Regulation of rat mammary-gland uptake of orally administered [1-¹⁴C]triolein by insulin and prolactin: evidence for bihormonal control of lipoprotein lipase activity

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The effects of insulin deficiency (streptozotocin-induced) or insulin plus prolactin deficiency on the disposal of orally administered [1-¹⁴C]triolein between oxidation to ¹⁴CO₂, uptake by mammary gland and transfer to suckling pups were studied. Insulin deficiency decreased mammary-gland total weight (by 40%), but caused no change in total tissue DNA. A greater proportion of the [1-¹⁴C]triolein was oxidized to ¹⁴CO₂ (120% increase) in the insulin-deficient rats, and there was a tendency for total transfer of [¹⁴C]lipid to mammary gland and suckling pups to be decreased. A parallel decrease in total mammary-tissue lipoprotein lipase activity occurred. Combined hormone deficiency caused more dramatic changes in all parameters measured. Replacement of insulin (24 h) in insulin-deficient rats decreased ¹⁴CO₂ production, increased the uptake of [¹⁴C]lipid into the mammary gland and tended to increase total lipoprotein

lipase activity. In contrast, administration of prolactin to insulin-deficient rats had no effect on any of the parameters measured. Replacement of insulin (24 h) in the combined hormone-deficient rats increased uptake of [¹⁴C]lipid and lipoprotein lipase approx. 3-fold, whereas prolactin again had no significant effects. Replacement of both hormones increased (6-fold) transfer of [¹⁴C]lipid to the pups, but did not increase overall uptake of [¹⁴C]lipid (mammary gland, milk clot and pups) above the value for insulin alone. It is concluded that insulin is the primary regulator of triacylglycerol uptake and of lipoprotein lipase activity in the lactating mammary gland of the rat and that the action of prolactin on these processes is indirect. Prolactin, but not insulin, appears to have a direct effect on milk lipid transfer to pups.

INTRODUCTION

The uptake of exogenous lipid by the mammary gland in the lactating rat is considered to be controlled by the activity of the enzyme lipoprotein lipase (Hamosh et al., 1970; Flint et al., 1981). During lactation the activity of lipoprotein lipase is high in the mammary tissue and decreases rapidly 24 h after removal of the litter (Hamosh et al., 1970; Flint et al., 1981; Oller do Nascimento and Williamson, 1986). Although it is generally assumed that prolactin regulates lipoprotein lipase activity in the mammary gland (see Scow and Chernick, 1987), some evidence indicates that the regulation may not be so simple. Firstly, in starved lactating rats the activity of lipoprotein lipase in the mammary gland decreases (Oller do Nascimento and Williamson, 1988), whereas plasma prolactin remains high (Robinson et al., 1978). Secondly, if plasma prolactin is decreased by treatment with bromocryptine (Seki et al., 1974) and then insulin is administered to lactating rats, the activity of lipoprotein lipase in the lactating mammary gland increases, as does the uptake of exogenous [¹⁴C]lipid (Da Costa and Williamson, 1993). Lipoprotein lipase is under insulin control in adipose tissue (see Eckel, 1987; Borensztajn, 1987; Braun and Severson, 1992), and the enzyme activity increases in the milk of women that undergo a euglycaemic/hyperinsulinaemic clamp (Neville et al., 1991), so the hormone may also play a role in the regulation of this enzyme in mammary tissue. In the present study we have examined the effects of insulin deficiency, and of insulin plus prolactin deficiency, on the uptake of exogenous [1-¹⁴C]triolein by the mammary gland and the activity of tissue lipoprotein lipase.

MATERIALS AND METHODS

Animals

Rats used in the study were from the Wistar strain. They were mated between 2 and 2.5 months of age, and on week 2 of pregnancy the animals were transferred to individual cages, where they delivered their litters. Room temperature (21 ± 2 °C) and dark/light cycle (12 h cycle, lights on at 08:00 h) were controlled. Commercial chow (No. 3; Special Diet Services, Witham, Essex, U.K.; containing 52% carbohydrate, 21% protein, 4% fat and residue of non-digestible material) and water were given *ad libitum*. On lactation day 4 or 5, rats, with litter sizes of 8–10 pups, were selected for daily weighing and measurement of food intake. Animals were taken for experiments only if litter growth was within the normal range established from 20 litters housed in the animal house. Treatment of lactating rats started on lactation day 7 or 8.

