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Evidence for conservation of dietary lipid in the rat during lactation and the immediate period after removal of the litter

Decreased oxidation of oral [1-^{14}C]triolein

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Production of $^{14}CO_2$ from an oral load of [1-^{14}C]triolein was greatly decreased (70%) in lactating rats or immediately after (24–48 h) removal of the litter, compared with virgin rats. This decreased oxidation of dietary lipid was accompanied by accumulation of ^{14}C-labelled lipid in lactating mammary gland or adipose tissue (after litter removal). No difference in $^{14}CO_2$ production between lactating and virgin rats was observed when [1-^{14}C]octanoate was administered. It is concluded that a major factor in this conservation of dietary triacylglycerol is the relative activity of lipoprotein lipase in the tissues.

INTRODUCTION

Lactation is characterized by an increased demand for metabolic substrates to provide the constituents of milk (e.g. lactose, lipid and protein), and this necessitates a number of metabolic adaptations in various tissues, such as mammary gland, adipose tissue, intestine and liver (see Bauman & Currie, 1980; Williamson, 1980). The lipid present in milk is derived from a number of sources: (*a*) fatty acids synthesized in the liver and mammary gland, (*b*) fatty acids mobilized from adipose tissue and (*c*) dietary fat. Triacylglycerols of the diet, after hydrolysis to non-esterified fatty acids, are absorbed in the intestine and are mainly transported in blood as components of chylomicrons. The uptake of this substrate is dependent on the lipoprotein lipase activity on the endothelial cell wall of peripheral tissues (see Robinson, 1970). This enzyme is important in milk fat production because it is responsible for the hydrolysis of chylomicrons or very-low-density lipoprotein (derived from liver) at the luminal surface of the mammary-gland endothelial cells (see Scow, 1977). Lipoprotein lipase activity is increased in mammary gland during lactation, whereas the activity decreases in adipose tissue just before parturition and remains low throughout lactation (Otway & Robinson, 1968; Hamosh *et al.*, 1970) and increases again on removal of pups (Hamosh *et al.*, 1970; Flint *et al.*, 1981). The net result of these reciprocal changes in lipoprotein lipase activity in mammary gland and adipose tissue is that triacylglycerols are directed (Williamson, 1973) to mammary tissue during lactation and are thus less available for storage or eventual oxidation. The present investigation was undertaken to provide direct information about the effects of lactation or premature removal of pups on the disposal of an oral load of [1-^{14}C]triolein between accumulation of ^{14}C-labelled lipid in tissues and production of $^{14}CO_2$.

EXERIMENTAL

Rats of the Wistar strain were fed on a diet consisting of approx. 52% carbohydrate, 21% protein and 4% fat (Special Diet Services, Witham, Essex, U.K.) and were maintained at an ambient temperature of 20–22 °C in a room with lights on from 07:30 h to 19:30 h. Lactating rats (225–355 g) with 10–12 pups were used after a lactation period of 10–14 days. A group of lactating rats had their pups removed at different intervals (24 h, 48 h or 7 days) before the experiment. Virgin rats weighing 150–240 g were used for comparison. All experiments were started between 09:00 h and 10:00 h. Radiochemicals were obtained from Amersham International.

