Tissue-specific effects of starvation and refeeding on the disposal of oral [1-¹⁴C]triolein in the rat during lactation and on removal of litter

Claudia M. OLLER DO NASCIMENTO and Dermot H. WILLIAMSON

Metabolism Research Laboratory, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE, U.K.

1. The effects of starvation and refeeding on the disposal of oral [¹⁴C]triolein between ¹⁴CO₂ production and ¹⁴C-lipid accumulation in tissues of virgin rats, lactating rats and lactating rats with pups removed were studied. 2. Starvation (24 h) increased ¹⁴CO₂ production in lactating rats and lactating rats with pups removed to values found in virgin rats. This increase was accompanied by decreases in ¹⁴C-lipid accumulation in mammary gland and pups of lactating rats and in white and brown adipose tissue of lactating rats with pups removed. 3. Short-term (2 h) refeeding *ad libitum* decreased ¹⁴CO₂ production in lactating rats and lactating rats with pups removed, and restored the ¹⁴C-lipid accumulation in mammary glands plus pups and in white and brown adipose tissue respectively. 4. Insulin deficiency induced with mannoheptulose inhibited the restoration of ¹⁴C-lipid accumulation in white adipose tissue on refeeding of lactating rats with pups removed, but did not prevent the restoration of ¹⁴C-lipid accumulation in mammary gland. 5. Changes in the activity of lipoprotein lipase in mammary gland and white adipose tissue paralleled the changes in ¹⁴C-lipid accumulation in these tissues. 6. It is concluded that ¹⁴C-lipid accumulation in mammary gland may not be affected by changes in plasma insulin concentration and that it is less sensitive to starvation than is lipogenesis or lactose synthesis. This has the advantage that the milk lipid content can still be maintained from hepatic very-low-density lipoprotein for a period after withdrawal of food. The major determinant of the disposal of oral ¹⁴C-triolein appears to be the total tissue activity of lipoprotein lipase. When this is high in mammary gland (fed lactating rats) or white adipose tissue (fed lactating rats with pups removed), less triacylglycerol is available for the muscle mass and consequently less is oxidized.

INTRODUCTION

During lactation in the rat, large amounts of circulating substrates are extracted (glucose, triacylglycerols, non-esterified fatty acids and amino acids) from the bloodstream by the mammary gland to provide the macronutrients of milk (lactose, lipid and protein) (see Williamson, 1980; Williamson *et al.*, 1984). This utilization of substrates is extremely sensitive to the hormonal and nutritional status of the rat. Starvation for 24 h decreases glucose extraction by mammary tissues (Robinson & Williamson, 1977; Page & Kuhn, 1986), and the rates of lipogenesis (Robinson *et al.*, 1978; Busmann *et al.*, 1984) and lactose synthesis *in vivo* (Busmann *et al.*, 1984). In addition, the arteriovenous differences across the gland for a wide spectrum of amino acids are decreased (Viña *et al.*, 1983). As a consequence milk production is considerably depressed, as judged by changes in pup weight (Brosnan *et al.*, 1982). All these alterations in the starved lactating rat can be reversed by refeeding chow *ad libitum* for 2–2.5 h (Robinson *et al.*, 1978; Viña *et al.*, 1983; Busmann *et al.*, 1984). In contrast, apart from a report that the arteriovenous difference for triacylglycerol across the lactating gland of the rat is decreased by 50% after 24 h starvation (Hawkins & Williamson, 1972), there appears to be no information on the effects of dietary status on triacylglycerol metabolism by mammary gland. It is well established that the gland extracts a considerable amount of chylomicrons (derived from dietary fat) for

milk lipid synthesis and that lipoprotein lipase plays a key role in this process (see Scow & Chernick, 1987). Previous experiments from our laboratory have shown that dietary lipid is conserved during lactation for utilization by the gland and, immediately after removal of the litter, for repletion of adipose-tissue depots (Oller do Nascimento & Williamson, 1986).

The aim of the present investigation was to examine whether starvation (24 h) or short-term refeeding (2 h) altered the disposal of an oral load of [1-¹⁴C]triolein between tissue accumulation and production of ¹⁴CO₂ in virgin rats, lactating rats and lactating rats after removal of the litter. In addition, the effects of short-term insulin deficiency on the fate of oral [1-¹⁴C]triolein have been examined.

EXPERIMENTAL

Materials

Rats. Female rats of the Wistar strain were fed on a diet consisting of approx. 52% carbohydrate, 21% protein and 4% fat; residue contained non-digestible material (Special Diet Services, Witham, Essex, U.K.). They were maintained at an ambient temperature of 20–22 °C in a room with lights on from 07:30 to 19:30 h. Virgin rats weighed 190–240 g, and lactating rats (10–14 days *post partum*) weighed 240–350 g and had eight to ten pups. A group of lactating rats had their pups removed for 24 h. Where indicated, rats were

starved for 24 h with free access to water. Groups of starved rats were allowed to refeed with the chow diet *ad libitum* for 2 h.

