Effects of exogenous insulin or vanadate on disposal of dietary triacylglycerols between mammary gland and adipose tissue in the lactating rat: insulin resistance in white adipose tissue

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The effects of exogenous insulin or vanadate (an insulin mimetic) on the disposal of dietary [^{14}C]lipid between oxidation to $^{14}CO_2$, deposition in adipose tissue or uptake by mammary gland and transfer to suckling pups were studied in virgin and lactating rats. After an oral load of [1-^{14}C]triolein, virgin rats treated with a supraphysiological dose of insulin over 24 h showed a decrease (58 %) in $^{14}CO_2$ production and increased accumulation of [^{14}C]lipid in carcass and white adipose tissue. There was a 2.5-fold increase in lipoprotein lipase activity in the latter. Chronic vanadate administration (12 days) had no effect on these parameters. In lactating rats, the stimulation of the deposition of [^{14}C]lipid in adipose tissue by exogenous insulin was about 10 % of that in virgin rats. In prolactin-deficient lactating rats there was no stimulation of [^{14}C]lipid deposition in adipose tissue by insulin. However, both insulin and vanadate treatment increased the accumulation of [^{14}C]lipid in mammary gland to the values seen in the mammary glands plus pups of normal lactating rats. Lipoprotein lipase activity in the gland was also restored to normal values. It is concluded that in lactation there is resistance to insulin stimulation of dietary lipid deposition in adipose tissue, and that this is not due to circulating prolactin. In addition, exogenous insulin plays a role in the regulation of lipoprotein lipase and hence of dietary lipid uptake into lactating mammary gland.

INTRODUCTION

The partitioning of triacylglycerol between mammary gland and adipose tissue is well documented (Hamosh et al., 1970; Flint et al., 1981; Oller do Nascimento and Williamson, 1986). During lactation, plasma triacylglycerols (chylomicrons or very-low-density lipoproteins) are directed to the mammary tissue (Oller do Nascimento and Williamson, 1986) in response to the reciprocal changes in the activity of the key enzyme, lipoprotein lipase, in mammary gland and adipose tissue (Hamosh et al., 1970; Flint et al., 1981). On removal of the pups (cessation of lactation), lipoprotein lipase activity rapidly declines in mammary gland and increases in adipose tissue (Hamosh et al., 1970), and the plasma triacylglycerols are directed to the latter to replenish lipid stores (Oller do Nascimento and Williamson, 1986).

It is generally considered that the activity of lipoprotein lipase in lactating mammary gland is regulated by the plasma prolactin (see Scow and Chernick, 1987), because the activity decreases in parallel with the decrease in the hormone on removal of the pups, treatment with bromocryptine (an inhibitor of prolactin secretion) or hypophysectomy. However, uptake of exogenous lipid into the gland does not necessarily correlate with the changes in lipoprotein lipase activity or plasma prolactin (Oller do Nascimento et al., 1989). Specifically, on starvation the activity of lipoprotein lipase in lactating mammary gland decreases, as does the uptake of exogenous triacylglycerols (Oller do Nascimento and Williamson, 1988) without any change in plasma prolactin concentration (Robinson et al., 1978). These findings suggest that other hormonal signals, in addition to prolactin, may influence lipoprotein lipase activity in mammary tissue.

In adipose tissue, prolactin cannot have a direct effect on lipid metabolism, because of the absence of prolactin receptors (Flint et al., 1981). It has therefore been suggested (Oller do Nascimento et al., 1989) that the low plasma insulin in the lactating rat (Robinson et al., 1978; Flint et al., 1979; Burnol et al., 1983; Jones et al., 1984; Madon et al., 1990) may in part be responsible for the depressed activity of lipoprotein lipase in white adipose tissue during lactation. Cessation of lactation is associated with a considerable increase in plasma insulin (Agius et al., 1979; Flint, 1982; Oller do Nascimento et al., 1989) and increased activity of lipoprotein lipase in adipose tissue (Flint et al., 1981; Oller do Nascimento and Williamson, 1986; Oller do Nascimento et al., 1989).