The rate of lipid oxidation *in vivo* was measured by the determination of the $^{14}CO_2$ produced after intragastric administration of [1-^{14}C]triolein (glycerol tri[1-^{14}C]oleate; about 0.7 g; 0.33 μCi per rat); no anaesthetic was used for the intubation. The rat and litter were placed in a large glass desiccator connected to a wash bottle fitted with a sintered-glass tube and containing 20 ml of Lumasorb (May and Baker) to absorb the CO_2. Air was drawn through the system at about 2 litres/min. The contents of the wash bottle were changed every 1 h for 5 h, and a portion of the Lumasorb was added directly to the scintillation fluid for measurement of radioactivity. The rate of $^{14}CO_2$ production was linear with time between 2 h and 5 h after the oral load (Fig. 1). After 5 h the rats were anaesthetized with Nembutal (60 mg/kg body wt.) and the whole of the intestinal tract and samples of parametrial adipose tissue, interscapular brown adipose tissue, liver, heart and mammary gland were taken. Another series of experiments were carried out in which [1-^{14}C]octanoate (about 0.7 g; 1.4 μCi) was administered. The rate of $^{14}CO_2$ production was linear between 0.5 and 5 h (results not shown). The intestinal tract was homogenized in 150 ml of 3% (w/v) $HClO_4$. Arterial blood was collected from the aorta into a heparinized syringe and was centrifuged for 10 min at 3000 rev./min to collect plasma. Samples of tissues (about 1 g; 0.1 g for brown adipose tissue), intestinal-tract homogenate (2–3 ml) and plasma (0.2 ml) were added to 3 ml of 30% (w/v) KOH, and the lipid was saponified and non-esterified fatty acids were extracted by the method of Stansbie *et al.* (1976). The extracted fatty acids were dissolved in 10 ml of scintillation fluid, and radioactivity was measured for determination of the ^{14}C-labelled lipid accumulated by the tissues and the

amount of ^{14}C-labelled lipid remaining in the intestinal tract. The absorption of ^{14}C-labelled lipid was determined by subtraction of the amount of radioactivity remaining in the intestinal tract from the amount administered. Plasma triacylglycerol was determined by a modification of the method of Eggstein & Kreutz (1966), and plasma non-esterified fatty acids were measured by the method of Shimizu *et al.* (1979). The lipoprotein lipase activities of parametrial adipose tissue, interscapular brown adipose tissue and mammary gland were measured in acetone/ether-dried preparations of the tissues (Nilsson-Ehle *et al.*, 1972). The dry tissue powders were solubilized with 0.2 M-Tris/HCl buffer, pH 8.1, containing 1 M-ethylene glycol. The assay system consisted of 0.1 ml of homogenate and 0.1 ml of substrate containing ^{3}H-labelled triolein prepared by the method of Nilsson-Ehle & Ekman (1977). Incubations were carried out at 37 °C for 60 min. The reaction was stopped by addition of methanol/chloroform/heptane (28:25:20, by vol.), and fatty acids were extracted as described by Nilsson-Ehle & Schotz (1976). The activity is expressed as nmol of fatty acid released/min per mg of acetone-dried tissue.

RESULTS AND DISCUSSION

Administration of an oral load of triolein should result in a constant delivery of chylomicrons into the circulation over a period of time and therefore represent the situation existing after a single high-fat meal. Support for this view comes from the linear production of $^{14}CO_2$ over the 2–5 h period after the oral load (Fig. 1). Evidence that the measured $^{14}CO_2$ originated from triacylglycerols (chylomicrons or very-low-density lipoproteins) rather than non-esterified fatty acids directly transported into the portal blood from the intestinal epithelial cells (Hyun *et al.*, 1967; McDonald *et al.*, 1980) is the considerable decrease (87%) in $^{14}CO_2$ production when triolein-loaded virgin rats were injected with Triton WR 1339, which prevents the removal of plasma triacylglycerol (Scanu, 1965) (results not shown).

