Re-examination of the putative roles of insulin and prolactin in the regulation of lipid deposition and lipogenesis *in vivo* in mammary gland and white and brown adipose tissue of lactating rats and litter-removed rats

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1. The effects of various treatments to alter either plasma prolactin (bromocryptine administration or removal of litter) or the metabolic activity of the mammary gland (unilateral or complete teat sealing) on the disposal of oral [^{14}C]lipid between $^{14}\text{CO}_2$ production and [^{14}C]lipid accumulation in tissues of lactating rats were studied. In addition, the rates of lipogenesis *in vivo* were measured in mammary gland, brown and white adipose tissue and liver. 2. Bromocryptine administration lowered plasma prolactin, but did not alter [^{14}C]lipid accumulation in mammary gland or in white and brown adipose tissue, nor the rate of lipogenesis in mammary gland or in white and brown adipose tissue. 3. In contrast, complete sealing of teats results in no change in plasma prolactin, but a 90 % decrease in [^{14}C]lipid accumulation in mammary gland and a 4-fold increase in white and brown adipose tissue. The rate of lipogenesis in mammary gland was decreased by 95 %, but there was no change in the rate in white and brown adipose tissue. Unilateral sealing of teats resulted in a decrease in [^{14}C]lipid accumulation in white adipose tissue. 4. Removal of the litter for 24 h (low prolactin) produced a similar pattern to complete teat sealing, except that there was a 6-fold increase in lipogenesis in white adipose tissue. Re-suckling for 5 h increased plasma prolactin, but did not alter the response seen in litter-removed lactating rats. 5. Changes in lipoprotein lipase activity and in plasma insulin paralleled the reciprocal changes in [^{14}C]lipid accumulation in white and brown adipose tissue and in mammary gland. 6. It is concluded that the plasma insulin is more important than prolactin in regulating lipid deposition in adipose tissue during lactation, and that any effects of prolactin must be indirect.

INTRODUCTION

It is well established that deposition of lipid (lipid uptake and synthesis *de novo*) is depressed in white adipose tissue of the rat during lactation, despite a high food intake (see Williamson, 1980; Vernon & Flint, 1983; Scow & Chernick, 1987). The changes include a decrease in lipoprotein lipase activity (Hamosh *et al.*, 1970), which is accompanied by decreased accumulation of exogenous [^{14}C]lipid (Oller do Nascimento & Williamson, 1986) in the tissue and a decrease in the rate of fatty acid synthesis (lipogenesis) both *in vivo* (Robinson *et al.*, 1978) and *in vitro* (Smith, 1973; Flint *et al.*, 1979). These changes are all reversed on removal of the litter for 24–48 h.

A key question is the signal which depresses lipid metabolism in adipose tissue during lactation. A possible candidate is plasma prolactin, which increases in concentration in response to the suckling stimulus. Treatments which decrease plasma prolactin, such as hypophysectomy (Zinder *et al.*, 1974), removal of the litter (Hamosh *et al.*, 1970; Flint *et al.*, 1981) or administration of bromocryptine for 48 h (Flint *et al.*, 1981), decrease the activity of lipoprotein lipase in mammary gland and increase it in adipose tissue. Administration of ovine or bovine prolactin can reverse these changes in lipoprotein lipase activity in hypophysectomized (Zinder *et al.*, 1974) and bromocryptine-treated rats (Flint *et al.*, 1981), but not in rats with litters removed (Flint *et al.*, 1981). Similarly, removal of the

litter increases the rate of lipogenesis *in vivo* (Agius *et al.*, 1979) and *in vitro* (Flint *et al.*, 1981) in white adipose tissue and decreases it in the mammary gland (Agius *et al.*, 1979). Prolactin deficiency (bromocryptine treatment) causes a similar increase in lipogenesis in white adipose tissue *in vitro* (Flint *et al.*, 1981). Administration of prolactin *in vivo* within 24 h reverses these changes in the rate of lipogenesis in white adipose tissue (Agius *et al.*, 1979; Flint *et al.*, 1981).

