Regulation of peripheral lipogenesis by glucagon

Inability of the hormone to inhibit lipogenesis in rat mammary acini *in vitro* in the presence or absence of agents which alter its effects on adipocytes

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1. The rate of lipogenesis in acini isolated from mammary glands of mid-lactating rats was studied by measuring the rate of incorporation of ^3H from $^3\text{H}_2\text{O}$ into total lipid and fatty acids, with glucose as substrate. 2. Glucagon did not affect the rate of lipogenesis in acini. 3. Glucagon did not antagonize the maximal stimulatory effect of insulin, nor did it alter the insulin dose–response curve. 4. Theophylline, at concentrations up to 20 mM, was a potent inhibitor of lipogenesis in acini. Glucagon did not augment the degree of inhibition of lipogenesis induced by 5 mM-theophylline. 5. The results suggest that mammary-gland acini do not respond to glucagon *in vitro* under conditions in which the hormone induces inhibition of lipogenesis (the present paper) and of individual key steps in the lipogenic pathway in adipocytes [Zammit & Corstorphine (1982) *Biochem. J.* **208**, 783–788; Green (1983) *Biochem. J.* **212**, 189–195]. 6. In agreement with these observations, we could detect only a minimal degree of specific binding of ^{125}I -labelled glucagon to acini which bound insulin normally. 7. This difference in responsiveness of mammary and adipose cell preparations *in vitro* to glucagon suggests that the two tissues may be differentially responsive to changes in the circulating insulin/glucagon concentration ratio *in vivo*. The significance of these findings for the regulation of substrate utilization for lipogenesis in the two tissues during lactation is discussed.

Adipose tissue and mammary gland are both insulin-sensitive tissues. Acute changes in insulin concentration result in rapid alterations in the rate of lipogenesis (Stansbie *et al.*, 1976; Agius *et al.*, 1980) and in the fraction of key lipogenic enzymes, such as acetyl-CoA carboxylase (EC 6.4.1.2) and pyruvate dehydrogenase (EC 1.2.4.1) in the active state in both tissues (Coore *et al.*, 1971; Baxter & Coore, 1978; McNeillie & Zammit, 1982). This similarity in response to acute (but unphysiological) changes in circulatory insulin concentrations raises the question as to the mechanism(s) whereby the lipogenic activity of the two tissues is differentially regulated during lactation. Thus, in the mid-lactating rat, the mammary gland is the major lipogenic tissue, whereas fatty acid synthesis in adipose tissue is suppressed (Robinson *et al.*, 1978; Agius *et al.*, 1979, 1980). This reciprocal behaviour of the lipogenic rate in the two tissues is partly mediated by long-term changes in enzyme concentrations (Smith, 1973; Mackall & Lane, 1977; Sinnott-Smith *et al.*, 1980; Martyn &

Hansen, 1981). However, adipose tissue of mid-lactating rats possesses substantial amounts of lipogenic enzymes [e.g. acetyl-CoA carboxylase and pyruvate dehydrogenase (Smith, 1973; Sinnott-Smith *et al.*, 1980)], which are likely to be under hormonal control in order to prevent competition between lipogenesis in adipose and mammary tissues. Thus the animal requires to maintain the circulatory insulin concentration at a value which is sufficiently high to enable maximal lipogenesis in the mammary gland, while simultaneously preventing the lipogenic effects of insulin on adipose tissue. One possible mechanism that has been suggested (Williamson, 1980, 1981) to account for the apparent selective sensitivity of mammary tissue to insulin during lactation is that a second hormone, which would act antagonistically to insulin, affects adipose tissue but not mammary tissue. Several hormones, particularly prolactin (Agius *et al.*, 1979; Williamson, 1980, 1981), have been suggested for such a role. However, these are likely to mediate such an antagonistic effect

through long-term changes in enzyme concentrations (McNeillie & Zammit, 1982).