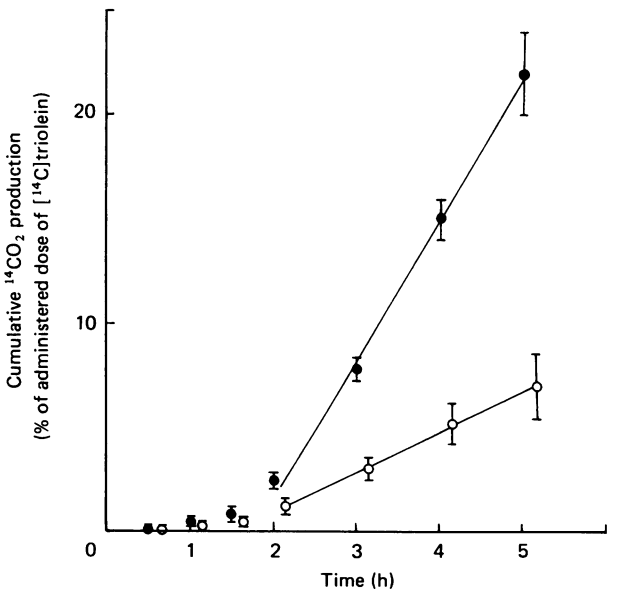

Fig. 1. Effects of lactation on $^{14}CO_2$ production from oral [1-^{14}C]triolein

For experimental details see the text. The results are means ± S.E.M. for the numbers of rats in parentheses: ●, virgin rats (6); ○, lactating rats (5).

Production of $^{14}CO_2$ after intragastric administration of [1-^{14}C]triolein was about 70% lower in lactating rats than in virgin rats when compared on the basis of percentage of dose administered (Fig. 1). There were no significant differences between lactating and virgin rats in the concentrations of plasma triacylglycerols or non-esterified fatty acids at the end of the experiment (results not shown). The rate of [1-^{14}C]triolein absorption was slightly higher in the lactating rats (Table 1), and consequently the other results in this paper are expressed on the basis of percentage of dose absorbed. Removal of the pups for 24 or 48 h did not result in a significant increase in the amount of $^{14}CO_2$ produced by the mothers; however, 7 days after removal of the pups the $^{14}CO_2$ production had returned to that found with virgin rats (Table 1). This latter finding suggests that age of the rats is not an important determinant of the rate of lipid oxidation.

A possible explanation for these findings is a decrease in whole-body oxidation of fatty acids in lactation. To examine this question, [1-^{14}C]octanoate, a medium-chain fatty acid which is directly absorbed into the portal blood (Hyun *et al.*, 1967) and is not esterified in rat tissues (Fritz, 1961), was administered to virgin and lactating rats. In contrast with the results with [1-^{14}C]triolein, there was no significant difference in $^{14}CO_2$ production between virgin and lactating rats (48.9 ± 3.7% versus 43.9 ± 3.0% of [1-^{14}C]octanoate absorbed/g; $n = 3$; means ± S.E.M.). These results suggest that there is no major impairment of fatty acid (or ketone-body) oxidation in tissues (e.g. liver, muscle, brown adipose tissue) of the lactating rat. As expected, injection of Triton WR 1339 did not significantly decrease $^{14}CO_2$ production in virgin rats when [1-^{14}C]octanoate was administered (results not shown).

The experiments with Triton WR 1339 indicate that the release of oleate by the action of lipoprotein lipase in peripheral tissues is the primary event leading to the eventual oxidation of the [1-^{14}C]oleate in liver and other tissues (e.g. muscle, brown adipose tissue). The [1-^{14}C]oleate is taken up by the tissue where the enzyme is sited and is then esterified, oxidized or released into the plasma. The accumulation of ^{14}C-labelled lipid (on a g wet wt. basis) was decreased in white and brown adipose tissue and liver in lactating rats compared with virgin rats, but it was high in mammary gland (Table 1); the latter value is an underestimate, because some ^{14}C-labelled lipid was found in the gastric contents of the pups. Removal of pups for 24 or 48 h decreased the accumulation of ^{14}C-labelled lipid in mammary gland by about 90% and increased the accumulation in white (10-fold) and brown (3-fold) adipose tissue; the value for white adipose tissue was approx. 4-fold higher than that in this tissue from virgin rats (Table 1). At 7 days after removal of pups, the accumulation in brown and white adipose tissue was not significantly different from that found with virgin rats (Table 1). There were no significant differences in the amount of ^{14}C-labelled lipid in plasma or heart in any of the situations studied. When [1-^{14}C]octanoate was administered, the largest accumulation of ^{14}C-labelled lipid was in lactating mammary gland (0.43 ± 0.012% of absorbed dose/g; $n = 3$; means ± S.E.M.). Accumulation in white adipose tissue (0.03

