flow quantitatively is the radioactive microsphere method, it has two major disadvantages; firstly it measures flow at a single time point rather than over a period of time, and secondly it is technically difficult to perform on conscious animals. The DDT method, which involves measuring the gradual accumulation of DDT in tissues, can be easily performed on conscious animals provided that an intravenous cannula is fitted, and gives an average measure of blood flow to a particular tissue over a defined time period. Although DDT accumulation may be influenced by the amount of neutral lipid in tissues, and is thus not a good measure of comparative flow to different tissues, it is a valid method for assessing relative flow to any one tissue, assuming that the lipid content is constant (as in the present study, Table 1). In the present study, blood flow and lipogenic rate were measured simultaneously in the starved and re-fed rats (during the 30–90 min re-feeding period). Blood flow to the mammary gland showed a modest increase during re-feeding (Table 1), which reached statistical significance when expressed per g of tissue (*P* < 0.05). However, in terms of insulin delivery to the tissue, such a small increase in blood flow would seem unlikely to be sufficient to provoke such a large stimulation of lipogenic activity. This comparatively small change in blood flow to the mammary gland during re-feeding agrees with previously published data on blood flow to the gland of re-fed lactating rats measured by the microsphere method (Jones & Williamson, 1984) in anaesthetized animals.

Thus the activation of lipogenesis over 30–90 min after re-feeding appears to occur without a major increase in the rate of insulin delivery to the gland. To assess whether the switch-on occurs as an asynchronous response to the preceding hyperinsulinaemia or if there

is continuing stimulation by the low insulin concentration during the period of measurement of lipogenesis, a group of starved-re-fed lactating animals was given streptozotocin (to inhibit pancreatic insulin release acutely; Schein *et al.*, 1971) 30 min after the start of re-feeding, i.e. after the insulin surge, at the same time as $^3\text{H}_2\text{O}$ administration. This procedure abolished the normal lipogenic switch-on in the mammary gland, the lipogenic rate in the streptozotocin-treated group being $15.3 \pm 4.4 \mu\text{mol}$ of $^3\text{H}_2\text{O}$ incorporated/h per g wet wt. (*n* = 4), which was significantly lower (*P* < 0.05) than the normal re-fed value shown in Table 1. Similar results were obtained when somatostatin, rather than streptozotocin, was infused between 30 and 90 min (15.2 , $12.9 \mu\text{mol}$ of $^3\text{H}_2\text{O}$ incorporated/h per g wet wt.; individual values).

These results provide clear evidence that the initial hyperinsulinaemic response to re-feeding (0–30 min) is not in itself sufficient to maintain activation of lipogenesis over the remainder of the 90 min experimental period. This strongly suggests that the insulin-sensitivity of the mammary gland is acutely increased during this time.

Time course of the lipogenic switch-on in the mammary gland during re-feeding

In view of the asynchronous nature of the plasma insulin response and the lipogenic response during the re-feeding, it was considered desirable to investigate more fully the time course of the re-activation of lipogenesis in the mammary gland. Lipogenesis was therefore measured in an additional group of lactating rats over 30 min periods during re-feeding (Table 2). There was very little re-activation of lipogenesis during the first 30 min after commencement of feeding, despite the large surge in circulating insulin during this time (Fig. 1a). Between 30 and 60 min after the start of re-feeding, by which time the plasma insulin had decreased dramatically, there was a large increase in lipogenic rate, to a value some 50-fold higher than the starved rate. Between 60 and 90 min the rate of lipogenesis decreased substantially, although it remained significantly above the 0–30 min value. At first sight these findings appear to

Table 2. Time course of lipogenesis in the mammary gland of starved lactating rats after re-feeding

For details see the text. Values that differ significantly by Student's *t* test from the 0–30 min group are indicated by: *P < 0.02; **P < 0.01. A significant difference between the 30–60 min group and the 60–90 min group is indicated by: †*P* < 0.02.

Time after start of re-feeding (5 g of chow)	Rate of lipogenesis <i>in vivo</i> (μmol of ³ H ₂ O incorporated/h per g wet wt.)
0–30 min	13.5 ± 4.6 (4)
30–60 min	93.9 ± 20.2 (6)**
60–90 min	33.4 ± 3.5 (4)†

conflict with those of Bussmann *et al.* (1984), who showed a large increase in lipogenesis 30 min after re-feeding, which then continued to rise over the next few hours. However, their rats were fed *ad libitum*, rather than with the standard amount (5g) of chow used in the present study, the rats were anaesthetized during the measurements, and the first period of measurement was between 30 and 60 min after the start of re-feeding (i.e. corresponding to the period of peak rate of lipogenesis in the present study).

The results of the present study emphasize the rapidity of changes in mammary-gland lipogenesis. Importantly, they clearly show that there is an asynchrony between the plasma insulin response to re-feeding with a limited amount of chow diet and the lipogenic response. That this lipogenic switch-on in the gland is prevented by inhibition of insulin secretion after the initial spike of insulin release suggests that limited re-feeding induces an acute increase in the sensitivity of the tissue to the stimulating influence of insulin (direct or indirect) on the lipogenic process. Such an association between sensitivity of the mammary gland to insulin and re-feeding with a carbohydrate meal explains why insulin infusion in the absence of re-feeding fails to re-activate lipogenesis fully in the gland of 24 h-starved lactating rats (Jones *et al.*, 1984). The decrease in lipogenic activity towards the end of the 90 min re-feeding period further supports the view

that the control of lipogenesis in the mammary gland is acutely tuned to the availability of carbohydrate.

The re-feeding model reported here may be of considerable value in delineating the precise temporal changes in key sites of lipogenic regulation or in the mammary gland during acute changes in substrate supply.

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