Acute regulation of the lipogenic rate in mammary and adipose tissues would be expected to be related to the nutritional status of the animal so as to allow rapid physiological responses to conditions such as short-term starvation and re-feeding as well as diurnal variations in the rate of food intake. Therefore the properties required of a hormone to fulfil this proposed functional role are that it should antagonize the action of insulin in adipose tissue, but not in mammary tissue, and that its circulatory concentrations should be related to the nutritional status of the animal. The possibility that glucagon satisfies these criteria for adipose tissue emerged through recent observations that, at near-physiological concentrations, glucagon inhibits key steps in the pathway of lipogenesis from glucose in isolated adipocytes *in vitro*. Thus the initial activity of acetyl-CoA carboxylase (Zammit & Corstorphine, 1982) and the transport of 2-deoxyglucose (in adipocytes from starved rats; Green, 1983) are markedly inhibited by glucagon. Inhibition of these steps is accompanied by an inhibition of lipogenesis in adipocytes (the present paper). The relationships between the effects of glucagon and insulin on adipocytes suggest that lipogenesis in adipose tissue *in vivo* could be very sensitive to changes in the circulatory insulin/glucagon concentration ratio (Zammit & Corstorphine, 1982). If lipogenesis in mammary tissue is not sensitive to such inhibition by glucagon and is acutely regulated *in vivo* by changes in circulatory insulin, rather than by the insulin/glucagon ratio, a mechanism would exist enabling the two tissues in effect to be differentially responsive to circulatory insulin concentrations.

In the present study we have investigated the effects of glucagon and other agents (which are known to affect lipid metabolism in adipose tissue) on the rates of lipogenesis in acini isolated from mammary glands of fed mid-lactating rats. The results suggest that glucagon has no effect on lipogenesis in mammary acini *in vitro*.

Materials and methods

Rats

The source and maintenance of rats was as described previously (Zammit, 1980, 1981). Rats were used 10–12 days *post partum*.

Preparation of acini and adipocytes

Rats were anaesthetized with pentobarbitone (60 mg/kg body wt.) at 09:30 h; all subsequent procedures were carried out in a warm room at 37°C. The inguinal/abdominal mammary glands were rapidly dissected and placed in oxygenated

(O₂/CO₂, 19:1) Krebs–Henseleit (1932) bicarbonate-buffered saline containing 5 mM-glucose (referred to below as 'saline'). The tissue was minced with scissors and finely chopped with razor blades. The resulting minced tissue was rinsed twice in 5 vol. of saline and resuspended in 30 ml of saline which contained 20 mg of fatty acid-poor albumin/ml, 50 mg of dialysed Ficoll/ml and 1 mg of collagenase/ml. The suspension was placed in a 250 ml plastic conical flask, which was incubated with shaking (180 strokes/min) for 60 min. The flask was gassed throughout with O₂/CO₂. The resulting digested tissue was strained, and the acini were washed three times and separated by light centrifugation from a medium containing 2% Ficoll in saline. Finally the acini were resuspended in 6.5 ml of saline containing 2% Ficoll and 40 mg of albumin/ml.

Adipocytes were prepared essentially as described for acini, except that Ficoll was omitted from the media.

Incubations of acini and adipocytes

Portions (1 ml) of the final suspensions of acini or adipocytes were dispensed into each of six 25 ml plastic conical flasks, which contained 4 ml of the same resuspension medium, either without or with addition of hormones and/or effectors. The flasks were gassed, stoppered and incubated, with shaking, for 15 min, after which time 1 mCi of ³H₂O (100 µl) was added to each flask and incubations were allowed to proceed for a further 45 min. Rates of ³H incorporation into lipid by acini were determined to be linear over this time interval. At the end of the incubations the acini were separated by centrifugation (400g) and homogenized in 6 ml of chloroform/methanol (2:1, v/v) with a Polytron tissue homogenizer (Kinematica, Basle, Switzerland). For adipocytes the incorporation of ³H₂O was performed over 15 min after 5 min of exposure to hormones.

Extraction of acini and quantification of lipid

After the first extraction of acini with chloroform/methanol, the solid matter was re-extracted with another 6 ml of the same solvent mixture. To the combined extracts (12 ml) were added 3 ml of 0.88% KCl and 0.1 ml of 6M-HCl. The mixture was shaken and spun (400g) to obtain two distinct phases. The lower phase was rinsed twice with 5 ml of methanol/0.88% KCl/chloroform (48:47:3, by vol.). Finally, the lower phase was filtered (Whatman no. 1 filter paper) and evaporated to dryness in a rotary evaporator. When required, a few drops of ethanol were added to ensure removal of traces of water. The dry residue was redissolved