The objective of the present experiments was to examine the effects of exogenous insulin or vanadate, an insulin mimetic in various insulin-sensitive tissues (see Shechter, 1990), on the disposal of dietary triacylglycerol during lactation and on removal of the pups. In particular, the question whether an increase in plasma insulin (or vanadate) can relieve the inhibition of lipid deposition in white adipose tissue of the lactating rat is addressed. Part of this work has been briefly reported (Da Costa and Williamson, 1992).

MATERIALS AND METHODS

Animals

Virgin and lactating Wistar rats were housed in individual cages and maintained in a controlled environment (ambient temperature 21 ± 2 °C; 12 h light/dark cycle). They had free access to a chow diet (Special Diet Services, Witham, Essex, U.K.) containing 52 % carbohydrate, 21 % protein, 4 % fat, and residue of non-digestible material. Virgin rats weighed between 180 g and 240 g. Litters contained nine or ten pups. Rats were randomly selected to receive vanadate in the drinking water [0.03 % (w/v) sodium orthovanadate] for 10–12 days and commencing at day 2 or 3 after parturition. Vanadate solutions were freshly prepared every 3 days. The rat and litter weights and food and water intakes were monitored in the group receiving vanadate solution and in the control group receiving normal drinking water. Rats were assigned to different treatments 24 h before experiments. A prolactin-deficient group received three subcutaneous injections of bromocryptine (10 mg/kg body wt. at 09:00, 17:00 and 09:00 h next day). Another group had the litter removed (24 h).

Exogenous insulin was administered where stated by subcutaneous injection of human isophane insulin (Novo Industri A/S, Denmark) in three doses each of 1 unit per rat in virgins (09:00 h, 17:00 h, and 09:00 h next day) and two doses of 1 unit per lactating rat at 09:00 and 17:00 h and one dose of 0.5 unit/rat at 09:00 h the next day (see the Results and discussion section). Lactating rats were used at 10–15 days of lactation.

Biochemicals and radioactive compounds

Bromocryptine mesylate was kindly provided by Dr. D. Römer (Sandoz Pharma, Basle, Switzerland); [1-^{14}C]triolein (glycerol tri[1-^{14}C]oleate) and glycerol tri[9,10(n)-^{3}H]oleate were from Amersham International, Amersham, Bucks, U.K. Triolein ($C_{18:1,cis-9}$), 99% pure, and lecithin (L-γ-phosphatidylcholine, type III-E, from egg yolk) and sodium orthovanadate (Na_3VO_4) were from Sigma, St. Loius, MO, U.S.A.

Measurements of disposal of [1-^{14}C]triolein load

The disposal of an oral load of [1-^{14}C]triolein (0.6 g, about 0.5 μCi per rat) was measured as described by Oller do Nascimento and Williamson (1986). After the gastric intubation of [1-^{14}C]triolein, the production of $^{14}CO_2$ was measured hourly over 5 h. Carcass values refer to the whole body minus intestinal tract, mammary gland and portions of tissues used for lipid extraction (Stansbie et al., 1976). Litters were killed under ether anaesthesia and the stomachs were dissected. Milk clots were carefully removed, weighed and well mixed, and the lipid content and radioactivity were measured. The remainder of the pup carcass was saponified and lipid was extracted as for the maternal carcass. Lipoprotein lipase activity in acetone-dried powders of parametrial and perirenal adipose tissue and mammary gland was determined by the method of Nilsson-Ehle and Schotz (1976).

RESULTS AND DISCUSSION

Insulin dosage

To eliminate possible interference from counter-regulatory hormones, the method of choice for administration of insulin is the euglycaemic/hyperinsulinaemic-clamp technique; however, it was considered unlikely that this could be maintained for 24 h in lactating rats. Consequently, it was decided to use divided doses of insulin administered subcutaneously. Although physiological doses of insulin are normally administered on a body-weight

basis, in the present experiments the insulin was given at supraphysiological doses (8–12 units/kg body wt.) on a per-rat basis (see also Kilgour and Vernon, 1987). The insulin used was of the 'intermediate acting' type, so that when the preparation is administered subcutaneously the plasma insulin increases rapidly and then decreases over the next 3–4 h. In preliminary experiments on virgin rats with an insulin dose of 1 unit at 09:00, 17:00, and 09:00 h the next day, it was found that the venous plasma glucose was decreased by 50–60% and plasma insulin increased to around 200 μ-units/ml for the last 4 h and then returned towards normal in the next 1 h. There were no overt signs of symptomatic hypoglycaemia. Administration of this dose to lactating rats resulted in symptomatic hypoglycaemia (excessive sweating, drowsiness, disorientation, and failure to nurse) and some deaths during the last 5 h. Consequently, the final dose of insulin (09:00 next day) was decreased to 0.5 unit per rat, and in preliminary experiments with this dose the plasma glucose remained low (around 2 mM) and plasma insulin increased (about 250 μ-units/ml) for 4 h.