It is, however, unlikely that prolactin acts directly on adipocytes, because Flint *et al.* (1981) were unable to demonstrate receptors for this hormone on adipocyte membranes. They suggested that the effects of prolactin were indirect and required a functional mammary gland. In addition, the present evidence for an indirect involvement of prolactin can be criticized on the following grounds: (a) hypophysectomy will decrease the plasma concentrations of other hormones (growth hormone, corticotropin) as well as prolactin; (b) the pharmacological doses of prolactin administered may contain other hormones as contaminants (Hwang *et al.*, 1972; Vorherr, 1978). Experiments with an antiserum to rat growth hormone have indicated that complete suppression of lactation, as measured by pup growth, in the rat requires the absence of both prolactin and growth hormone, although deficiency of growth hormone alone has no effect (Madon *et al.*, 1986).

We have previously demonstrated that the switch-on of exogenous [^{14}C]lipid accumulation in white adipose tissue of lactating rats on removal of the litter is

dependent on feeding and on an increase in plasma insulin (Oller do Nascimento & Williamson, 1988). In view of this finding and the uncertainties regarding the role of prolactin discussed above, we have measured the disposal of exogenous [^{14}C]lipid between $^{14}\text{CO}_2$ production and tissue accumulation (a measure of lipoprotein lipase activity; Oller do Nascimento & Williamson, 1986) and the rates of lipogenesis *in vivo* in lactating rats treated to alter the endogenous prolactin secretion and/or the functional activity of the gland. In addition, we have examined the effects of milk stasis (sealing of teats) on these processes in mammary gland.

EXPERIMENTAL

Rats

All rats (Wistar strain) were fed *ad libitum* on a chow diet consisting of 52% carbohydrate, 21% protein and 4% fat (the residue was non-digestible material; Special Diet Services, Witham, Essex, U.K.) with free access to drinking water, and were maintained at an ambient temperature of $21 \pm 2^\circ\text{C}$ with a 12 h-light/12 h-dark cycle (light from 07:00 h). Lactating rats with eight to ten pups weighed 270–350 g and were 10–12 weeks old when mated. The experiments were carried out 10–12 days after the commencement of lactation, and care was taken to check that the litters were gaining weight satisfactorily (about 1.8 g per pup per day). Food intake of the rats was measured in the 24 h period before death. Prolactin deficiency was induced by subcutaneous injection of bromocryptine (10 mg/kg body wt.; Seki *et al.*, 1974) at 09:00 h and 17:00 h. Mammary-gland teats were sealed, where stated, with a drop of Histacryl (BraunMelsungen A.G., Melsungen, Germany); effectiveness of this treatment was monitored by failure of pups to gain weight over the 24 h period in the all-teats-sealed group. In one group of rats teats were sealed unilaterally and tissue was sampled for [^{14}C]lipid accumulation, for lipogenesis and for lipoprotein lipase activity from both sides. Measurements of lipogenesis and disposal of oral [^{14}C]lipid were commenced between 09:00 and 10:00 h.

Biochemicals and radioactive compounds

All enzymes and coenzymes were from Boehringer Corp. (London), Lewes, Sussex, U.K. Bromocryptine (2-bromo- α -ergocryptine mesylate) was given by Professor E. Flückiger (Sandoz, Basle, Switzerland). $^3\text{H}_2\text{O}$, [$1\text{-}^{14}\text{C}$]triolein {glycerol tri[$1\text{-}^{14}\text{C}$]oleate and glycerol tri[9,10(n)- ^3H]oleate} were obtained from Amersham International, Amersham, Bucks., U.K.

Methods

The lipogenic (fatty acid synthesis *de novo*) rate *in vivo* was determined by using $^3\text{H}_2\text{O}$ as previously described (Robinson *et al.*, 1978). Tissues were saponified and fatty acids extracted by the method of Stansbie *et al.* (1976).

The metabolic fate of an orally administered [$1\text{-}^{14}\text{C}$]triolein load [about 0.7 g (0.33 μCi) per rat] was examined as described by Oller do Nascimento & Williamson (1986, 1988). The values for the carcass refer to whole body minus intestinal tract and portions of tissue used for lipid extraction. Duplicate samples of each tissue were taken and the results averaged. Total tissue accumulation was calculated by multiplication of this value by whole tissue (or carcass) weight.