Chemicals

Streptozotocin, ovine luteotrophic hormone (prolactin), triolein (C_{18:1, cis-9}; 99% pure) and lecithin (L-γ-phosphatidylcholine, type III-E from egg yolk) were purchased from Sigma, St. Louis, MO, U.S.A. Isophane insulin was from Novo Nordisk, Copenhagen, Denmark. Bromocryptine mesylate was kindly provided by Dr. D. Römer (Sandoz Pharma, Basle, Switzerland). [1-¹⁴C]Triolein (glycerol tri[1-¹⁴C]oleate) was from Amersham International, Amersham, Bucks., U.K. Glycerol tri[9,10(n)-³H]oleate was from Dupont, Boston, MA, U.S.A. All enzymes

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and coenzymes were purchased from Boehringer Corp. (London), Lewes, Sussex, U.K. Rat prolactin radioimmunoassay kit was obtained from IDS Ltd., Boldon, Tyne and Wear, U.K.

Treatments of the animals

On lactation day 7 or 8 a group of rats received an intravenous (tail-vein) injection of streptozotocin (50 mg/kg body wt., solution in 0.01 M citrate buffer pH 4.5) to suppress insulin secretion by the pancreatic β -cells (Schein et al., 1971). Control animals received an intravenous injection of saline. At 48 h after the injection of streptozotocin, when the diabetic state was fully established and confirmed by urine glucose measurement, a group of animals were treated for 24 h with bromocryptine (Seki et al., 1974), to lower plasma prolactin levels. Bromocryptine was given by subcutaneous injections at a dose of 10 mg/kg body wt. [solution in 10% (v/v) ethanol containing 1% (w/v) tartaric acid], every 8 h, starting at 09:00 h. Animals that received saline through the tail vein were treated with subcutaneous injection of saline (control) or bromocryptine (prolactin-deficient) as above. Hormone replacement with isophane insulin and/or prolactin was performed as subcutaneous injections. Rats replaced with hormones for 24 h received injections at 09:00 h and 17:00 h. A third injection was given just after the oral [14 C]lipid load (09:00 h next day). Animals replaced for 5 h received only the last injection. Insulin dosage was 4.5 i.u./24 h, divided as two injections of 2.0 i.u. and one of 0.5 i.u. All intravenous injections were made under light ether anaesthesia. Prolactin dosage consisted of three injections of 20 i.u. each.

Measurements of disposal of [14 C]triolein load and lipoprotein lipase activity

The procedure used has been described in detail by Oller do Nascimento and Williamson (1986). Briefly, the disposal of an oral load of [14 C]triolein (0.6 g, $\sim 0.5 \mu\text{Ci}/\text{rat}$), which gives an indication of functional lipoprotein lipase activity, was measured. After the gastric intubation of [14 C]triolein, the oxidation of the oral lipid load ($^{14}\text{CO}_2$ production) was measured hourly over 5 h. Samples of tissues (mammary gland, white adipose tissue, liver etc.) were extracted for saponified-lipid determination (Stansbie et al., 1976). The stomachs of the pups were dissected, and the milk clot was removed, weighed, and the lipid content and radioactivity were measured. The remainder of the pup carcass and the maternal carcass (excluding the pieces of tissues taken for extraction, the total mammary gland and the intestinal tract) were saponified and lipid was extracted. Lipoprotein lipase activity in acetone-dried powders of mammary gland and parametrial adipose tissue was determined by the method of Nilsson-Ehle and Schotz (1976).

Blood metabolites, plasma prolactin and DNA in mammary tissue

Blood was obtained from the abdominal aorta 5 h after the oral [14 C]triolein load. Whole-blood glucose was determined by the method of Slein (1963), ketone bodies were determined by that of Williamson et al. (1962) and plasma triacylglycerol was determined by that of Eggstein and Kreutz (1966). DNA was determined in the mammary-tissue acetone-dried powders by the colorimetric method of Giles and Myers (1965). Plasma prolactin was measured by radioimmunoassay. Prolactin was not measured in the groups replaced with ovine prolactin, because of the low cross-reactivity of the rat anti-prolactin antibody.

Statistical analysis and data presentation

Statistical analysis was performed by one-way analysis of variance (ANOVA) for repeated measurements combined with Tukey test, using CSTAT and SAS programmes. Results are given as means $\pm$ S.E.M. for the number of observations (n) indicated. Data on pup weight change over the period studied (Figure 1) were compared by Student's t test for independent samples.