Table 1. Effects of lactation or premature removal of litter on whole-body $^{14}CO_2$ production and tissue accumulation of ^{14}C-labelled lipid after intragastric administration of [1-^{14}C]triolein

For experimental details see the text. The results are means ± 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 virgin rats are shown by $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, and for those for lactating rats are shown by $\dagger P < 0.05$, $\dagger\dagger P < 0.01$, $\dagger\dagger\dagger P < 0.001$.

Experimental group		Absorption of ^{14}C-labelled lipid (%)	$^{14}CO_2$ production (% of absorbed dose)	Tissue ^{14}C-labelled lipid accumulation (% of absorbed dose/g of tissue)					
				Mammary gland	White adipose tissue	Brown adipose tissue	Liver	Heart	Plasma
Lactating	(5)	82.5 ± 3.2*	8.4 ± 1.9***	1.36 ± 0.23	0.17 ± 0.06*	1.02 ± 0.57***	0.32 ± 0.05*	0.17 ± 0.05	0.06 ± 0.01
Pups removed (24 h)	(4)	73.3 ± 2.4†	5.1 ± 0.4***	0.13 ± 0.04†††	1.57 ± 0.16***,†††	3.98 ± 0.63††	0.53 ± 0.10	0.21 ± 0.04	0.11 ± 0.04
Pups removed (48 h)	(5)	79.0 ± 2.4	8.5 ± 1.1***	0.23 ± 0.07†††	1.79 ± 0.39**,††	3.01 ± 0.67*	0.33 ± 0.03**	0.16 ± 0.04	0.05 ± 0.01
Pups removed (7 days)	(3)	75.6 ± 4.9	29.8 ± 2.8†††	0.17 ± 0.08††	0.55 ± 0.18	3.26 ± 0.90	0.53 ± 0.06†	0.17 ± 0.03	0.07 ± 0.005
Virgin	(6)	73.2 ± 2.0†	30.2 ± 2.8†††	—	0.41 ± 0.10†	5.50 ± 0.60†††	0.73 ± 0.09†	0.15 ± 0.02	0.07 ± 0.01

± 0.01 versus 0.11 ± 0.02) and brown adipose tissue (0.30 ± 0.15 versus 1.05 ± 0.46) was decreased compared with virgin rats.

As expected, the reciprocal changes in the accumulation of ^{14}C-labelled lipid from [1-^{14}C]triolein in mammary gland and white adipose tissue of lactating rats and rats whose litters had been removed were accompanied by reciprocal alterations in the activity of lipoprotein lipase (Table 2). However, the increased accumulation of ^{14}C-labelled lipid in brown adipose tissue on removal of litters for 48 h was not accompanied by a comparable increase in lipoprotein lipase activity (Table 2). In this tissue, other factors, such as changes in blood flow (Foster & Frydman, 1979) may be involved in the relatively large accumulation of ^{14}C-labelled lipid in the non-lactating rats. Conversely, the decreased blood flow to mammary gland on removal of the pups (Hanwell & Linzell, 1973) will play a role in diverting triacylglycerol from this tissue. The alterations in lipoprotein lipase activity in mammary gland and white adipose tissue on removal of the litter were qualitatively similar to those described by others (Hamosh *et al.*, 1970; Flint *et al.*, 1981), although the rapidity of the changes in mammary gland was less. One reason for this discrepancy may be that other workers have used rats early in lactation (5–7 days), and in the present experiments the rats were given an oral load of fat. Others have not noted the 'overshoot' of activity of lipoprotein lipase in white adipose tissue (compared with virgin or 7 days post-removal of pups) on immediate removal of the pups (Table 2).