in 6 ml of chloroform/methanol (9:1, v/v), transferred to a scintillation vial and evaporated to dryness under a stream of nitrogen. The residue was redissolved in 2 ml of toluene. A portion of this toluene solution (0.95 ml) was taken for measurement of ^3H in total lipid. Another sample was taken for extraction of fatty acids as follows. To 0.95 ml of the toluene solution was added 2 ml of methanolic H_2SO_4 (1 vol. of concn. H_2SO_4 in 100 vol. of methanol); the mixture was heated at 50°C overnight in a stoppered glass tube, cooled and extracted with 4 ml of n-heptane and 4 ml of water. The upper phase was retained. The lower phase was washed again with n-heptane. The combined organic phases were washed with 3 ml of water, and the radioactivity was measured to obtain a value for the incorporation of ^3H into fatty acids. Radioactivity in a sample of the combined lower phase and washings was measured to obtain a value for the incorporation of ^3H into acylglycerol glycerol. This procedure is considerably more involved and lengthy than those used by other workers (see, e.g., Robinson & Williamson, 1977b). However, it was designed specifically for studying lipogenesis in mammary tissue, since it prevents the loss of short-chain fatty acids, which form a substantial proportion of the fatty acids synthesized by mammary tissue (Strong & Dils, 1972).

Quantification of lipogenesis by adipocytes

This was performed as described by Stansbie *et al.* (1976) after separation of adipocytes from the medium by light centrifugation. NADP⁺-malate dehydrogenase activity was measured as described previously (Zammit & Corstorphine, 1982).

Chemicals

Fatty acid-poor albumin, adenosine deaminase (EC 3.5.4.4), aprotinin, bacitracin, antipain, leupeptin, pepstatin, crystalline bovine insulin, Ficoll 400, theophylline and pentobarbitone were from Sigma (Poole, Dorset, U.K.). Crystalline pig glucagon was generously given by Lilly Research Laboratories (Indianapolis, IN, U.S.A.). Collagenase (EC 3.4.24.3) was type CLS III from Worthington (Flow Laboratories, Irvine, Scotland, U.K.).

Albumin and Ficoll were dialysed exhaustively (against water) before use. Stock solutions of insulin (1 mg/ml) and glucagon (1 mg/ml) were stored in acid solution in small batches at -20°C and used as required. Adenosine deaminase was dialysed against 0.9% NaCl, and its activity was measured immediately before use (Zammit & Corstorphine, 1982). One unit of activity refers to the utilization of $1\text{ }\mu\text{mol}$ of adenosine/min at 25°C .

Results and discussion

The absolute rates of ^3H incorporation into fatty acids by acini in the absence of any additions were similar to the rates of fatty acid synthesis reported previously by others workers (Robinson & Williamson, 1977b; Munday & Williamson, 1981). Experiments in which the incorporation of ^3H from $^3\text{H}_2\text{O}$ into lipid was measured as a function of time demonstrated that rates of lipogenesis were linear between 5 and 60 min. Subsequently rates were measured routinely between 15 and 60 min, the initial 15 min period being used to ensure the attainment of the full response to the effectors added. The incorporation of label into acylglycerol glycerol was about 20% of that into fatty acids under all conditions studied.

Incubation of acini with glucagon had no effect on the rates of synthesis of total lipid or fatty acids (Table 1). The concentration of glucagon used in the present study was about 100-fold higher than those found to give a maximal inhibitory response on acetyl-CoA carboxylase activity in adipocytes (Zammit & Corstorphine, 1982). This high concentration was used to ensure that degradation of the hormone by acini did not significantly deplete the incubations of glucagon. The complete lack of effect of glucagon on the rate of lipogenesis in mammary acini was in contrast with the inhibition of lipogenesis observed in adipocytes (Table 2) and in agreement with results of two determinations reported by Williamson *et al.* (1983) while the present paper was in preparation.

Inclusion of insulin in the incubation medium resulted in a marked stimulation (50–60%) of lipogenesis in mammary acini (Table 1). This stimulation was much larger than that observed previously (Robinson & Williamson, 1977a; Agius & Williamson, 1980) for lactating rats fed on a comparable diet, although a similar degree of stimulation by insulin was reported by the same workers when they used acini isolated from rats fed on a high-energy diet (Agius & Williamson, 1980). Incubation of acini with both insulin and glucagon in the incubation medium did not have any significant effect on the rate of lipogenesis in acini, compared with the rates obtained when insulin alone was added, contrary to the antagonistic effects of the two hormones in adipocytes (Zammit & Corstorphine, 1982; Green, 1983). Moreover, glucagon did not affect the sensitivity to insulin of lipogenesis by acini, as judged by its lack of effect on the dose-response curve for insulin (Fig. 1).