When the rats were killed at 5 h after the end of the triolein-load experiment, none of the insulin-treated groups had mean arterial plasma glucose values below 4 mM. The plasma insulin at this time was variable, and therefore the differences between
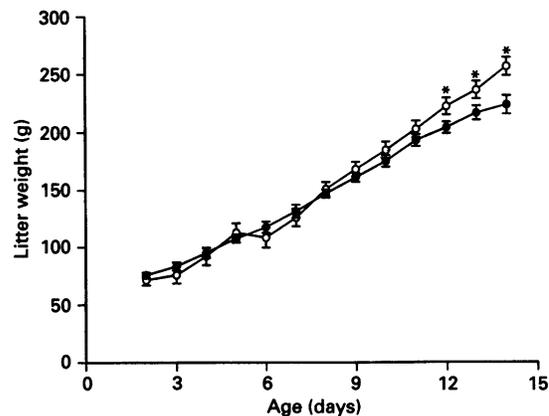

Figure 1   Effects of vanadate on litter weight gain

Dams in the control group (O) received normal drinking water, and dams in the vanadate group (●) received a 0.03% (w/v) solution of orthovanadate. Results are mean ± S.E.M. expressed in g litter wt. for the control ($n = 12$) and vanadate-treated ($n = 21$) groups. A significant difference by Student's t test between the control and vanadate-treated groups is indicated: *$P < 0.05$.

Table 1   Effects of insulin or vanadate on the disposal of oral [1-^{14}C]triolein between $^{14}CO_2$ production and [1-^{14}C]lipid accumulation in carcass, white and brown adipose tissue of virgin rats

See the text for experimental details. The results are mean ± S.E.M. for five observations. Values that are significantly different by Student's t test from the virgin control group are shown: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

| Experimental group | $^{14}CO_2$ (% of administered dose/5 h) | Tissue [^{14}C]lipid accumulation (% of absorbed dose/5 h) | | | Lipoprotein lipase (nmol of fatty acid released/ min per mg of acetone-dried tissue) |
		Total carcass	White adipose tissue (per g)	Brown adipose tissue (per g)	White adipose tissue
Virgin	20.0 ± 2.4	31.4 ± 4.2	0.63 ± 0.09	7.74 ± 1.37	1.84 ± 0.31
+ insulin	8.4 ± 1.2**	45.1 ± 0.8*	1.41 ± 0.11***	7.72 ± 1.58	4.54 ± 0.14***
+ vanadate	16.9 ± 3.5	33.9 ± 4.4	0.73 ± 0.31	7.44 ± 0.92	2.43 ± 0.68

Table 2   Effects of insulin or vanadate on the disposal of oral [1-¹⁴C]triolein between ¹⁴CO₂ production and [1-¹⁴C]lipid accumulation in carcass, white and brown adipose tissue of lactating, prolactin-deficient lactating and 24 h litter-removed rats

See text for experimental details. The results are mean ± S.E.M. for observations. Values for insulin or vanadate that are significantly different from the respective control group by Student's *t* test are shown: *$P < 0.05$, **$P < 0.01$.

