Regulation of lactating-rat mammary-gland lipogenesis by insulin and glucagon *in vivo*

The role and site of action of insulin in the transition to the starved state

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1. Starvation for 6 h and 24 h caused an 80% and 95% decrease in the rate of mammary-gland lipogenesis respectively in conscious lactating rats. 2. Plasma insulin concentrations decreased and circulating ketone-body concentrations increased with the length of starvation. 3. The inhibition of lipogenesis after 24 h starvation was accompanied by increased concentrations of glucose, glucose 6-phosphate and citrate in the mammary gland. Qualitatively similar changes were observed after 6 h starvation. 4. Infusion of insulin at physiological concentrations caused a 100% increase in the rate of lipogenesis in fed animals and partially reversed the inhibition of lipogenesis caused by starvation. 5. Infusion of insulin tended to reverse the changes seen in intracellular metabolite concentrations. 4. Infusion of glucagon into fed rats caused no change in the rates of lipogenesis in mammary gland, liver or white adipose tissue. 7. It is concluded that (a) insulin acts physiologically to regulate lipogenesis in the mammary gland, (b) hexokinase and phosphofructokinase are important regulatory enzymes in the short-term control of lipogenesis in the mammary gland, which are under the influence of insulin, and (c) the unresponsiveness of mammary-gland lipogenesis *in vivo* to infusions of glucagon is consistent with an adaptive mechanism which diverts substrate towards the lactating mammary gland and away from other tissues.

Previous work has shown that metabolic processes in the lactating mammary gland are very sensitive to the availability and composition of the diet. Withdrawal of food for periods as short as 6 h decreases the rates of lipogenesis (Williamson *et al.*, 1983) and lactose synthesis (Carrick & Kuhn, 1978) in the lactating-rat mammary gland. Both processes are rapidly re-activated (in 2 h) by re-feeding, implying that they are regulated by a factor(s) or metabolite(s), the concentration of which is altered by food ingestion or absorption (Wilde & Kuhn, 1979; Williamson *et al.*, 1983). After longer periods of starvation (24 h), insulin has been implicated as a potential signal of food availability, since plasma insulin concentrations decrease on starvation and increase again in parallel with the rates of lipogenesis on re-feeding (Robinson *et al.*, 1978). Furthermore, treatment of

the 24 h-starved rat with large doses of insulin partially restores the rate of lipogenesis, probably via re-activation of pyruvate dehydrogenase (Baxter & Coore, 1978) and acetyl-CoA carboxylase (McNeillie & Zammit, 1982; Munday & Williamson, 1982) and stimulation of the rate of glucose transport into the tissue (Threadgold & Kuhn, 1984).

Experiments *in vitro* also support a role for insulin in the regulation of mammary-gland metabolism, although the concentrations of hormone required are high. Addition of insulin to acini from 24 h-starved animals stimulates lipogenesis, probably through an effect on acetyl-CoA carboxylase (Munday & Williamson, 1981). Similarly, in acini from 6 h-starved animals glucose removal and its conversion into lipid are stimulated by insulin (Williamson *et al.*, 1983).

Significant alterations in plasma insulin concentrations have not been detected *in vivo* at short periods of starvation, although there is a tendency to lower values (Williamson *et al.*, 1983). This may be because the changes in insulin concentration required to regulate the mammary gland are small and therefore difficult to measure accurately in the anaesthetized animal. Alternatively, factors other than insulin may be involved. For example, a factor, released from the gastrointestinal tract when food intake is decreased, could inhibit mammary-gland metabolism.

The studies described here were conducted on conscious lactating rats in order to assess the role of insulin and glucagon *in vivo* in the short-term regulation of mammary-gland metabolism in response to food withdrawal.

Methods

Lactating Wistar rats with nine or ten pups were used at peak lactation (10–16 days). Non-lactating controls were rats whose pups had been weaned 3–4 weeks previously. Under general anaesthesia (diethyl ether) polyethylene cannulae (internal diam. 0.63 mm, external diam. 0.96 mm) were inserted into the right carotid artery for blood sampling, and into the right external jugular vein for intravenous infusions. The cannulae were exteriorized and sutured to the skin over the scapulae, and the animals allowed to recover for 48 h. By this time the animals had recovered their normal food intake (40–50 g/day).

All experiments were conducted with animals in their home cage and with the pups still present. The cannulae were extended to reach outside the cage to facilitate blood sampling, and thus minimal disturbance was caused to the behaviour of the mother and to suckling by the pups.