Whole-blood glucose was determined by the method of Slein (1963).

Parametrial-adipose-tissue and mammary-gland lipoprotein lipase activities were determined by the technique of Nilsson-Ehle & Schotz (1976) and Nilsson-Ehle & Ekman (1977).

Plasma insulin was determined by radioimmunoassay with a rat insulin standard (Albano *et al.*, 1972). Plasma prolactin was determined by double-antibody radioimmunoassay with materials supplied by Dr. D. J. Flint (see Madon *et al.*, 1986).

RESULTS AND DISCUSSION

Oxidation and tissue accumulation of an oral lipid load

Decrease of plasma prolactin by either treatment with bromocryptine or removal of the litter for 24 h had no effect on the low rate of production of $^{14}\text{CO}_2$ (Tables 1 and 2) associated with lactation or the immediate period after cessation of lactation (Oller do Nascimento & Williamson, 1986, 1988; Evans & Williamson, 1988). However, sealing all the teats but allowing suckling to continue caused a significant increase (approx. 100%) in $^{14}\text{CO}_2$ production (Table 1). None of the treatments significantly affected the accumulation of lipid in the carcass (Table 1). Inhibition of prolactin secretion with bromocryptine did not decrease lipid accumulation in mammary gland or significantly increase it in adipose tissue (Table 1). This is surprising, in view of a previous report that bromocryptine administration increased lipoprotein lipase activity in white adipose tissue (4-fold) and decreased the activity in mammary gland (50%) (Flint *et al.*, 1981); however, the treatment extended over 48 h rather than 24 h as in the present experiment, and it is possible that measured activity did not represent functional lipoprotein lipase. Prolactin deficiency did, however, cause a considerable decrease in the transfer of [^{14}C]lipid to the suckling pups (Table 1), although there was no evidence of milk stasis. Assuming that transfer of [^{14}C]lipid can be equated with milk secretion, this finding would be in agreement with the smaller increase in pup weight in the 24 h after bromocryptine treatment [before treatment 1.9 ± 0.12 g/pup per 24 h, versus after treatment 0.77 ± 0.4 g/pup per 24 h; $n = 5$, $P < 0.001$; see also Madon *et al.* (1986)] and the 50% decrease in milk yield (Madon *et al.*, 1986). Sealing half the teats did not alter accumulation of [^{14}C]lipid in the total mammary tissue or its transfer to the pups (Table 1), but there was a significant decrease in [^{14}C]lipid accumulation in white adipose tissue. We have no explanation for this unexpected finding. There were no significant differences in the sum of lipid accumulation in mammary gland plus pups between control, bromocryptine-treated and half-teats-sealed groups. Sealing all the teats but allowing suckling to continue maintained plasma prolactin (Table 2), but decreased [^{14}C]lipid accumulation in the mammary gland by 90% and increased it 4-fold in white and brown adipose tissue (Table 1). Removal of the litter for 24 h caused a similar decrease in [^{14}C]lipid accumulation in the mammary gland and a tendency to a higher increase in white adipose tissue (Table 1). Re-suckling for 5 h after litter removal for 24 h increased plasma prolactin (Table 2), but did not significantly alter the pattern of change in [^{14}C]lipid accumulation brought about by removal of the litter (Table 1). Although plasma prolactin was only measured at the end of the 5 h period, others

Table 1. Effects of various treatments on the disposal of oral [$1-^{14}\text{C}$]tri-olein between $^{14}\text{CO}_2$ production and [^{14}C]lipid accumulation in carcass, mammary gland, white and brown adipose tissue of lactating rats

