Impaired Lipogenesis in Mammary Glands of Lactating Rats Fed on a Cafeteria Diet

REVERSAL OF INHIBITION OF GLUCOSE METABOLISM *IN VITRO* BY INSULIN

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In lactating rats fed on a cafeteria diet (chow plus palatable high-energy foods) the decreased glucose uptake and lipogenesis *in vitro* in acini correlated with the depressed mammary-gland lipogenesis *in vivo*. Insulin *in vitro* restored the rate of glucose uptake and its conversion to lipid to values approaching those for acini from rats fed on the chow diet alone.

Cafeteria feeding, in which the animals are offered a variety of palatable high-energy foods in addition to their normal chow diet, induces hyperphagia, and the eventual development of obesity (Sclafani & Springer, 1976; Rolls *et al.*, 1979).

Experiments on lactating rats given cafeteria food from parturition indicate that the growth rate of the litter is depressed relative to that of controls fed on chow only, even though the weight of the mother increases more than that of the controls. This poor litter performance is associated with a decreased rate of mammary-gland lipogenesis *in vivo* (B. J. Rolls, E. A. Rowe, S. Fahrback, L. Agius & D. H. Williamson, unpublished results). In chow-fed lactating rats short-term insulin withdrawal *in vivo* results in a decreased rate of mammary-gland lipogenesis (Robinson *et al.*, 1978). Starvation (24 h) of lactating rats in the presence of the pups also depresses mammary-gland lipogenesis *in vivo* and this is rapidly reversed on re-feeding, when plasma insulin increases (Robinson *et al.*, 1978). However, addition of insulin *in vitro* does not relieve the inhibition of lipogenesis observed in acini isolated from starved lactating rats (Robinson & Williamson, 1977a).

The aim of the present experiments was to test whether the inhibition of mammary-gland lipogenesis in the rats fed on a cafeteria diet could be demonstrated *in vitro* using preparations of acini isolated from the mammary gland and to examine the possible effects of insulin on glucose metabolism and lipogenesis.

Experimental

Female hooded Lister rats (about 280 g) were fed

on a normal chow diet throughout pregnancy until parturition. They were then divided into two groups: one was fed on only chow, and the other was given an assortment of palatable supermarket foods (potato crisps, chocolate chip cookies and cheese crackers) in addition to the chow. For the approximate energy contents and composition of the foods, see Rolls *et al.* (1979). The litters were culled to eight pups after delivery and the experiments were performed between 8 and 12 days after parturition.

Measurements of the rates of lipogenesis *in vivo* were made with $^3\text{H}_2\text{O}$ as described previously (Robinson *et al.*, 1978). All experiments were carried out between 09:30 and 11:30 h. The rats were injected (intraperitoneal) with 5 mCi (0.5 ml) of $^3\text{H}_2\text{O}$ and were anaesthetized with pentobarbital (60 mg/kg body wt.; solution in 0.9% NaCl) about 50 min after injection of $^3\text{H}_2\text{O}$. The litters were then removed and at 60 min duplicate samples (about 1 g) of the inguinal mammary gland were removed. The weighed tissue was added to 3 ml of 30% KOH, and lipid was saponified and extracted by the method of Stansbie *et al.* (1976b).

The rest of the mammary gland was used for the preparation of acini (Robinson & Williamson, 1977a). The incubation conditions were as described by Robinson & Williamson (1977a). [1- ^{14}C]Glucose (5 mM) was added as substrate and the concentration of insulin used was 50 m-i.u./ml. The following metabolites were determined in the neutralized HClO_4 extracts of the incubation medium by standard enzymic methods: glucose (Slein, 1963), lactate and pyruvate (Hohorst *et al.*, 1959). The conversion of [1- ^{14}C]glucose into $^{14}\text{CO}_2$ and lipid was measured as described by Robinson & Williamson (1977a).

Crystalline insulin free from glucagon was a gift from Dr. N. Z. Lazarus (Wellcome Research Laboratories, Beckenham, Kent, U.K.). All enzymes, including collagenase (grade II) and coenzymes were obtained from Boehringer Corp. (London) Ltd., London W.5, U.K. $^3\text{H}_2\text{O}$ and $[1-^{14}\text{C}]\text{glucose}$ were obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Rates of glucose utilization and lactate and pyruvate production by acini were obtained from the graphical plots of the 20, 40 and 60 min values. Rates of conversion of $[1-^{14}\text{C}]\text{glucose}$ into $^{14}\text{CO}_2$ and incorporation into the lipid fraction were obtained in the same way. Measurement of rates excluded possible interference from the presence of ^3H -labelled lipid in the acini, and tests showed that contribution of $[^3\text{H}]$ lipid to the total radioactivity in the lipid fraction of the acini after incubation with $[1-^{14}\text{C}]\text{glucose}$ was small.