In experiments with adipocytes, incubation with theophylline results in inhibition of lipogenesis (see Trost & Stock, 1979). Therefore, in order to test whether glucagon might have an inhibitory effect on lipogenesis in mammary acini by aug-

Table 1. *Effects of glucagon, insulin or glucagon plus insulin on the rates of incorporation of ^3H from $^3\text{H}_2\text{O}$ into total lipid, fatty acid and acylglycerol glycerol in acini from mammary glands of mid-lactating rats*

Acini were incubated as described in the Materials and methods section for 15 min either in the absence of any additions or in the presence of glucagon (2 $\mu\text{g}/\text{ml}$) or insulin (1 $\mu\text{g}/\text{ml}$) or a combination of the two hormones. After 15 min, $^3\text{H}_2\text{O}$ (100 μl , containing 1 mCi) was added and the incubations were allowed to proceed for a further 45 min. The acini were separated from the medium by centrifugation and extracted as described in the Materials and methods section. Values are means $\pm$ S.E.M. for determinations on separate preparations of acini (numbers shown in parentheses). Levels of statistical significance (paired *t* test) for the differences in rates obtained under different conditions from those obtained in the absence of additions are denoted by **P* < 0.01, and †*P* < 0.05.

Additions	Incorporation of ^3H from $^3\text{H}_2\text{O}$ ($\mu\text{mol}/\text{min}$ per 100 mg defatted dry wt.)		
	Total lipid	Fatty acid	Acylglycerol glycerol
None (11)	0.543 $\pm$ 0.023	0.429 $\pm$ 0.026	0.113 $\pm$ 0.007
Glucagon (11)	0.561 $\pm$ 0.027	0.432 $\pm$ 0.033	0.129 $\pm$ 0.017
Insulin (11)	0.812 $\pm$ 0.042*	0.668 $\pm$ 0.046*	0.146 $\pm$ 0.011
Glucagon plus insulin (3)	0.907 $\pm$ 0.110*	0.774 $\pm$ 0.160†	0.133 $\pm$ 0.015

Table 2. *Effects of insulin and glucagon on the rate of ^3H incorporation from $^3\text{H}_2\text{O}$ into fatty acids by adipocytes from parametrial adipose tissue*

Adipocytes were prepared and incubated in a manner identical with that described for mammary acini (see the Materials and methods section), except that Ficoll was omitted from the media. Adipocytes were incubated for 5 min in the absence or presence of insulin or glucagon, after which 1 mCi of $^3\text{H}_2\text{O}$ was added (100 μl) and the rate of lipogenesis measured after a further 15 min of incubation as described by Stansbie *et al.* (1976). Values are means $\pm$ S.E.M. for three separate determinations and are expressed as ^3H incorporation into fatty acids per munit of NADP⁺-malate dehydrogenase activity at 30°C in the infranatant of a sample of adipocytes disrupted by vortex-mixing in a glass tube. Values significantly different from controls (*P* < 0.01) are indicated by *.

Additions	Incorporation of ^3H from $^3\text{H}_2\text{O}$ ($\mu\text{mol}/\text{min}$ per munit of malate dehydrogenase activity)
None	13.3 $\pm$ 0.3
Insulin (1 $\mu\text{g}/\text{ml}$)	17.5 $\pm$ 0.3*
Glucagon (2 $\mu\text{g}/\text{ml}$)	9.0 $\pm$ 0.2*

menting the potential effects of theophylline, experiments were performed in which either glucagon or insulin was added to the incubation medium simultaneously with theophylline (5 mM). Theophylline at this concentration inhibited the rate of lipogenesis by about 15% (Table 3, experimental series I). Glucagon did not augment this effect. However, insulin still had a stimulatory effect on lipogenic rates in the presence of theophylline (Table 3).