The following metabolites were measured enzymically: glucose (Slein, 1963), lactate and pyruvate (Hohorst, 1963), ketone bodies (Williamson *et al.*, 1962), citrate (Dagley, 1963), glucose 6-phosphate and ATP (Lamprecht & Trautschold, 1963). Before analysis, the neutralized acid extract of mammary tissue were filtered through 0.45 µm-pore-size type HA Millipore filters (Millipore S.A., Molsheim, France) to remove milk-fat globules. Insulin was determined by radioimmunoassay with a rat insulin standard (Albano *et al.*, 1972), and rates of lipogenesis *in vivo* were measured by the incorporation of $^3\text{H}_2\text{O}$ into non-esterified fatty acids as previously described (Robinson *et al.*, 1978).

The intracellular concentration of glucose in mammary gland was calculated by assuming an extracellular glucose space of 20% (Threadgold & Kuhn, 1984) and that the extracellular glucose was

in free equilibrium with the plasma. Thus:

Intracellular glucose =

$$\text{Total glucose} - \frac{\text{Plasma glucose}}{5}$$

If the equilibrium between plasma and extracellular glucose were to be altered from unity by changes in the rate of glucose utilization, then this correction would tend to exaggerate the differences in intracellular glucose.

Low-dose insulin infusion

Conscious unrestrained lactating rats were infused intravenously for 90 min with either 0.9% (w/v) NaCl containing 1% (w/v) bovine serum albumin or a solution of insulin (pig, soluble, ACTRAPID MC; NOVO, Copenhagen, Denmark) in 0.9% NaCl containing 1% bovine serum albumin. The insulin concentration was 60 munits/ml and rats were infused at a rate equivalent to 120 munits/kg body wt. per h. At 30 min they were given an intraperitoneal injection of $^3\text{H}_2\text{O}$. At 90 min they were anaesthetized with sodium pentobarbital, and samples of mammary gland and liver were quickly taken and frozen in liquid N_2 for subsequent analysis of tissue metabolites (Wollenberger *et al.*, 1960). Samples of mammary gland, liver and white adipose tissue were also taken for the measurement of lipogenic rates. Blood samples (0.4 ml) were taken at 0, 15, 30, 60 and 90 min to measure changes in concentrations of circulating metabolites and plasma insulin. Blood removed was replaced by an equivalent volume of 0.9% (w/v) NaCl. Three groups of animals were studied: fed, 6h-starved and 24h-starved.

Low-dose glucagon infusion

In a separate study, fed, conscious, unrestrained lactating rats were infused intravenously for 90 min with either 0.9% (w/v) NaCl containing 1% (w/v) bovine serum albumin or a solution of glucagon (crystalline; Sigma, Poole, Dorset, U.K.) in 0.9% NaCl containing 1% bovine serum albumin. The glucagon concentration was 150 ng/ml and rats were infused at a rate equivalent to 300 ng/kg per h. At 30 min they were given an intraperitoneal injection of $^3\text{H}_2\text{O}$. Samples of mammary gland, liver and white adipose tissue were also taken for the measurement of lipogenic rates. Blood was taken from the aorta for the measurement of circulating metabolite and insulin concentrations.

Statistics

Results are presented as means $\pm$ S.E.M. Statistical differences were tested by Student's unpaired *t* test.

Results and discussion

The decrease in lipogenic rate (88%) in 6h-starved rats demonstrates the extreme sensitivity of lactating-rat mammary gland to the nutritional status of the animal (Carrick & Kuhn, 1978; Williamson *et al.*, 1983). Insulin concentrations measured in the basal state showed a significant and progressive decrease as the period of starvation increased (Table 1 and Fig. 1). In previous studies insulin concentrations were found to be higher and more variable (Williamson *et al.*, 1983); this emphasizes the need to avoid stress when

measuring hormones in labile and sensitive endocrine systems. Paradoxically, basal glucose concentrations rose on starvation, perhaps reflecting the decreased overall demand for glucose when mammary-gland lipogenesis is inhibited. This implies that glucose cannot be the over-riding influence on insulin secretion in lactation and that other stimuli must be acting. It is well established that hormones released from the gut can stimulate the pancreatic B-cell and augment insulin secretion in response to food intake, the so-called incretin effect (McIntyre *et al.*, 1964; Rubenstein, 1979). In view of the high rate of food intake in lactation (Cripps & Williams,

Table 1. *Blood metabolite and plasma insulin concentrations for fed rats and for rats starved for 6 and 24 h*
Results are expressed as means $\pm$ S.E.M. Numbers of rats in each group are shown in parentheses. Values significantly different from fed controls are shown: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$.