For experimental details see the text. The results are mean values $\pm$ S.E.M., with the numbers of rats shown in parentheses. Values that are significantly different by the Student's *t* test from those for lactating rats are shown by * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Treatment of rats	$^{14}\text{CO}_2$ (% of absorbed dose/5 h)	Tissue [^{14}C]lipid accumulation (% of absorbed dose/total tissue per 5 h)			Tissue [^{14}C]lipid accumulation (% of absorbed dose/g of tissue per 5 h)	
		Carcass	Mammary gland	Pups	White adipose tissue	Brown adipose tissue
Lactating						
Lactating (bromocryptine-treated; 24 h)	8.4 $\pm$ 1.9 (5)	46.2 $\pm$ 3.6 (5)	34.7 $\pm$ 3.0 (5)	15.1 $\pm$ 3.8 (5)	0.17 $\pm$ 0.06 (5)	1.02 $\pm$ 0.57 (5)
Lactating (half teats sealed; 24 h)	7.8 $\pm$ 1.5 (3)	52.1 $\pm$ 3.1 (4)	40.4 $\pm$ 6.2 (4)	1.8 $\pm$ 0.6 (4)*	0.29 $\pm$ 0.08 (4)	0.42 $\pm$ 0.15 (4)
Lactating (all teats sealed; 24 h)	7.6 $\pm$ 0.81 (5)	35.5 $\pm$ 4.9 (4)	37.3 $\pm$ 2.1 (7)	17.9 $\pm$ 3.1 (7)	0.03 $\pm$ 0.01 (7)*	0.13 $\pm$ 0.02 (7)
Lactating (litter removed 24 h)	17.5 $\pm$ 2.3 (4)*	42.0 $\pm$ 3.3 (6)	3.70 $\pm$ 0.83 (5)***	—	0.81 $\pm$ 0.22 (6)*	5.10 $\pm$ 0.92 (6)**
Lactating (litter removed 24 h; re-suckled 5 h)	5.1 $\pm$ 0.4 (4)	48.6 $\pm$ 2.6 (4)	3.46 $\pm$ 1.09 (4)***	—	1.57 $\pm$ 0.16 (4)***	3.98 $\pm$ 0.63 (4)**
	10.4 $\pm$ 2.4 (4)	40.8 $\pm$ 2.0 (4)	2.35 $\pm$ 0.57 (4)***	1.7 $\pm$ 0.42*	1.08 $\pm$ 0.23 (4)**	6.45 $\pm$ 0.71 (4)***

have shown that re-suckling after a 12 h removal of the litter rapidly increases plasma prolactin (within 30 min) to values found in maintained suckling (Amenomori *et al.*, 1970).

Lipoprotein lipase activity

The increase in lipoprotein lipase activity in white adipose tissue in the immediate period (24 h) after removal of the pups from lactating rats is dependent on the food intake and is suppressed by starvation (Oller do Nascimento & Williamson, 1988). In the present experiments the only treatment group which showed a significant depression of food intake (30%) was the litter-removed group, and this group had the highest activity of lipoprotein lipase in parametrial adipose tissue (Table 3). It should, however, be emphasized that all groups of lactating rats had increased food intake compared with normal female rats of similar body weight (results not shown). Inhibition of prolactin secretion with bromocryptine for 24 h did not increase lipoprotein lipase activity in white adipose tissue, but decreased the activity in mammary gland by 56%. After treatment for 48 h with bromocryptine, Flint *et al.* (1981) observed a similar decrease in activity in the mammary gland and a 4-fold increase in activity in white adipose tissue. Unilateral sealing of the teats significantly decreased the activity in white adipose tissue, whereas complete sealing resulted in a 100% increase in activity in this tissue (Table 3), despite the high plasma prolactin (Table 2). Removal of the litter for 24 h decreased the activity of lipoprotein lipase in mammary gland by 59%, but increased the activity in adipose tissue 6-fold (Table 3; see also Flint *et al.*, 1981). Thus a similar decrease in plasma prolactin (Table 2) on treatment with bromocryptine or removal of the pups produced widely different changes in lipoprotein lipase activity in white adipose tissue, although the decreases in activity in mammary gland were similar. A short-term increase in plasma prolactin by re-suckling for 5 h did not significantly decrease lipoprotein lipase activity in adipose tissue or increase it in mammary gland (Table 3). There was a good correlation (coefficient 0.94; $P < 0.005$; $n = 28$) between the value for lipoprotein lipase activity and for [^{14}C]lipid accumulation in white adipose tissue in the various situations (Tables 1 and 3). Similarly, Cryer *et al.* (1974) have shown a correlation in male rats between the activity of lipoprotein lipase activity in the epididymal fat-body and the uptake of intravenously administered chylomicron triacylglycerol labelled in the fatty acid moiety with ^{14}C .