A similar series of experiments was performed

Fig. 1. *Dose-response curve for the effect of insulin on the rate of lipogenesis by mammary-gland acini in the absence (○) or presence (●) of glucagon*

Concentrations of hormones were as given in the legend to Table 1. Values are means $\pm$ S.E.M. for three separate determinations.

with adenosine and adenosine deaminase, which, in adipose tissue, induce lipogenic and antilipogenic effects respectively (Trost & Stock, 1979; Honnor & Saggerson, 1980). Neither adenosine nor adenosine deaminase affected the lipogenic rates of mammary acini (Table 3, series II). Glucagon added in combination with adenosine deaminase also did not result in any inhibition of the rate of lipogenesis (Table 3). Both adenosine and adenosine deaminase were added to acini at con-

Table 3. Effects of glucagon and insulin on the rates of incorporation of ³H from ³H₂O into total lipid, fatty acid and acylglycerol glycerol in the presence of theophylline or adenosine deaminase in acini from mammary glands of lactating rats. Acini were incubated for 15 min in the presence of hormones and/or effectors (see Table 1); ³H₂O was added and incubations were terminated after a further 45 min. Values are means ± s.e.m. for two separate series of experiments (I and II; n = 4 and 3 respectively). See Table 1 for statistical treatment of data (*P < 0.01, †P < 0.05).

Series of experiments . . .	Incorporation of ³ H from ³ H ₂ O (μmol/min per 100mg defatted dry wt.)					
	Total lipid		Fatty acid		Acylglycerol glycerol	
	I	II	I	II	I	II
None	0.64 ± 0.15	0.52 ± 0.03	0.51 ± 0.14	0.40 ± 0.03	0.14 ± 0.01	0.12 ± 0.02
Glucagon (2 μg/ml)	0.65 ± 0.10	0.56 ± 0.05	0.47 ± 0.10	0.41 ± 0.12	0.18 ± 0.03	0.15 ± 0.05
Insulin (1 μg/ml)	1.03 ± 0.31*	0.79 ± 0.05*	0.72 ± 0.18*	0.62 ± 0.06*	0.17 ± 0.01	0.18 ± 0.02
Theophylline (5 mM)	0.56 ± 0.13†	—	0.45 ± 0.11†	—	0.12 ± 0.06	—
Theophylline + glucagon	0.55 ± 0.14†	—	0.42 ± 0.14†	—	0.13 ± 0.01	—
Theophylline + insulin	0.81 ± 0.20	—	0.63 ± 0.20†	—	0.18 ± 0.04	—
Adenosine deaminase (1 unit/ml)	—	0.55 ± 0.06	—	0.42 ± 0.06	—	0.13 ± 0.01
Adenosine deaminase + glucagon	—	0.55 ± 0.06	—	0.41 ± 0.05	—	0.14 ± 0.02
Adenosine (50 μM)	—	0.56 ± 0.08	—	0.41 ± 0.06	—	0.15 ± 0.04

Fig. 2. Effects of theophylline on the rate of incorporation of ³H from ³H₂O into total lipid (●) and fatty acids (■) by rat mammary-gland acini

Acini were incubated with the appropriate concentration of theophylline for 15 min, and the rate of incorporation of ³H was measured over the following 45 min after addition of 1 mCi of ³H₂O.

amounts of cell protein in these incubations were much higher than those involved in adipocyte incubations (see, e.g., Zammit & Corstorphine, 1982). Therefore rates of production and/or destruction of adenosine in incubations of acini could be much higher, thus tending to mask the effects of exogenously added adenosine or adenosine deaminase.

The observation that theophylline, when added to the incubation medium at a concentration of 5 mM, gave a modest but significant inhibition of lipogenesis in acini prompted us to study the dependence of the extent of inhibition on increasing theophylline concentrations (Fig. 2). The results suggest that theophylline, in the concentration range normally used in studies on isolated adipocytes to increase intracellular concentrations of cyclic AMP and stimulate lipolysis (Mosinger & Vaughan, 1967; Steinfeldt & Joost, 1982), is a potent inhibitor of lipogenesis in mammary acini. Although direct measurements of cyclic AMP concentrations were not performed on acini in the present study, it is presumed that the inhibitory effects observed with theophylline on lipogenic rates were accompanied by a rise in the concentration of the cyclic nucleotide. The persistence of the lipogenic effect of insulin under these conditions would be compatible with the known ability of insulin to reverse modest increases in intracellular concentrations of cyclic AMP in isolated adipocytes (Manganiello *et al.*, 1971) and hepatocytes (Pilkis *et al.*, 1975).