	Fed	Starved (6h)	Starved (24h)
Insulin (μ units/ml)	27.5 $\pm$ 4.0 (12)	14.2 $\pm$ 2.2 (11)**	10.0 $\pm$ 1.5 (8)***
Glucose (μ mol/ml)	4.25 $\pm$ 0.12 (12)	5.20 $\pm$ 0.23 (15)***	5.03 $\pm$ 0.28 (8)*
Lactate (μ mol/ml)	1.45 $\pm$ 0.20 (12)	1.08 $\pm$ 0.14 (15)	0.75 $\pm$ 0.04 (8)***
Pyruvate (μ mol/ml)	0.160 $\pm$ 0.020 (12)	0.136 $\pm$ 0.016 (15)	0.085 $\pm$ 0.013 (8)**
Acetoacetate (μ mol/ml)	0.089 $\pm$ 0.011 (12)	0.166 $\pm$ 0.022 (15)***	0.196 $\pm$ 0.024 (8)**
β -Hydroxybutyrate (μ mol/ml)	0.077 $\pm$ 0.014 (12)	0.155 $\pm$ 0.035 (13)*	0.293 $\pm$ 0.033 (8)***
Ketone bodies (μ mol/ml)	0.166 $\pm$ 0.020 (12)	0.315 $\pm$ 0.057 (13)*	0.489 $\pm$ 0.054 (8)***

Fig. 1. *Changes in concentrations of plasma insulin, glucose and ketone bodies in conscious fed, 6h-starved and 24h-starved rats during intravenous infusion of saline (—) or insulin (---)*

For experimental details see the Methods section. Results are shown as means $\pm$ S.E.M.. Each point represents the mean of measurements for at least four animals, except where S.E.M. bars are omitted, where only two measurements were made.

1975) and the intestinal hypertrophy (Chatwin *et al.*, 1969), such hormones released from the gut acting in this way might play an important role in signalling food availability and could increase the sensitivity of response of the system regulating mammary-gland metabolism in the starvation/re-feeding transition.

Using non-physiological concentrations (3000 munits/kg per h) of insulin in the anaesthetized post-absorptive lactating rat, Burnol *et al.* (1983) have concluded that lipogenesis in the mammary gland is responsive to insulin. In the present experiments low-dose insulin infusion (120 munits/kg per h) to achieve plasma concentrations approx. 2–4-fold higher than basal values stimulated mammary-gland lipogenesis in the fed animal and partially reversed the inhibition in lipogenesis after 6 h and 24 h starvation (Table 2), demonstrating the insulin-sensitivity of this tissue *in vivo*. Combined with the changes in insulin concentration caused by starvation, this finding supports the hypothesis that insulin acts as a physiological regulator of the mammary gland to signal carbohydrate availability (Robinson *et al.*, 1978).

It is noteworthy that for the same infusion rate larger increments in plasma insulin were observed in starved than in fed animals. Since the mammary gland actively concentrates insulin (Jones *et al.*, 1984) and blood flow to the gland is lower when metabolic activity is decreased (Hanwell & Linzell, 1973; Jones & Williamson, 1984), it is possible that this difference represents decreased insulin clearance in starvation.

The failure of insulin infusion to re-activate lipogenesis completely after periods of starvation could be due to a number of factors. Firstly, in both 6 h- and 24 h-starved animals carbohydrate reserves (i.e. hepatic glycogen) were depleted (Table 3). Since it has previously been suggested that in fed animals hepatic glycogen is an important store of precursors for mammary-gland lipid synthesis (Munday & Williamson, 1983), the supply of substrate for lipogenesis *de novo* would be limited. Inhibition of mammary-gland metabolism on starvation is probably an adaptive mechanism which conserves carbohydrate reserves, and indeed insulin stimulation of lipogenesis in the mammary gland of fed, 6 h- and 24 h-starved animals led to a decrease in hepatic glycogen, although this was only significant in the fed state (Table 3). This is also reflected in the greater degree of hypoglycaemia produced by insulin infusion in the 24 h-starved animals (Fig. 1).