Radiochemicals and biochemicals. [1-¹⁴C]Triolein (glycerol tri[1-¹⁴C]oleate) was obtained from Amersham International. All biochemicals were purchased from Boehringer Corp. (London). Mannoheptulose was purchased from Sigma Chemical Co.

Methods

Measurement of ¹⁴C-lipid oxidation *in vivo*. The method was essentially that described by Oller do Nascimento & Williamson (1986) in which the ¹⁴CO₂ produced after intragastric administration of [1-¹⁴C]-triolein (about 0.7 g; 0.33 μ Ci/rat) is measured. All experiments were commenced between 08:00 and 09:00 h. After intubation of the [1-¹⁴C]triolein without anaesthetic, the rat (plus litter in the case of lactating rats) was placed in a large desiccator connected to a wash bottle fitted with a sintered-glass tube and containing 20 ml of Lumasorb (May and Baker) to absorb the CO₂. 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.

Measurement of ¹⁴C-lipid absorption from intestine and accumulation in tissue. After 5 h the rats were anaesthetized with Nembutal (60 mg/kg body wt.) and blood was collected from the abdominal aorta into a heparinized syringe. The whole intestinal tract was removed together with samples of parametrial adipose tissue, interscapular brown adipose tissue and mammary gland. The intestinal tract was homogenized in 150 ml of 3% (w/v) HClO₄ in a Waring blender. The carcass (body without intestinal tract or portions of tissue used for lipid extraction) was autoclaved to soften bones and then homogenized with 350 ml water in a Waring blender. Samples of tissues (about 1 g; 0.1 g for brown adipose tissue), intestinal-tract homogenate (2–3 g) and carcass homogenate (3–4 g) were added to 3 ml of 30% (w/v) KOH. 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 ¹⁴C-labelled lipid accumulated by the tissues and the amount of ¹⁴C-labelled lipid remaining in the intestinal tract. Subtraction of the amount of radioactivity remaining in the intestinal tract from the amount administered was considered to equal the amount absorbed. The fat content of the tissues and carcass was determined by a gravimetric method.

Measurement of tissue lipoprotein lipase activity. 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 ³H-labelled triolein prepared by the method of Nilsson-Ehle & Ekman (1977). Incubations were

Table 1. Effects of starvation and refeeding on absorption of ¹⁴C-lipid, ¹⁴CO₂ production and accumulation of ¹⁴C-labelled lipid in carcasses of virgin, lactating and lactating rats with pups removed

Experimental group	Virgin			Lactating			Pups removed (24 h)		
	Absorption of ¹⁴ C-lipid (%)	¹⁴ CO ₂ production (% of absorbed dose)	Carcass ¹⁴ C-lipid (% of absorbed dose)	Absorption of ¹⁴ C-lipid (%)	¹⁴ CO ₂ production (% of absorbed dose)	Carcass ¹⁴ C-lipid (% of absorbed dose)	Absorption of ¹⁴ C-lipid (%)	¹⁴ CO ₂ production (% of absorbed dose)	Carcass ¹⁴ C-lipid (% of absorbed dose)
Chow-fed	73.0 $\pm$ 2.0 (6)	30.2 $\pm$ 2.8 (4)	34.8 $\pm$ 2.5 (5)	82.5 $\pm$ 3.2 (5)	8.4 $\pm$ 1.9 (5)	46.2 $\pm$ 3.6 (5)	73.3 $\pm$ 2.4 (4)	5.1 $\pm$ 0.4 (4)	48.6 $\pm$ 2.6 (4)
Starved (24 h)	71.9 $\pm$ 3.0 (4)	18.9 $\pm$ 2.6***	—	78.1 $\pm$ 3.8* (4)	22.6 $\pm$ 2.7** (4)	39.5 $\pm$ 2.0 (4)	72.9 $\pm$ 2.8 (4)	31.8 $\pm$ 1.9*** (4)	20.0 $\pm$ 1.4*** (4)
Refed chow (2 h <i>ad libitum</i>)	68.5 $\pm$ 7.8 (6)	33.5 $\pm$ 3.2 (5)	32.8 $\pm$ 2.0 (5)	89.7 $\pm$ 0.49 (4)	12.5 $\pm$ 1.1 (4)	49.6 $\pm$ 5.0 (4)	83.6 $\pm$ 2.0** (5)	17.7 $\pm$ 0.8*** (5)	42.2 $\pm$ 1.4 (5)
Refed chow (1.5 g/100 g body wt.)	—	—	—	—	—	—	79.8 $\pm$ 2.1 (4)	28.3 $\pm$ 1.8*** (4)	27.3 $\pm$ 1.5*** (4)
Refed chow (2 h) + mannoheptulose	—	—	—	66.5 $\pm$ 3.3** (4)	21.3 $\pm$ 2.6** (4)	36.2 $\pm$ 2.3* (4)	71.8 $\pm$ 3.9 (7)	27.1 $\pm$ 3.5*** (7)	25.8 $\pm$ 2.8*** (7)