Lipogenesis *in vivo*

Prolactin deficiency induced by bromocryptine treatment did not decrease lipogenesis in mammary tissue, but significantly increased it in liver (about 100%, Table 4; see also Agius *et al.*, 1979). The increase in lipogenesis in white adipose tissue was not significant (see also Agius *et al.*, 1979). Sealing all the teats dramatically decreased lipogenesis in the gland, but had no significant effect on liver or adipose tissue. Litter removal produced a similar decrease in lipogenesis in mammary gland and a 6-fold increase in white adipose tissue. This pattern was not altered by re-suckling for 5 h. It can be argued that any indirect effect of prolactin via the mammary gland may be impaired on re-suckling because of the loss of mammary-gland prolactin receptors

Table 2. Blood glucose and plasma insulin and prolactin in lactating rats after various treatments

For experimental details see the text. The results are mean values $\pm$ S.E.M., with the numbers of rats in parentheses. The plasma prolactin values for the lactating group are taken from Robinson *et al.* (1978). Values that are significantly different by the Student's *t* test from those for lactating rats are shown by **P* < 0.05, ****P* < 0.001.

Experimental group	Blood glucose (μ mol/ml)	Plasma insulin (μ units/ml)	Plasma prolactin (ng/ml)
Lactating	5.10 $\pm$ 0.66 (6)	23.4 $\pm$ 7.1 (6)	381 $\pm$ 58 (9)
Lactating (bromocryptine-treated; 24 h)	6.30 $\pm$ 0.24 (4)	49.1 $\pm$ 9.7 (4)	12 $\pm$ 1.8 (4)***
Lactating (half teats sealed; 24 h)	5.38 $\pm$ 0.28 (6)	38.6 $\pm$ 3.6 (9)	460 $\pm$ 129 (5)
Lactating (all teats sealed; 24 h)	6.22 $\pm$ 0.64 (6)	64.8 $\pm$ 4.7 (5)***	409 $\pm$ 134 (6)
Lactating (litter removed 24 h)	7.33 $\pm$ 0.58 (4)*	122.8 $\pm$ 15.1 (4)***	32 $\pm$ 19 (3)***
Lactating (litter removed 24 h; re-suckled 5 h)	6.14 $\pm$ 0.24 (5)	107.9 $\pm$ 17.5 (4)***	562 $\pm$ 99 (7)

Table 3. Effects of various treatments on the food intake and on the activity of lipoprotein lipase in white adipose tissue and mammary gland of lactating rats

For experimental details see the text. The results are mean values $\pm$ S.E.M., with the numbers of rats in parentheses. Values that are significantly different by the Student's *t* test from those for lactating rats are shown by **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

Treatment of rats		Food intake (g in previous 24 h)	Lipoprotein lipase activity (nmol of fatty acid released/min per mg of acetone-dried tissue)	
			Mammary gland	Parametrial adipose tissue
Lactating	(5)	47 $\pm$ 3.2	0.90 $\pm$ 0.15	0.23 $\pm$ 0.04
Lactating (bromocryptine-treated; 24 h)	(4)	50 $\pm$ 4.5	0.40 $\pm$ 0.07*	0.35 $\pm$ 0.04
Lactating (half teats sealed; 24 h)	(6)	50 $\pm$ 2.0	0.80 $\pm$ 0.05	0.12 $\pm$ 0.01*
Lactating (all teats sealed; 24 h)	(5)	39 $\pm$ 3.4	0.17 $\pm$ 0.04**	0.46 $\pm$ 0.03**
Lactating (litter removed 24 h)	(4)	33 $\pm$ 4.2*	0.37 $\pm$ 0.05*	1.41 $\pm$ 0.3**
Lactating (litter removed 24 h; re-suckled 5 h)	(4)	35 $\pm$ 4.8	0.26 $\pm$ 0.03**	0.82 $\pm$ 0.04***