RESULTS AND DISCUSSION

Effects of insulin deficiency, or combined insulin plus prolactin deficiency on pup weights

After administration of streptozotocin on lactation day 7, the pups started to decrease body-weight gain, and 48 h later there was a marked and significant decrease in weight gain compared with the control group (Figure 1; see also Walters and McLean, 1968; Martin and Baldwin, 1971; Ikawa et al., 1992). In the group where dams were insulin + prolactin-deficient for 24 h, the pups lost weight (Figure 1). The food intake (g) for the last 24 h was slightly decreased ($P < 0.05$) in all treated groups: control, 63.9 ± 2.3 (6); insulin-deficient, 50.9 ± 2.1 (6); prolactin-deficient, 48.2 ± 1.2 (6); combined deficiency, 50.8 ± 2.0 (5). If the results are expressed as g/100 g body wt., then only the prolactin-deficient group is significantly decreased ($P < 0.05$).

Blood metabolites and plasma prolactin

The diabetic rats had marked hyperglycaemia, but no increase in blood ketone bodies or plasma triacylglycerols (Table 1). The insulin + prolactin-deficient rats had increased blood ketone bodies and a tendency to hypertriglyceridaemia (Table 1). The diabetic rats had similar plasma prolactin values to control animals (Table 1). This is in contrast with the finding of a decrease in plasma prolactin in short-term (2 h) streptozotocin treatment (Robinson et al., 1978) and in the long-term streptozotocin-diabetic lactating rats (Ikawa et al., 1992).

Changes in mammary-gland weight, lipid content and milk secretion

Insulin deficiency decreased (40%) the fresh weight of mammary gland (see also Walters and McLean, 1968), whereas prolactin

Figure 1 Effects of hormone deficiency on pup weight change

Treatment of dams is described in the text. Control (○), insulin-deficient (□) and insulin + prolactin-deficient (▽) groups (six rats in each group) were studied. Arrow (a) indicates the intravenous injection of streptozotocin to induce insulin-deficiency; arrow (b) indicates the subcutaneous injection of bromocryptine to make the dams prolactin-deficient. The control rats received saline injections. Differences by Student's t test are indicated: ** $P < 0.01$, *** $P < 0.001$, compared with the control group.

Table 1 Concentrations of blood glucose and ketone bodies, plasma triacylglycerols and prolactin

For experimental details see the text. The results are expressed as means $\pm$ S.E.M. for n observations. The difference among the groups was determined by analysis of variance and group comparison performed by Tukey test. Significant difference is indicated by * $P < 0.05$ compared with the control group.

Experimental group	n	Glucose ($\mu\text{mol/ml}$)	Ketone bodies ($\mu\text{mol/ml}$)	Triacylglycerol ($\mu\text{mol/ml}$)	Prolactin (ng/ml)
Control	5	5.86 ± 0.72	0.30 ± 0.05	0.89 ± 0.27	110 ± 4.9
Insulin-deficient	6	$16.60 \pm 2.80^*$	0.51 ± 0.05	1.04 ± 0.24	95 ± 15.0
Prolactin-deficient	5	6.27 ± 0.08	0.30 ± 0.03	1.03 ± 0.19	—
Insulin + prolactin-deficient	5	$18.50 \pm 0.60^*$	$2.66 \pm 1.18^*$	8.35 ± 5.65	$1.9 \pm 0.4^*$

Table 2 Total weight and percentage of fat in mammary gland and milk clot

For experimental detail see the text. The results are expressed as means $\pm$ S.E.M. for n observations. The difference among the groups was determined by analysis of variance and group comparison performed by Tukey test. Significant difference is indicated by * $P < 0.05$ compared with the control group and † $P < 0.05$ compared with the prolactin-deficient group.