Physiological considerations

The most significant finding of the present study was that glucagon had no effect on lipogenesis in

centrations in excess of those required to elicit their respective responses in adipose tissue. However, the apparent unresponsiveness of acini to these agents has to be interpreted with caution, since the

Table 4. *Specific binding of ^{125}I -labelled insulin, but not of ^{125}I -labelled glucagon, to rat mammary acini*

Specific binding and degradation of ^{125}I -labelled insulin (sp. radioactivity 50–70 $\mu\text{Ci}/\mu\text{g}$) were determined in the presence of bacitracin (600 units/ml) exactly as described by Flint (1982), except that the pH was 7.6 and the time of incubation was 24 h. The results obtained were similar to those reported by Flint (1982) for mammary acini of 10-day lactating rats. Identical protocols were followed to determine the binding and degradation of ^{125}I -labelled glucagon (2.5 ng/ml). The results of three separate experiments, each done with a single preparation of acini, are given. Three ^{125}I -labelled glucagon preparations, with different specific radioactivities, were used as follows: Expt. 1, 52 $\mu\text{Ci}/\mu\text{g}$; Expt. 2, 346 $\mu\text{Ci}/\mu\text{g}$; Expt. 3, 295 $\mu\text{Ci}/\mu\text{g}$. Those used in Expts. 1 and 2 were prepared from native glucagon by the chloramine-T method, whereas that used in Expt. 3 was a monoiodinated commercial product (NEN Chemicals, Dreieich, Germany). Control experiments (not shown) demonstrated that neither by shortening the duration of the incubation (1 h, 18°C) nor by diminishing the extent of glucagon degradation (by using aprotinin, 2.5 units/ml; pepstatin, 0.5 mM; leupeptin, 0.5 mM; and antipain, 0.5 mM, singly or in various combinations) was it possible to increase the measurable specific glucagon binding: indeed, both modifications resulted in decreases in binding.

Expt. no.	^{125}I -labelled insulin		^{125}I -labelled glucagon	
	Specific binding (c.p.m./100 μg of DNA)	Degradation (%)	Specific binding (c.p.m./100 μg of DNA)	Degradation (%)
1	3571	5	164	60
2	7239	10	559	42
3	5601	5	432	33

mammary acini: it neither antagonized the stimulatory effect of insulin nor enhanced the inhibitory effect of theophylline. In addition it caused no change in the dose–response curve obtained for the degree of stimulation of lipogenesis by increasing concentrations of insulin. Nevertheless, the ability of acini to respond *in vitro* to other effectors suggests that the preparation procedure did not impair the potential regulatory mechanisms involved in the stimulation or inhibition of the rate of lipogenesis. Indeed, the degree of stimulation of lipogenesis in acini produced by insulin was very similar to the extent of stimulation of lipogenesis or acetyl-CoA carboxylase activity observed in insulin-treated adipocytes (Table 2; see also Brownsey *et al.*, 1979; Zammit & Corstorphine, 1982). Consequently, the lack of effect of glucagon on acini (but not on adipocytes) leads us to suggest that mammary secretory cells do not respond to glucagon. In this respect it is noteworthy that we could not detect significant specific binding of glucagon to preparations of acini which showed normal specific binding of insulin (Table 4). Therefore it is suggested that, unlike that in adipose tissue, mammary tissue lipogenesis is not likely to be sensitive to circulatory glucagon *in vivo*.

If this suggestion is valid, it would have important physiological implications for the reciprocal regulation of the rates of lipogenesis in adipose tissue and mammary gland in the lactating rat. Thus, whereas the concentration of insulin in rat plasma during lactation is somewhat lower than that in unmated animals (Kuhn, 1977; Robinson *et al.*, 1978; Flint *et al.*, 1979), the circulatory insulin/glucagon ratio in lactating rats is about half that in virgin animals (Robinson *et al.*, 1978).

Consequently, for a tissue that is sensitive to the insulin/glucagon ratio (such as adipose tissue) the hormone-sensitive steps of the lipogenic pathway will be relatively inhibited, whereas in a tissue that is not sensitive to glucagon (mammary gland) the activity of the lipogenic pathway would not be markedly affected. Hence the differential responsiveness of the two tissues to circulatory insulin during lactation would be achieved.

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