Results and Discussion

There was a positive correlation (coefficient 0.93) between the rate of mammary-gland lipogenesis *in vivo* and the rate of glucose utilization *in vitro* by acini isolated from the same rats for both the control

and the cafeteria-diet-fed group (Fig. 1). The rate of glucose utilization by acini from cafeteria-diet-fed rats was decreased by 46% compared with control acini, and this was accompanied by a similar decrease in the rate of conversion of $[1-^{14}\text{C}]\text{glucose}$ to $^{14}\text{CO}_2$ and of its incorporation into lipid (Table 1). The proportion of glucose utilized that accumulates in the medium as lactate was increased by 85% in acini from cafeteria-diet-fed rats. These results suggest that the decreased lipogenesis observed *in vivo* may be related to the defects in glucose metabolism found *in vitro*. Both the rates of lipogenesis *in vivo* and the rate of glucose utilization by acini *in vitro* reported in Table 1 for the hooded Lister rats fed on a chow diet are similar to those found in this laboratory for Wistar rats (Robinson & Williamson, 1977a,b; Robinson *et al.*, 1978; Agius *et al.*, 1979).

Addition of insulin only had a small effect on glucose removal (16% increase; $P < 0.025$) in acini from chow-fed rats, whereas in acini from the cafeteria-diet-fed rats it increased the removal of glucose by 79% ($P < 0.005$) to give values similar to the control group. In acini from cafeteria-diet-fed rats insulin also increased the conversion of $[1-^{14}\text{C}]\text{glucose}$ to $^{14}\text{CO}_2$ and its incorporation into lipid

Fig. 1. Relationship between glucose utilization in isolated acini and mammary gland lipogenesis *in vivo*. For details see the Experimental section. The rates of glucose utilization in acini are expressed as $\mu\text{mol}/\text{min}$ per 100mg defatted dry wt. and the rates of mammary-gland lipogenesis *in vivo* as μmol of $^3\text{H}_2\text{O}$ incorporated into lipid/h per g wet wt. of tissue. The correlation coefficient $r = 0.93$ ($n = 14$). Chow-fed rats (●) and cafeteria-diet-fed rats (○).

Table 1. *Effects of insulin on [1-¹⁴C]glucose metabolism in acini isolated from lactating mammary glands of chow- and cafeteria-diet-fed rats*

For details see the Experimental section. Where indicated, insulin (50 m.i.u./ml) was added to the incubation medium. The results are mean values $\pm$ s.d. expressed as $\mu\text{mol/min}$ per 100 mg defatted dry wt. with the number of observations in parentheses. Values obtained in the presence of insulin that are significantly different (Student's *t*-test) from those obtained in its absence are indicated by: *P < 0.05; **P < 0.005.

	Normal (8)		Cafeteria-diet-fed (7)	
		+Insulin		+Insulin
Glucose removal	1.29 $\pm$ 0.17	1.49 $\pm$ 0.18*	0.70 $\pm$ 0.32	1.25 $\pm$ 0.27**
[1- ¹⁴ C]Glucose $\rightarrow$ ¹⁴ CO ₂	0.44 $\pm$ 0.09	0.51 $\pm$ 0.07	0.23 $\pm$ 0.06	0.42 $\pm$ 0.07**
[1- ¹⁴ C]Glucose $\rightarrow$ ¹⁴ C-labelled lipid	0.32 $\pm$ 0.04	0.45 $\pm$ 0.05*	0.16 $\pm$ 0.04	0.37 $\pm$ 0.05**
Lactate formation	0.46 $\pm$ 0.22	0.55 $\pm$ 0.25	0.35 $\pm$ 0.13	0.71 $\pm$ 0.30*
Pyruvate formation	0.13 $\pm$ 0.04	0.12 $\pm$ 0.04	0.10 $\pm$ 0.03	0.12 $\pm$ 0.04