Experimental group	n	Mammary gland		Milk clot	
		Weight (g)	Fat (%)	Weight (g)	Fat (%)
Control	6	21.0 ± 1.0	12.8 ± 0.9	4.7 ± 0.6	25.3 ± 0.7
Insulin-deficient	6	$13.1 \pm 1.0^*$	10.4 ± 0.6	$2.3 \pm 0.4^*$	20.3 ± 1.2
Prolactin-deficient	5	19.6 ± 0.8	$22.9 \pm 1.3^*$	$0.5 \pm 0.1^*$	18.8 ± 2.6
Insulin + prolactin-deficient	5	$11.4 \pm 1.4^*$	$13.2 \pm 1.8^\dagger$	$0.8 \pm 0.2^*$	$8.8 \pm 3.2^\dagger$

Table 3 Effects of insulin-, prolactin- or insulin + prolactin-deficiency on the oxidation of an oral [$1\text{-}^{14}\text{C}$]triolein load and accumulation of [^{14}C]lipid in carcass and liver

For experimental details see the text. The results are expressed as means $\pm$ S.E.M. for n observations. The difference among the groups was determined by analysis of variance and group comparison performed by Tukey test. Significant difference is indicated by * $P < 0.05$ compared with control.

Experimental group	n	Radioactivity (% of absorbed dose/5 h)		
		$^{14}\text{CO}_2$	Total carcass	Liver
Control	6	7.9 ± 1.9	9.8 ± 0.6	2.49 ± 0.36
Insulin-deficient	6	$17.4 \pm 1.4^*$	10.1 ± 0.6	2.35 ± 0.29
Prolactin-deficient	5	15.9 ± 2.9	$13.4 \pm 2.9^*$	$7.93 \pm 0.9^*$
Insulin + prolactin-deficient	5	$34.7 \pm 2.8^*$	12.1 ± 1.1	4.46 ± 0.68

deficiency did not cause any significant change (Table 2). Combined hormone deficiency did not significantly decrease the tissue weight below that found with insulin deficiency alone (Table 2). Measurements of DNA showed that there was an increase in the diabetic group [control, 2.36 ± 0.26 ($n = 6$) mg of DNA/g wet wt. of tissue; insulin-deficient group, 3.28 ± 0.12 ($n = 6$) mg of DNA/g wet wt. of tissue; $P = 0.009$]; however, after correction for the total tissue mass the two groups had similar DNA content [control, 48.2 ± 3.34 ($n = 6$) mg of DNA/total gland; insulin-deficient group, 42.8 ± 2.69 ($n = 6$) mg of DNA/total gland]. Thus the change in mammary-gland weight

in the insulin-deficient group is due to loss of content (milk and water) and not a decrease in cell number. This finding underlines a potential problem in expressing the results per g of fresh tissue (or of acetone-dried powder for lipoprotein lipase) when changes in total mass of tissue occur. Where appropriate, we have therefore expressed the results per total mammary gland, which gives an indication of whole-body capacity of the mammary tissue (see also Walters and McLean, 1968). The RNA content per total gland is approximately halved in alloxan-diabetes (Walters and McLean, 1968), but unchanged on a per-g-of-tissue basis (Martin and Baldwin, 1971).

The fat content of the mammary gland significantly increased (79%) in the prolactin-deficient group (Table 2), and this may be due to decreased transfer of milk to pups (Table 4; Da Costa and Williamson, 1993). Insulin-, prolactin- and combined hormone-deficiency decreased milk clot weight. Only when both plasma prolactin and insulin concentrations were decreased was the lipid content of the milk clot affected (Table 2).

Partitioning of an oral [$1\text{-}^{14}\text{C}$]triolein load to $^{14}\text{CO}_2$ and carcass [^{14}C]lipid accumulation

Insulin-deficient lactating rats showed a 120% ($P < 0.05$) increase in the amount of $^{14}\text{CO}_2$ exhaled after the [^{14}C]lipid load (Table 3). The increase in the amount of [^{14}C]lipid oxidized was not accompanied by any change in the amount of [^{14}C]lipid incorporated into the carcass (minus liver, gastrointestinal tract and mammary gland). Insulin + prolactin-deficient lactating rats had a much higher increase (4-fold) in $^{14}\text{CO}_2$ production after the oral [^{14}C]lipid load, with no significant increase in the [^{14}C]lipid accumulated into the maternal carcass, although the amount of [^{14}C]lipid accumulated in the liver increased (79%). On the other

Table 4 Effects of insulin, prolactin- or insulin + prolactin-deficiency on the accumulation of [^{14}C]lipid in the mammary gland, milk clot and litter carcasses

For experimental details see the text. The results are expressed as means $\pm$ S.E.M. for the n observations. The difference among the groups was determined by analysis of variance and group comparison performed by Tukey test. Significant difference is indicated by $*P < 0.05$ compared with the control group. Abbreviation: NEFA, non-esterified fatty acids.