Experimental group	n	¹⁴CO₂ (% of administered dose/5 h)	Tissue [¹⁴C]lipid accumulation (% of absorbed dose/5 h)			Lipoprotein lipase (nmol of fatty acid released/min per mg of acetone-dried tissue)
			Total carcass	White adipose tissue (per g)	Brown adipose tissue (per g)	White adipose tissue
Lactating	10	6.6±1.2	12.4±0.7	0.04±0.009	0.13±0.02	1.59±0.38
+ insulin	4	4.3±0.1	16.5±1.1**	0.10±0.02**	0.45±0.18	1.66±0.59
+ vanadate	6	5.1±0.5	11.5±1.8	0.04±0.008	0.10±0.02	1.36±0.27
Lactating, prolactin-deficient	6	9.6±2.0	23.6±2.2	0.33±0.11	–	2.39±0.41
+ insulin	5	5.3±0.8	18.1±0.7	0.15±0.05	0.43±0.11	1.75±0.41
+ vanadate	6	5.5±1.4	16.6±1.0*	0.06±0.01*	0.23±0.05	0.79±0.11*
Litter-removed	6	8.7±1.6	56.3±3.5	2.47±0.33	4.43±0.68	6.14±0.74
+ insulin	6	9.8±1.1	48.0±3.9	2.03±0.37	6.37±0.98	3.55±0.53*
+ vanadate	7	8.2±1.0	53.6±2.8	2.27±0.49	3.82±0.36	4.02±0.60*

Table 3   Effects of insulin or vanadate on [1-¹⁴C]lipid accumulation in the mammary gland, milk clot and pup carcass and on mammary tissue lipoprotein lipase activity of normal and prolactin-deficient lactating rats

See the text for experimental details. The results are mean ± S.E.M. for *n* observations. Values for insulin or vanadate that are statistically different from the respective controls are indicated by *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Experimental group	n	Total [¹⁴C]lipid accumulation (% of absorbed dose/5 h)				Lipoprotein lipase activity (nmol of fatty acid released/min per mg of acetone-dried tissue)
		Mammary gland (a)	Milk clot (b)	Pup carcass (c)	Sum (a+b+c)	Mammary gland
Lactating	10	38.3±4.4	16.4±2.3	5.3±1.0	60.0±3.3	3.93±0.51
+ insulin	4	48.0±4.4	15.4±4.2	3.0±0.2	66.4±1.4	3.38±0.44
+ vanadate	6	38.2±4.6	17.3±4.6	6.4±1.9	61.9±2.4	5.82±0.33*
Lactating, prolactin-deficient	6	37.5±5.5	3.4±2.7	1.1±0.8	42.0±6.8	1.44±0.40
+ insulin	5	60.3±3.3**	5.9±2.6	2.7±1.0	68.9±1.4*	6.28±1.15*
+ vanadate	6	55.4±5.8*	4.0±1.2	2.7±1.0	62.1±5.6*	4.36±0.49***

groups did not reach statistical significance (results not shown). This variability is likely to be a combination of (a) the decreasing concentration of exogenous insulin, (b) use of anaesthesia and (c) the absence of food over the experimental period.

The pattern of insulin administration had no significant effects on pup weight gain over the 24 h, and tended to decrease the food intake only in the prolactin-deficient group ($P < 0.056$) (results not shown).

Effects of vanadate on food and water intake and pup growth

Chronic vanadate administration in the drinking water decreased the mean food intake of virgin [control rats, 21 ± 4.8 g/day (6); vanadate-treated, 17 ± 0.7 g/day (9); $P < 0.01$] and of lactating rats [control rats, 55 ± 1.8 g/day (12); vanadate-treated, 50 ± 1.2 g/day (21); $P < 0.05$]. Vanadate also decreased the water intake of virgin rats [control rats, 30 ± 1.4 ml/day (6); vanadate-treated, 21.2 ± 1.4 (9) ml/day; $P < 0.001$] and lactating rats [control, 82 ± 3.2 (11); vanadate-treated, 70 ± 2.3 ml/day (21); $P < 0.01$]. The intake of vanadate calculated from the water consumption was 0.17 mmol/day per kg body wt. in virgin rats and 0.44 mmol/day per kg body wt. in lactating rats. The litters

of the lactating rats given vanadate weighed significantly less ($P < 0.05$) from day 12 of lactation (Figure 1), possibly because of the slight decrease in maternal food intake. Others have reported decreased litter weights after daily oral administration of vanadate to lactating rats (Domingo et al., 1986).