Secondly, other hormones, such as the gut hormones proposed above, may act synergistically with insulin to regulate the mammary gland (Carrick & Kuhn, 1978; Williamson *et al.*, 1983). The absence of such factors from preparations of

mammary-gland acini *in vitro* may explain their poor response (30% stimulation) even to high doses of insulin (Williamson *et al.*, 1983).

Finally, as starvation progresses, it is possible that changes occur within the gland, making it less responsive to acute stimulation by insulin. Both pyruvate dehydrogenase (Baxter & Coore, 1978) and acetyl-CoA carboxylase (McNeillie & Zammit, 1982; Munday & Williamson, 1982) are inactivated by 24 h starvation, and their full re-activation may require factors other than insulin or a longer period of insulin stimulation than in the present experiments (90 min). After 6 h of starvation the activation state of acetyl-CoA carboxylase activity is unchanged in mammary gland (Williamson *et al.*, 1983).

The decrease in hepatic glycogen seen with insulin infusion in fed and starved animals (Table 3) demonstrates the high priority of the mammary gland for glucose supply compared with other tissues. Although the mechanism leading to the decrease in hepatic glycogen is not certain, it could in part be explained by the small decrease in blood glucose (Fig. 1) leading to catecholamine and glucagon release. Adrenaline treatment of lactating rats does not affect rates of mammary-gland lipogenesis, though it apparently decreases the contribution of glucose to this process (Bussmann *et al.*, 1984). The fact that the fall in hepatic glycogen occurs in the presence of a raised plasma insulin concentration sufficient to stimulate mammary-gland lipogenesis implies that in the lactating rat inhibition of hepatic glycogen breakdown is relatively less sensitive to insulin, as inferred from previous studies of insulin concentrations (Robinson *et al.*, 1978), insulin receptors (Flint, 1982) and insulin metabolism (Jones *et al.*, 1984) during lactation. Similarly, the failure of insulin infusion to re-activate hepatic lipogenesis which is depressed by starvation (Table 3) points to the differential insulin-sensitivity of lipogenesis in liver and mammary gland in lactation. As expected, the hepatic glucose concentration decreased on infusion of insulin, but [glucose 6-phosphate] did not alter, and citrate was decreased only in the fed state (Table 3).

Infusion of glucagon into fed lactating rats, at a rate sufficient to cause a 5–6-fold increase in plasma concentration of the hormone (Soman & Felig, 1978), caused no change in the rates of lipogenesis in liver, mammary gland or adipose tissue (Table 4). The lack of inhibition of mammary-gland lipogenesis by glucagon is in accord with findings *in vitro* (Williamson *et al.*, 1983; Robson *et al.*, 1984), and is consistent with the view that the increased glucagon/insulin ratio observed in lactation acts to direct substrates towards the mammary gland and away from other tissues (Robinson *et al.*,

Table 2. Rates of lipogenesis and metabolite concentrations in mammary glands of lactating rats infused with saline or insulin

For experimental details and correction of [glucose] see the Methods section. Rates of lipogenesis are expressed as $\mu\text{mol of } ^3\text{H}_2\text{O}$ incorporated into saponified lipid/h per g wet wt. of tissue. Metabolite concentrations are expressed as $\mu\text{mol/g wet wt. of tissue}$. Numbers of rats in each group are shown in parentheses. Values significantly different from matched saline controls are shown: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$. Values significantly different from fed controls are shown: † $P < 0.05$; †† $P < 0.01$; ††† $P < 0.005$.

	Fed		Starved (6h)		Starved (24h)	
	Saline	Insulin	Saline	Insulin	Saline	Insulin
Lipogenesis	73 ± 19 (8)	146 ± 22 (5)*	8.0 ± 1.5 (8)††	54 ± 11 (8)***	2.7 ± 0.5 (4)†††	21.5 ± 9.0 (6)
Glucose	1.15 ± 0.17 (8)	0.44 ± 0.13 (5)**	1.76 ± 0.10 (6)††	1.16 ± 0.22 (5)*	2.02 ± 0.14 (6)†††	0.76 ± 0.21 (6)**
Glucose (corrected)	0.17 ± 0.15 (7)	-0.25 ± 0.10 (5)*	0.63 ± 0.17 (6)	0.33 ± 0.17 (5)	1.14 ± 0.23 (3)††	0.16 ± 0.16 (6)*
Glucose 6-phosphate	0.117 ± 0.018 (8)	0.067 ± 0.016 (5)	0.164 ± 0.026 (6)	0.110 ± 0.015 (5)	0.190 ± 0.012 (4)††	0.060 ± 0.010 (6)***
Citrate	0.044 ± 0.006 (7)	0.031 ± 0.001 (3)	0.087 ± 0.075 (5)†	0.075 ± 0.013 (5)	0.119 ± 0.019 (3)††	0.098 ± 0.010 (4)
ATP	1.22 ± 0.109 (8)	1.19 ± 0.300 (5)	1.50 ± 0.146 (6)	1.14 ± 0.077 (5)	1.03 ± 0.190 (4)	0.70 ± 0.140 (6)