by 83% and 132% respectively, whereas the corresponding increases in acini from chow-fed rats were 16% and 40% respectively. The proportion of glucose accumulating in the medium as lactate was greater (*P* < 0.05) for the acini from the cafeteria-diet-fed rats, suggesting a possible depression of pyruvate dehydrogenase activity which was not reversed by insulin *in vitro* (Table 1; see also Robinson & Williamson, 1977a). Formation of pyruvate was similar in acini from chow- and cafeteria-diet-fed rats and was not greatly affected by addition of insulin. These experiments *in vitro* indicate that the impaired metabolism of glucose in acini from cafeteria-diet-fed rats can be virtually restored to normal by the addition of insulin. It is of interest to compare this situation with that of short-term starvation (24 h) of lactating rats where the rate of lipogenesis in mammary gland *in vivo* is inhibited by 98% (Robinson *et al.*, 1978), and addition of insulin *in vitro* to acini does not restore the rate of lipogenesis to normal (Robinson & Williamson, 1977a). One reason for this difference between the findings *in vitro* with acini from starved and cafeteria-diet-fed rats may be related to the fact that the formation of lactate is considerably higher in the acini from starved rats.

Variations in composition of dietary intake influence milk-fat composition (Garton, 1963; Coniglio & Bridges, 1966), and consequently would be expected to influence mammary-gland lipogenesis. Lactating rats eating the cafeteria foods have a high fat intake (35% by energy) compared with chow-fed rats (6% by energy; B. J. Rolls, E. A. Rowe, S. Fahrbach, L. Agius & D. H. Williamson, unpublished results). High-fat diets have been shown to decrease mammary gland and also liver lipogenesis *in vivo* in lactating mice (Romsos *et al.*, 1978), as well as incorporation of acetate into lipid in rat mammary-gland slices *in vitro* (Coniglio & Bridges, 1966). However, others have found little or

no effect of high-fat diet on lipogenesis in mouse or rat mammary gland *in vitro* (Smith *et al.*, 1969; Farid *et al.*, 1978). It is of interest that the cafeteria foods do not depress hepatic lipogenesis *in vivo* during lactation (B. J. Rolls, E. A. Rowe, S. Fahrbach, L. Agius & D. H. Williamson, unpublished results). Stansbie *et al.* (1976a) have shown that feeding rats a diet containing 40% fat for about 20 days results in a substantial decrease in total pyruvate dehydrogenase activity in adipose tissue and a diversion of glucose metabolism away from fatty acid synthesis to lactate output. If a similar decrease occurred in the mammary glands of the cafeteria-diet-fed rats this might explain the increased accumulation of lactate in the medium. In contrast with the present results the defects in glucose metabolism in adipocytes from rats fed on a high-fat diet (70% fat) are not corrected *in vivo* by addition of insulin (Lavau *et al.*, 1979).

There is considerable evidence that composition of the diet can affect the sensitivity of various tissues to insulin (Beck-Nielsen *et al.*, 1978; Lavau *et al.*, 1979; Wigand *et al.*, 1979). It is possible that in the present experiments the hyperphagia induced by the palatability of the cafeteria diet may eventually lead to decreased insulin secretion or decreased sensitivity of the mammary gland to insulin. Alternatively, in the cafeteria-diet-fed rat, insulin *in vitro* may relieve the inhibition by an intracellular regulator that suppresses lipogenesis *in vivo*. We have observed a higher fat content in the mammary gland of rats fed on the cafeteria diet (B. J. Rolls, E. A. Rowe, S. Fahrbach, L. Agius & D. H. Williamson, unpublished results), but we have not established whether this excess fat is in the acini or in the adipocytes of the mammary tissue. Whatever the mechanism for the impaired lipogenesis and its reversal by insulin observed in the present experiments it would seem reasonable for the rate of lipid synthesis *de novo* in mammary gland to be de-

creased when excess fat is present in the diet. In this way, glucose might be spared for lactose synthesis in the gland and for utilization by other tissues, without necessarily altering the fat content of the milk (Coniglio & Bridges, 1966; Farid *et al.*, 1978). Lactose synthesis in rat mammary gland does not appear to be regulated, at least in the short-term, by insulin (Wilde & Kuhn, 1979). However, decreased dietary intake affects lactose synthesis *in vivo* (Wilde & Kuhn, 1979), and it would be important to establish whether a change in composition of the diet can also influence this process.

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