Effects of insulin or vanadate on [¹⁴C]lipid disposal to adipose tissue in virgin rats

Virgin rats treated for 24 h with exogenous insulin showed a large decrease (58 %) in the ¹⁴CO₂ production after the load of [1-¹⁴C]triolein (Table 1). This was accompanied by a parallel increase in the amount of [¹⁴C]lipid deposited in the carcass (Table 1). The activity of lipoprotein lipase increased approx. 2.5-fold in the parametrial adipose tissue of the insulin-treated rats, and the accumulation of [¹⁴C]lipid in this depot increased about 2-fold (Table 1). The vanadate-treated rats showed no significant changes in these parameters. Thus in virgin rats exogenous insulin increases deposition of dietary lipid in adipose tissue, whereas vanadate does not. It is possible that absence of a vanadate effect might be due to the decreased (20 %) food intake.

Effects of insulin or vanadate on [^{14}C]lipid disposal to adipose tissue in lactating rats

Neither insulin nor vanadate had a significant effect on the low $^{14}CO_2$ production of lactating rats (Table 2). Insulin, but not vanadate, increased the deposition of [^{14}C]lipid in carcass and white adipose tissue by 33 % and 150 % respectively (Table 2). However, in absolute terms the increases were appreciably smaller (at least an order of magnitude) than those seen in carcass and white adipose tissue of insulin-treated virgin rats (Table 1) or untreated litter-removed rats (Table 2). Although the results presented refer to parametrial white adipose tissue, we have also obtained similar values for [^{14}C]lipid accumulation and lipo-protein lipase activity in perirenal adipose tissue (results not shown). Thus exogenous insulin alone (in the presence of an active mammary gland) cannot reproduce the effects of litter removal (24 h) on lipid deposition (50-fold increase) in adipose tissue. Insulin tended to decrease [^{14}C]lipid accumulation in carcass and white adipose tissue in prolactin-deficient lactating rats and vanadate treatment caused significant decreases (Table 2), reinforcing the view that prolactin does not directly influence insulin action on white adipose tissue. Clearly, compared with adipose tissue of the virgin rat, that of the lactating rat is resistant to the action of insulin to stimulate lipoprotein lipase activity and hence deposition of dietary lipid. The high rate of deposition of [^{14}C]lipid in the carcass and white and brown adipose tissue after removal (24 h) of the litter was not further increased by insulin or vanadate, suggesting that maximum stimulation had occurred.

Effects of insulin or vanadate on [^{14}C]lipid disposal to mammary gland

In normal lactating rats, treatment with insulin or vanadate had no effect on [^{14}C]lipid accumulation in mammary gland, or its transfer to the milk or pups (Table 3) Similarly, there was no change in lipoprotein lipase activity in mammary gland with insulin, but the activity significantly increased with vanadate (48 %; Table 3). As expected from previous work from this laboratory (Oller do Nascimento et al., 1989), prolactin deficiency (induced by bromocryptine) did not affect the accumulation of [^{14}C]lipid in the gland, but decreased the transfer of [^{14}C]lipid to the milk and the pups (Table 3). The net result was a 30 % decrease in lipid transferred from the plasma to mammary gland plus pups (Table 3). Insulin treatment significantly increased (60 %) the accumulation of [^{14}C]lipid in the mammary gland of prolactin-deficient lactating rats without relieving the depressed transfer to the pups (Table 3). The effects of vanadate were similar to those of insulin (Table 3). Parallel with the increased deposition of [^{14}C]lipid in mammary gland on treatment with insulin or vanadate, there were higher activities of mammary-tissue lipoprotein lipase (approx. 4-fold and 3-fold respectively; Table 3). These findings raise the question why insulin and vanadate only increased [^{14}C]lipid uptake by the mammary gland in the prolactin-deficient state and not in normal lactation. One possibility is that, because of the high sensitivity of the mammary gland to insulin (Burnol et al., 1983; Jones et al., 1984) and the increased number of insulin receptors (Flint, 1982), the lipo-protein lipase activity and [^{14}C]lipid uptake is maximally stimu-lated in normal lactation. Prolactin deficiency, however, results in a decrease in the number of insulin receptors on mammary tissue (Flint, 1982), and therefore this decrease, if accompanied by a change in insulin sensitivity, might explain the present findings. Such a change could occur by a preferential loss of apparent high-affinity insulin receptors, although this appears