Table 3. Rates of lipogenesis and metabolite concentrations in livers of lactating rats infused with saline or insulin

For experimental details see the Methods section. Rates of lipogenesis are expressed as $\mu\text{mol of } ^3\text{H}_2\text{O}$ incorporated into saponified lipid/h per g wet wt. of tissue. Metabolite concentrations are expressed as $\mu\text{mol/g wet wt. of tissue}$. Numbers of rats in each group are shown in parentheses. Values significantly different from matched saline controls are shown: * $P < 0.05$; ** $P < 0.01$. Values significantly different from fed saline controls are shown: † $P < 0.05$; †† $P < 0.01$.

	Fed		Starved (6h)		Starved (24h)	
	Saline	Insulin	Saline	Insulin	Saline	Insulin
Lipogenesis	14.8 ± 1.1 (8)	16.8 ± 3.4 (5)	10.8 ± 2.0 (6)	9.3 ± 1.3 (8)	4.0 ± 1.3 (4)††	3.0 ± 0.6 (6)
Glycogen	80.5 ± 12.8 (8)	37.7 ± 9.5 (5)*	27.5 ± 7.8 (6)†	10.8 ± 2.8 (5)	8.0 ± 3.0 (4)††	3.9 ± 1.9 (6)
Glucose	5.51 ± 0.24 (8)	4.20 ± 0.42 (5)*	6.60 ± 0.48 (6)	5.16 ± 0.37 (5)*	6.00 ± 0.089 (3)	4.90 ± 1.02 (4)
Glucose 6-phosphate	0.282 ± 0.036 (8)	0.263 ± 0.072 (5)	0.300 ± 0.050 (6)	0.247 ± 0.024 (5)	0.220 ± 0.089 (4)	0.181 ± 0.068 (6)
Citrate	0.231 ± 0.032 (8)	0.134 ± 0.020 (3)*	0.220 ± 0.040 (6)	0.182 ± 0.048 (5)	0.181 ± 0.014 (4)	0.197 ± 0.052 (4)
ATP	1.96 ± 0.087 (8)	1.87 ± 0.066 (5)	2.07 ± 0.080 (6)	1.99 ± 0.099 (5)	1.60 ± 0.364 (4)	1.53 ± 0.250 (6)

Table 4. *Rates of lipogenesis in liver and mammary gland of lactating rats after infusion of saline or glucagon*
For experimental details see the Methods section.
Rates of lipogenesis are expressed as μmol of $^3\text{H}_2\text{O}$ incorporated/h per g wet wt. of tissue.

	Saline	Glucagon
Liver	18.4 ± 3.2 (7)	14.9 ± 1.7 (5)
Mammary gland	83.5 ± 24.7 (7)	90.9 ± 23.6 (5)
White adipose tissue	1.77 ± 0.81 (7)	0.88 ± 0.32 (5)

1978; Jones *et al.*, 1984; Robson *et al.*, 1984). That no significant inhibition of white-adipose-tissue lipogenesis was observed is not surprising, as this process is known to be inactivated in lactation (Smith, 1973; Robinson *et al.*, 1978; Flint *et al.*, 1979).

Possible site of insulin action

Five main sites of regulation of the mammary-gland lipogenic pathway have been suggested in the literature: glucose transport (Threadgold *et al.*, 1982), hexokinase and phosphofructokinase (Martin & Baldwin, 1971; Williamson *et al.*, 1975; Sochor *et al.*, 1984), pyruvate dehydrogenase (Baxter & Coore, 1978) and acetyl-CoA carboxylase (McNeillie & Zammit, 1982; Munday & Williamson, 1982), all of which may show sensitivity to insulin. The questions of whether one site predominates in the overall regulation of the pathway or whether and how control at the different sites is co-ordinated remain open.