on cessation of lactation (Hayden & Smith, 1981). In previous experiments from our laboratory, removal of pups also significantly increased hepatic lipogenesis (Agius *et al.*, 1979), but this was not observed in the present experiments. In general, the changes in white-adipose-tissue [14 C]lipid accumulation and the rate of lipogenesis moved in parallel in the various situations (Tables 1 and 4), the notable exception being the lactating rats with sealed teats (see below). There was a similar parallelism between the two measurements in mammary gland. There were no significant changes in the rate of lipogenesis in brown adipose tissue in response to the various treatments (Table 4).

Plasma insulin

Bromocryptine treatment (24 h) of lactating rats has been reported previously to increase plasma insulin (Agius *et al.*, 1979), but in the present experiments there was no significant difference (Table 2). On the other hand, sealing all teats increased plasma insulin 3-fold despite no change in plasma prolactin (Table 2). Removal of the litter produced an even greater increase in plasma insulin (6-fold, Table 2; see also Agius *et al.*, 1979; Flint, 1982). Administration of exogenous ovine prolactin to bromocryptine-treated or litter-removed lactating rats decreased plasma insulin (Agius *et al.*, 1979). In contrast, an increase in endogenous prolactin secretion on

re-suckling for 5 h (litter removed for 24 h) did not significantly decrease the high plasma insulin (Table 2). Overall, there was a good correlation (coefficient 0.96; *P* < 0.003; *n* = 28) between plasma insulin and [14 C]lipid accumulation in white adipose tissue. The increase in plasma insulin in the lactating rats with teats sealed or on removal of the litter (Table 2) is likely to be due to a number of factors, including: (a) increase in blood glucose; (b) decrease in mammary-gland blood flow; (c) decrease in the number of insulin receptors in mammary tissue. Flint (1982) has shown that prolactin deficiency induced by removal of the litter or by bromocryptine administration decreases the number of insulin receptors in mammary gland, and that this effect can be reversed by exogenous prolactin. It is possible that this is the mechanism whereby prolactin exerts its indirect effects on adipose-tissue lipid metabolism.

Effects of partial milk stasis

Flint and his colleagues have suggested that any regulatory actions of prolactin on mammary gland (direct effect) or adipose tissue (indirect effect) require that the mammary tissue should be actively synthesizing milk (Flint *et al.*, 1981; Flint, 1982). The finding that complete sealing of all teats with concomitant milk accumulation decreased the deposition of [14 C]lipid in mammary tissue and increased the deposition in white adipose tissue,

Table 4. Effects of various treatments on the rate of lipogenesis *in vivo* in the liver, mammary gland and white and brown adipose tissue of lactating rats

For experimental details see the text. The results are mean values $\pm$ S.E.M., with the numbers of rats shown in parentheses. Values for lactating rats are taken from Table 3 of Evans & Williamson (1988). Values that are significantly different by Student's *t* test from those for lactating rats are shown by **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

Treatment of rats		Rate of lipogenesis (μ mol of $^3\text{H}_2\text{O}$ incorporated into saponified lipid/h per g of tissue)			
		Liver	Mammary gland	White adipose tissue	Brown adipose tissue
Lactating	(8)	22.0 $\pm$ 3.2	112.8 $\pm$ 24.4	2.9 $\pm$ 0.9	33.9 $\pm$ 24.1
Lactating (bromocryptine-treated; 24 h)	(5)	45.9 $\pm$ 5.9**	136.4 $\pm$ 24.3	4.65 $\pm$ 2.7	49.8 $\pm$ 4.5
Lactating (half teats sealed; 24 h)	(5)	30.9 $\pm$ 6.6	108.7 $\pm$ 30.4	3.4 $\pm$ 1.2	24.5 $\pm$ 5.5
Lactating (all teats sealed; 24 h)	(6)	27.2 $\pm$ 6.5	5.91 $\pm$ 1.13***	4.7 $\pm$ 0.9	38.1 $\pm$ 13.4
Lactating (litter removed 24 h)	(6)	21.7 $\pm$ 7.0	3.23 $\pm$ 0.51***	17.2 $\pm$ 4.5**	72.6 $\pm$ 19.0
Lactating (litter removed 24 h; re-suckled 5 h)	(6)	14.8 $\pm$ 2.6	3.5 $\pm$ 0.51***	18.4 $\pm$ 1.9***	85.7 $\pm$ 18.9