not to be the case after 48 h of bromocryptine treatment (Flint, 1982). Flint (1982) has suggested that the decrease in the number of insulin receptors in prolactin deficiency or on cessation of lactation ensures that the accompanying increase in plasma insulin does not stimulate mammary-gland metabolism. Irres-pective of the mechanism, our results suggest that this decrease in insulin receptors, and presumably insulin sensitivity, can be overcome in prolactin deficiency by exogenous insulin at supra-physiological dose. Of interest is the finding that insulin or vanadate cannot restore the depressed transfer of [^{14}C]lipid to the pups in the prolactin-deficient rats. This suggests that prolactin has a specific role in the secretion of milk lipid from the mammary gland. Concomitant administration of bromocryptine and sheep prolactin (20 units per dose) restored pup weight gain over 24 h, mammary-gland lipoprotein lipase activity and milk transfer (results not shown). These findings suggest that bromocryptine is unlikely to have a direct pharmacological effect on the neuro-endocrine ejection reflex at the dose administered.

Concluding remarks

Previous work has shown that the synthesis of fatty acids *de novo* in adipose tissue of the lactating rat is resistant to insulin action both *in vivo* (Burnol et al., 1983; Jones et al., 1984) and *in vitro* (Burnol et al., 1986). This resistance may in part be due to impairment of the ability of supraphysiological doses of insulin to stimulate pyruvate dehydrogenase activity *in vivo* (Kilgour and Vernon, 1987), a key enzyme in lipogenesis. The stimulatory effect of insulin on the utilization of glucose by adipocytes is not affected by lactation (Burnol et al., 1986), which indicates that the site of insulin resistance must be distal to the glucose transporter. The present work extends the previous findings to show that the major pathway for lipid accretion in adipose tissue in the chow-fed rat (see Williamson, 1990), namely uptake of exogenous lipid (supplied as chylomicrons or very-low-density lipoprotein) is also resistant to stimulation by insulin in lactation. Here the key factor is the absence of stimulation of lipoprotein lipase activity by the hormone, as occurs in the virgin rat (Tables 1 and 2).

At first sight the failure of vanadate to stimulate lipoprotein lipase in adipose tissue of virgin rats (Table 1) would seem contrary to a previous report that vanadate increases activity of the enzyme in epididymal fat-pads from rats (Sera et al, 1990). However, the concentrations used (0.5–2 mM) were considerably higher than that likely to occur in the plasma of the rat in the present experiments (about 20 μM; Meyerovitch et al., 1987). In addition, it is possible that vanadate only acts *in vivo* where there is insulin insufficiency. Some support for this view is that chronic vanadate administration via the drinking water does not alter the blood glucose concentration in normal female rats, but rapidly causes normoglycaemia in the hyperglycaemic streptozotocin-diabetic rat (Heyliger et al., 1985; Blondel et al., 1989; Pugaz-henthi and Khandelwal, 1990). However, vanadate also improves glucose homoeostasis in hyperinsulinaemic obese rats (Brichard et al., 1989).

The intriguing finding that insulin (or vanadate) can increase lipoprotein lipase activity and [^{14}C]lipid accumulation in the mammary gland of the prolactin-deficient lactating rat (Table 3) suggests that prolactin and insulin may act synergistically to regulate mammary-tissue lipoprotein lipase activity. This would then explain the decrease in activity in the starved lactating rat (Oller do Nascimento and Williamson, 1988) when prolactin is high and insulin is low. However, if the two hormones act synergistically, an increase in lipoprotein lipase activity would be expected in lactating rats treated with insulin, but this did not

occur. Alternatively, it is possible that insulin is the primary regulator of the enzyme and that prolactin deficiency by decreasing the number of insulin receptors on mammary tissue 'unmasks' the requirement for insulin. Although this postulate would be contrary to accepted dogma that prolactin is the primary regulator of lipoprotein lipase activity in the mammary gland, it must be emphasized that all the available evidence is indirect and is based on the correlation between changes in plasma prolactin and enzyme activity (see Scow and Chernick, 1987). As mentioned above, this correlation does not hold in starvation (Oller do Nascimento and Williamson, 1988). The recent finding that a euglycaemic/hyperinsulinaemic clamp increases the lipoprotein lipase activity in human milk (Neville et al., 1991) lends some support to the view that insulin can regulate the enzyme activity, as does the stimulatory effect of vanadate on the enzyme in mammary gland of normal and prolactin-deficient lactating rats.

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