Experimental group	n	Radioactivity (% of absorbed dose/5 h)				Total activity (μmol of NEFA released/min) Mammary-gland lipoprotein lipase
		Total mammary gland (a)	Total milk clot (b)	Litter (c)	Total transfer (a + b + c)	
Control	6	47.6 $\pm$ 3.6	19.2 $\pm$ 2.4	5.5 $\pm$ 0.4	72.3 $\pm$ 5.9	12.4 $\pm$ 1.7
Insulin-deficient	6	42.6 $\pm$ 3.5	11.4 $\pm$ 2.0*	3.6 $\pm$ 0.5	57.6 $\pm$ 2.5	7.3 $\pm$ 0.8*
Prolactin-deficient	5	43.2 $\pm$ 2.8	0.2 $\pm$ 0.2*	0.7 $\pm$ 0.3*	44.1 $\pm$ 3.3*	4.6 $\pm$ 1.1*
Insulin + prolactin-deficient	5	17.2 $\pm$ 6.5*	1.2 $\pm$ 1.1*	0.9 $\pm$ 0.7*	19.3 $\pm$ 7.8*	2.7 $\pm$ 1.4*

Table 5 Effects of hormone replacement on insulin- or combined insulin + prolactin-deficient rats

For experimental details see the text. The results are expressed as means $\pm$ S.E.M. for n observations. The difference among the groups was determined by analysis of variance and group comparison performed by Tukey test. Significant difference is indicated by $*P < 0.05$ compared with the appropriate group without hormone replacement. Abbreviation: NEFA, non-esterified fatty acids.

Experimental group	Replacement	n	Mammary-gland weight (g)	Radioactivity (% of absorbed dose/5 h)					Total activity (μmol of NEFA released/min) Mammary-gland lipoprotein lipase
				$^{14}\text{CO}_2$	Total mammary gland (a)	Total milk clot (b)	Litter (c)	Total transfer (a + b + c)	
Insulin-deficient	None	6	13.2 $\pm$ 1.0	17.4 $\pm$ 1.4	42.6 $\pm$ 3.5	11.4 $\pm$ 2.0	3.6 $\pm$ 0.5	57.6 $\pm$ 2.5	7.3 $\pm$ 0.8
	+ Insulin (24 h)	5	18.3 $\pm$ 1.4	5.1 $\pm$ 0.46*	73.0 $\pm$ 2.7*	7.2 $\pm$ 3.4	2.3 $\pm$ 1.0	82.6 $\pm$ 4.2*	10.3 $\pm$ 1.8
	+ Prolactin (24 h)	5	13.8 $\pm$ 1.3	18.1 $\pm$ 1.5	44.3 $\pm$ 4.6	5.0 $\pm$ 2.2	2.6 $\pm$ 0.7	51.9 $\pm$ 6.7	3.6 $\pm$ 1.1 (4)
Insulin + prolactin-deficient	None	5	11.4 $\pm$ 1.4	34.7 $\pm$ 2.8	17.2 $\pm$ 6.5	1.2 $\pm$ 1.1	0.9 $\pm$ 0.7	19.3 $\pm$ 7.8	2.7 $\pm$ 1.4
	+ Insulin (24 h)	5	17.3 $\pm$ 1.3*	6.3 $\pm$ 0.9*	63.8 $\pm$ 4.0*	2.0 $\pm$ 1.1	0.6 $\pm$ 0.2	66.4 $\pm$ 4.0*	9.2 $\pm$ 1.2*
	+ Prolactin (24 h)	5	13.5 $\pm$ 0.6	23.9 $\pm$ 4.7	32.8 $\pm$ 6.1	1.7 $\pm$ 0.8	0.8 $\pm$ 0.4	35.3 $\pm$ 6.6	3.4 $\pm$ 0.6
	+ Prolactin + insulin (24 h)	5	15.1 $\pm$ 1.1	11.2 $\pm$ 2.0*	57.4 $\pm$ 7.5*	8.1 $\pm$ 2.9*	1.6 $\pm$ 0.5	67.1 $\pm$ 5.2*	11.2 $\pm$ 0.8*

hand, prolactin deficiency caused no change in the amount of $^{14}\text{CO}_2$ exhaled after the [^{14}C]lipid load, but increased (37 %) the amount of [^{14}C]lipid incorporated into the carcass and caused a 3-fold increase in the amount of [^{14}C]lipid accumulated into the liver. We have included this new group of prolactin-deficient rats in the present study to allow direct comparison with the other experimental groups and for measurement of mammary-gland lipid content.