Table 5. Effects of unilateral sealing of teats on [^{14}C]lipid accumulation, activity of lipoprotein lipase and rate of lipogenesis in lactating mammary gland

For experimental details see the text. The results are mean values $\pm$ S.E.M., with the numbers of rats shown in parentheses. Values for sealed side of gland that are significantly different from the unsealed side are shown by ***P* < 0.01, ****P* < 0.001.

Side of gland	[^{14}C]Lipid accumulation (% of absorbed dose/g of tissue per 5 h)	Lipoprotein lipase activity (nmol of fatty acid released/min per mg of acetone-dried tissue)	Rate of lipogenesis (μ mol of $^3\text{H}_2\text{O}$ incorporated into saponified lipid/h per g of tissue)
Unsealed teats	1.92 $\pm$ 0.21 (7)	0.80 $\pm$ 0.05 (6)	109 $\pm$ 30 (5)
Sealed teats	0.55 $\pm$ 0.10 (7)***	0.35 $\pm$ 0.07 (6)***	9.6 $\pm$ 3.3 (5)**

despite a high plasma prolactin concentration (Tables 1 and 2), is in agreement with this view. There is a considerable body of evidence from studies on lactating rats with unilateral sealing of teats to indicate that milk stasis itself is mainly responsible for the decline in mammary-tissue synthetic activity (McLean, 1964; Jones, 1968). More recent experiments have identified a protein in goat milk fractions which inhibits synthesis of milk constituents by rabbit mammary explants (Wilde *et al.*, 1987). It would seem reasonable to predict from the postulate of Flint and colleagues (Flint *et al.*, 1981; Flint, 1982) that sealing half the teats would result in an intermediate stimulation of white-adipose-tissue lipid metabolism; this, however, did not occur (Tables 1 and 4). The expected decrease in mammary-tissue lipid metabolism (McLean, 1964; Jones, 1968) did occur, in that the [^{14}C]lipid accumulation, lipoprotein lipase activity and rate of lipogenesis were all significantly lower in the mammary gland from the side with sealed teats compared with the contralateral tissue (Table 5).

Conclusions

The evidence in the present paper indicates that short-term (5–24 h) changes in plasma prolactin after bromocryptine treatment or re-suckling do not alter lipid metabolism (exogenous [^{14}C]lipid accumulation or lipogenesis) in mammary gland or adipose tissue (Tables 1

and 4). In addition, the reciprocal changes in [^{14}C]lipid accumulation in mammary gland and white adipose tissue on removal of the litter can be, to a large extent, reproduced in the presence of a high endogenous plasma prolactin concentration (sealed-teat experiment; Table 1). The absence of a significant increase in the rate of lipogenesis in the sealed-teat experiment (Table 4) may be due to a difference in time taken to 'switch on' lipoprotein lipase and lipogenesis in adipose tissue. Another factor which must be considered in the regulation of lipogenesis in white adipose tissue is the prevailing insulin/glucagon ratio, because glucagon is an inhibitor of fatty acid synthesis *de novo* in isolated adipocytes (Robson *et al.*, 1984). It has been suggested that this ratio may play a key role in lactation (Robson *et al.*, 1984) when plasma insulin is decreased. The parallelism between plasma insulin, and [^{14}C]lipid accumulation and lipoprotein lipase activity in white adipose tissue (Tables 1–3), suggests that the plasma insulin is more important than prolactin in controlling lipid deposition in adipose tissue during lactation and in the immediate period after cessation of lactation. The metabolic activity of the mammary gland is a major factor in regulating plasma insulin (Jones *et al.*, 1984); prolactin may have an indirect effect via altering the number of insulin receptors in mammary tissue (Flint, 1982).

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