These results indicate that dietary lipid is conserved during the lactating and the immediate post-weaning periods to provide milk fat or to replenish depleted adipose-tissue stores respectively. For example, the mean total weight of mammary gland in lactating rats from our animal house is 14 g, and therefore the total accumulation of ^{14}C-labelled lipid was 19% (14 × 1.36%; Table 1) of the dose of [1-^{14}C]triolein absorbed, which represents a high proportion (87%) of the decrease in $^{14}CO_2$ production observed between virgin and lactating rats. The fat content of virgin rats on standard defined diets (high in carbohydrate and low in fat) is around 10% of body wt., and decreases to 4% by the end of lactation (Kanto & Clawson, 1980; Steingrimsdottir *et al.*, 1980; Naismith *et al.*, 1982; Moore & Brasel, 1984). Assuming a body wt. of 250 g, it can be calculated that a virgin rat would accumulate 10.2% (25 × 0.41%) of the absorbed dose of [1-^{14}C]triolein in white adipose tissue, whereas a lactating rat would accumulate only 1.7% (10 × 0.17%). On removal of the pups for 48 h the accumulation would be 17.9% (10 × 1.79%). This, of course, assumes that all adipose-tissue depots accumulate ^{14}C-labelled lipid at the same rate. Clearly, these calculations do not provide an absolute balance, but they suggest that the major mechanism involved in this conservation (i.e. decreased oxidation) is the uptake and accumulation by the lactating mammary gland or the adipose tissue (after removal of the litter) of fatty acids released from triacylglycerol by the action of lipoprotein lipase.

An additional mechanism for conserving lipid in lactation is the decreased rate of ketogenesis and increased esterification observed with hepatocytes of lactating rats (Whitelaw & Williamson, 1977). For mammary gland it is unlikely that fatty acids removed from the circulation can be returned to it; however, this

Table 2. Effects of lactation or premature removal of litter on lipoprotein lipase activity in parametrial adipose tissue, interscapular brown adipose tissue and mammary gland

For experimental details see the text. The results are means ± 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 virgin rats are shown by $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, and for those for lactating rats are shown by $\dagger P < 0.05$, $\dagger\dagger P < 0.01$, $\dagger\dagger\dagger P < 0.001$.

Experimental group		Lipoprotein lipase activity (nmol of fatty acid released/min per mg of acetone-dried tissue)		
		White adipose tissue	Brown adipose tissue	Mammary gland
Lactating	(8)	0.228 ± 0.040***	0.329 ± 0.060	0.901 ± 0.148
Pups removed (24 h)	(6)	1.41 ± 0.32*, ††	0.707 ± 0.123†	0.367 ± 0.052††
Pups removed (48 h)	(4)	2.58 ± 0.89*, †	0.452 ± 0.086	0.345 ± 0.069††
Pups removed (7 days)	(3)	0.354 ± 0.068	—	0.053 ± 0.020†††
Virgin	(4)	0.493 ± 0.040†††	0.490 ± 0.076	—

is not the case in white adipose tissue, where lipolysis occurs. The decreased rate of noradrenaline-stimulated lipolysis in adipocytes from lactating rats 48 h after the litter was removed (Vernon & Finley, 1986) and the increased rate of fatty acid esterification in these adipocytes (Cubero *et al.*, 1983) presumably also play a role in the replenishment of adipose-tissue stores. Another factor responsible for the 70% increase in adipocyte volume 7 days after removal of the pups (Vernon & Finley, 1986) is the increased rate of lipogenesis (Agius *et al.*, 1979; Flint *et al.*, 1981).

The signals responsible for the transient increases in lipid accumulation in adipose tissue on removal of the pups may be the decrease in plasma prolactin and the temporary increase in plasma insulin (Agius *et al.*, 1979; Flint *et al.*, 1981), although other factors may be involved (Vernon & Finley, 1986).

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