Mammary-gland [^{14}C]lipid uptake and transfer to pups

The insulin-deficient state did not cause any difference in the amount of [^{14}C]lipid accumulated in the mammary gland (Table 4), but there was a decrease in the accumulation of radioactivity in the milk clot and a tendency ($P < 0.06$) for the total [^{14}C]lipid transferred through the mammary gland (mammary gland plus milk clot plus litter) to decrease. In this group, total mammary-gland lipoprotein lipase activity decreased by 41 % (Table 4). Similarly, prolactin deficiency did not alter the amount of [^{14}C]lipid transferred to the mammary gland, but virtually suppressed [^{14}C]lipid milk secretion (Table 4; Da Costa and Williamson, 1993). The total transfer of [^{14}C]lipid through the mammary gland decreased by 40 %, compared with a decrease in lipoprotein lipase activity of 63 % (see also Flint et al., 1981; Oller do Nascimento et al., 1989; Da Costa and Williamson, 1993).

Treatment of insulin-deficient rats with bromocryptine (24 h

prolactin-deficiency) caused a more dramatic change in all parameters. Mammary-gland [^{14}C]lipid accumulation decreased (62 %), and the stomachs of the pups contained only traces of milk clot together with bile secretion, indicating a considerable decrease in milk transfer (93 %). Total [^{14}C]lipid transfer through the mammary gland was decreased (73 %) and lipoprotein lipase activity decreased (78 %) (Table 4). These results indicate that deficiency of both insulin and prolactin is required to demonstrate appreciable impairment of [^{14}C]lipid uptake by the mammary gland and its transfer to the litter.

Hormone replacement

In the short-term insulin replacement (5 h), there was a tendency for insulin to decrease $^{14}\text{CO}_2$ production, and to increase ^{14}C lipid accumulation and lipoprotein lipase activity in mammary gland (results not shown). After 24 h treatment with insulin these changes were significant, except for the increase in whole mammary-tissue lipoprotein lipase (Table 5). Plasma prolactin was not decreased by insulin deficiency (Table 1), and administration of ovine prolactin to insulin-deficient lactating rats caused no change in the partitioning of the [^{14}C]triolein compared with the insulin-deficient rats (Table 5). This indicates that excess prolactin cannot stimulate lipoprotein lipase activity and/or uptake of exogenous [^{14}C]lipid by mammary gland in the insulin-deficient rat, whereas insulin can stimulate both in the prolactin-deficient rat (Da Costa and Williamson, 1993).

The effects of insulin replacement for 24 h were even more dramatic in the lactating rats with combined insulin and prolactin deficiency (Table 5). Production of $^{14}\text{CO}_2$ decreased by 82 %, the accumulation of ^{14}C lipid in the total mammary gland increased approx. 4-fold and lipoprotein lipase activity increased 3-fold; there was, however, no increase in [^{14}C]lipid in the milk clot (Table 5). Prolactin (24 h) replacement alone did not significantly change any of the parameters measured, whereas insulin + prolactin replacement gave a picture similar to that of insulin replacement alone, except that the [^{14}C]lipid in the milk clot increased nearly 7-fold (Table 5). The weights of milk clot (g) were: combined deficiency, 0.82 ± 0.25 (5); insulin replacement, 1.48 ± 0.45 (5); insulin plus prolactin replacement, 4.2 ± 0.71 (5); the last was significantly different ($P < 0.001$) from the combined deficiency.