From the results in Table 2 it is clear that the initial steps in the glycolytic pathway are acutely modulated by food intake. There is a significant inverse correlation between concentrations of glucose 6-phosphate and lipogenesis *in vivo* in the control animals ($r = 0.59$, $n = 18$, $P < 0.005$). This indicates that a step beyond glucose 6-phosphate is inhibited when glucose utilization by the gland is decreased. Preliminary results (not shown) indicate that fructose biphosphate concentrations decrease when lipogenesis is inhibited, pointing to inhibition of phosphofructokinase as the primary cause of the increase in glucose 6-phosphate. Modulation of phosphofructokinase activity as a point of regulation in the lactating mammary gland has been suggested previously from work on tissue slices (Williamson *et al.*, 1975) and in diabetic rats (Martin & Baldwin, 1971) and from measurements of fructose 2,6-bisphosphate concentrations (Sochor *et al.*, 1984). Its mode of regulation, as in other tissues, may be linked to ketone-body metabolism through changes in citrate concentrations (Randle *et al.*, 1964). Inhibition of glucose utilization by ketone bodies in isolated acini can be reversed by insulin, with concomitant lowering of

citrate concentrations (Robinson & Williamson, 1977). The findings in the present study suggest that this mechanism may occur *in vivo*, since starvation causes an increase in circulating ketone-body concentrations (Table 1) and a rise in citrate concentrations in the mammary gland (Table 2). Insulin infusion tended to reverse these effects, most notably in the 24h-starved animals. ATP concentrations, in contrast, were unchanged on starvation, were unaffected by insulin infusion and are therefore not likely to have a significant effect on phosphofructokinase activity in this situation. Information on the changes in the concentrations of fructose 2,6-bisphosphate in mammary gland in response to starvation would be of considerable interest.

The increase in mammary-gland intracellular glucose and glucose 6-phosphate concentrations induced by starvation make it unlikely that plasma-membrane glucose transport is the predominant regulatory site in the mammary-gland lipogenic pathway. This conclusion contrasts with the report (Threadgold & Kuhn, 1984) which concluded, on the basis of measurements of glucose transport by using 2-deoxyglucose and 3-O-methylglucose, that transport was a rate-limiting step for glucose utilization by lactating mammary gland on transition from the fed to the starved state. If transport was the predominant regulatory step, then intracellular glucose and glucose 6-phosphate concentrations would be expected to decrease on starvation, unless one postulates a feed-forward signal which causes inhibition of phosphofructokinase when transport is decreased.

A possible reason for the different emphasis placed by these two studies (Threadgold & Kuhn, 1984; the present paper) on the importance of glucose transport as a point of regulation may relate to inhibition of hexokinase. Uptake measurements using 2-deoxyglucose assume that the tracer is trapped by phosphorylation, and the degree of phosphorylation should remain constant if valid comparison are to be made. In the present study the percentage of intracellular glucose carbon in the phosphorylated state decreases as starvation proceeds (fed, 40%; starved 6h, 21%; starved 24h, 10%), suggesting inhibition of hexokinase. Such a decrease could clearly lead to an underestimate of glucose transport by using 2-deoxyglucose. This finding contrasts with the data of Threadgold & Kuhn (1984), where the percentage phosphorylation of deoxyglucose was constant and high (70%) in fed and starved (16h) animals.

Thus it seems that not all regulatory steps in the lipogenic pathway are inhibited simultaneously. From the present study, hexokinase and phosphofructokinase appear to be inhibited by 6h starvation, when pyruvate dehydrogenase [as judged

from arterio-venous differences (Williamson *et al.*, 1983)] and acetyl-CoA carboxylase (Williamson *et al.*, 1983) are still active. After 24 h of starvation, pyruvate dehydrogenase (Baxter & Coore, 1978) and acetyl-CoA carboxylase are inactivated (McNeillie & Zammit, 1982; Munday & Williamson, 1982), together with inhibition of glucose transport (Threadgold & Kuhn, 1984). It is not at present known whether glucose transport is inhibited *in vivo* after 6 h starvation. Re-feeding after starvation for both 6 h and 24 h rapidly re-activates lipogenesis, which may mean that re-activation in these two situations occurs by the same mechanisms. Whether changes in insulin and ketone-body concentrations are the sole signals to regulate glucose utilization *in vivo* by the lactating gland in starvation remains an open question.

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