Concluding remarks

Previous work from this laboratory has shown that mammary-gland uptake of exogenous lipid and tissue lipoprotein lipase activity are stimulated by insulin when prolactin secretion is inhibited by bromocryptine (24 h) (Da Costa and Williamson, 1993). It was suggested either that insulin was the primary regulator or that it acted in combination with prolactin. At first sight the comparatively small decrease (20 %) in total uptake (mammary gland, milk clot plus pups) of exogenous lipid in insulin deficiency (72 h) would appear to be against the view that it is a primary regulator. However, this decrease was paralleled by a 40 % decrease in total mammary-gland lipoprotein lipase activity (Table 4). It must also be emphasized that the dose of streptozotocin used would be expected to give a moderate diabetic state with residual insulin secretion (Schein et al., 1971). Confirmation of this is that the diabetic rats were not maintained on exogenous insulin. Indeed, it is likely that lactation protects against some of the lipid abnormalities seen in diabetes; for example, virtual cessation of lactation in the combined hormone-deficient rats was accompanied by ketonaemia and tendency to hypertriglyceridaemia (Table 1). A similar pattern occurs if the pups are removed for 24 h (results not shown).

Excess prolactin given to the diabetic rats or the rats with combined hormone deficiency was unable to restore either lipoprotein lipase activity or uptake of exogenous lipid by the mammary gland (Tables 3 and 5) in the absence of normal plasma insulin, and this suggests that prolactin does not act directly or synergistically. The dose of bromocryptine (10 mg/kg body wt.) used in the present study was higher than that used by other workers (Agius et al., 1979; Flint et al., 1981; Barber et al., 1992). Nevertheless, administration of prolactin (60 i.u./rat per day) restored pup weight gain (15.2 ± 1.6 g, $n = 4$), total clot weight (2.9 ± 0.4 g, $n = 3$) and lipoprotein lipase activity in mammary gland (9.0 ± 0.8 μmol of non-esterified fatty acid released/min per total mammary gland, $n = 4$) in bromocryptine-treated rats that were insulin-sufficient to values comparable with those of control rats. What then is the explanation for the decrease in lipoprotein lipase activity and total uptake (mammary gland, milk clot plus pups) of exogenous lipid in the prolactin-deficient rat (Table 4; Da Costa and Williamson, 1993) when plasma insulin is known to increase (Agius et al., 1979)? One possibility is the decrease in the number of insulin receptors on mammary tissue in prolactin deficiency (Flint, 1982). Another possible mechanism is the large decrease in milk transfer to the pups in the prolactin-deficient rats (Table 4; Da Costa and Williamson, 1993) that results in an increase in mammary-gland lipid content (Table 2). It is known that milk accumulation in the lactating gland causes a decrease in lipoprotein lipase activity

and in uptake of exogenous lipid (Oller do Nascimento et al., 1989), possibly via an autocrine factor (Wilde et al., 1987).

At first sight the increase in the plasma of triacylglycerols in the combined hormone-deficient rats (Table 1) would be expected to decrease the specific radioactivity of the plasma triacylglycerols and thus the apparent uptake of [^{14}C]lipid into mammary tissue (Table 4). However, the major determinant of the plasma triacylglycerol pool in these experiments is the entry rate of ^{14}C -labelled chylomicrons and the uptake of [^{14}C]lipid by the mammary gland. If the activity of lipoprotein lipase decreases as in the combined hormone-deficient rats (Table 4), [^{14}C]triacylglycerols accumulate in the plasma at approximately the same specific radioactivity as in the control rats (results not shown). Likewise, dilution of the incoming [^{14}C]lipid by the existing pool of mammary-tissue triacylglycerol will alter the apparent transfer of [^{14}C]lipid to the pups. However, the doubling of the lipid pool in mammary tissue of prolactin-deficient rats (Table 2) can only partially explain the large decrease (99 %) in [^{14}C]lipid in the milk clot of this group.

We conclude from the present experiments and our previous work (Da Costa and Williamson, 1993) that insulin is the primary regulator of triacylglycerol uptake and of lipoprotein lipase activity in mammary tissue during lactation and that prolactin acts indirectly. A primary role for insulin would explain the decrease in exogenous uptake of lipid by the mammary gland in starvation and the restoration of the uptake on re-feeding (Oller do Nascimento and Williamson, 1988). There is now good evidence that both pathways for the production of milk lipid, the synthesis of fatty acids *de novo* (lipogenesis) and the exogenous uptake of preformed lipid (chylomicrons or very-low-density lipoproteins), are controlled by insulin (see Williamson and Da Costa, 1993). The effects of the hormone on mammary-gland lipogenesis are rapid (Robinson et al., 1978), whereas those on the activity of lipoprotein lipase and the exogenous uptake pathway are somewhat slower (the present work). The present evidence suggests that prolactin, but not insulin, acts to stimulate lipid transfer from the mammary gland to the suckling pups.

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