For experimental details see the text. The results are mean values $\pm$ s.e.m. for the numbers of rats shown in parentheses. Values that are significantly different by Student's *t* test from those for the appropriate chow-fed control group are shown by **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

Table 2. Effects of starvation and refeeding on accumulation of ¹⁴C-lipid in mammary gland of lactating rats and lactating rats with pups removed

For experimental details see the text. The values for total mammary gland include ¹⁴C-lipid transferred to the pups. The results are means $\pm$ S.E.M. for the numbers of rats in parentheses. Values that are significantly different by Student's *t* test from those for the appropriate fed group are shown by ***P* < 0.01.

Experimental group	Mammary-gland ¹⁴ C-lipid accumulation			
	Lactating		Pups removed (24 h)	
	(% of absorbed dose/g of tissue)	(% of absorbed dose per total tissue)	(% of absorbed dose/g of tissue)	(% of absorbed dose per total tissue)
Chow-fed	1.36 $\pm$ 0.23 (5)	34.7 $\pm$ 3.0 (5)	0.13 $\pm$ 0.04 (4)	3.46 $\pm$ 1.09 (4)
Starved (24 h)	1.41 $\pm$ 0.05 (5)	14.5 $\pm$ 0.41 (5)**	0.19 $\pm$ 0.04 (4)	4.10 $\pm$ 1.17 (4)
Refed chow (2 h <i>ad libitum</i>)	2.68 $\pm$ 0.25 (4)**	39.4 $\pm$ 3.2 (4)	0.14 $\pm$ 0.03 (6)	2.23 $\pm$ 0.43 (6)
Refed chow (2 h) + mannoheptulose	2.71 $\pm$ 0.35 (4)	38.2 $\pm$ 0.82 (4)	0.17 $\pm$ 0.03 (8)	2.26 $\pm$ 0.32 (8)

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.

Induction of insulin deficiency with mannoheptulose. Administration of mannoheptulose causes a transient diabetes by inhibition of glucose utilization by the pancreatic β -cells (Simon *et al.*, 1972). Rats were injected subcutaneously with mannoheptulose (2 g/kg body wt.; 50 % solution in 0.9 % NaCl) before refeeding and 4 h after commencement of refeeding. The latter injection was to prevent the rebound phenomenon described by Simon & Kraicer (1966). Preliminary experiments showed that this protocol maintained a low plasma insulin concentration throughout the 7 h period of the experiments. Use of streptozotocin to induce short-term insulin deficiency was not practicable in these experiments, because 3 h after treatment there is an increase in plasma insulin, owing to destruction of β -cells and release of contents (Schein *et al.*, 1971).

Measurements of blood glucose and plasma insulin. Whole-blood glucose (Slein, 1963) was determined by a standard enzymic method. Plasma insulin was measured by radioimmunoassay (Albano *et al.*, 1972).

RESULTS AND DISCUSSION

¹⁴CO₂ production and ¹⁴C-lipid accumulation in carcass

The absorption of oral ¹⁴C-lipid during the 5 h period ranged from approx. 70 to 90 %, and the values for each group of animals were not markedly affected by starvation or refeeding (Table 1). However, to correct for differences in absorption between experimental groups, all results for ¹⁴CO₂ production and ¹⁴C-lipid accumulation in tissues are expressed as a percentage of the absorbed dose (see Oller do Nascimento & Williamson, 1986).

Starvation (24 h) decreased the high rate of oxidation of oral ¹⁴C-lipid (as measured by ¹⁴CO₂ excretion) in the

chow-fed virgin rats by 37 %, and refeeding *ad libitum* (2 h) returned the rate to that for the chow-fed rats (Table 1). In contrast, starvation increased the low rate of ¹⁴CO₂ production by lactating rats (169 %) and by lactating rats with the pups removed (523 %) (Table 1). For the lactating rats, this increase in ¹⁴CO₂ production was not accompanied by a significant decrease in the accumulation of ¹⁴C-lipid in the carcass; however, there was a 70 % decrease in the transfer of ¹⁴C-lipid to the suckling pups (Table 1), which accounted for a high proportion of the increased ¹⁴CO₂ production. Starvation decreased (by 59 %) the ¹⁴C-lipid accumulation in the carcasses of lactating rats with pups removed, and this decrease accounted for the increase in ¹⁴CO₂ production. Refeeding *ad libitum* for 2 h reversed these changes in starved lactating rats and starved lactating rats with the pups removed, so that the values for lipid accumulation in the carcass (or in pups) were not significantly different from those of chow-fed lactating rats and chow-fed lactating rats with pups removed (Table 1). In the latter group, the ¹⁴CO₂ production on refeeding was still significantly higher than in the fed group (247 %; *P* < 0.001). If starved lactating rats with pups removed were refed with a restricted amount of chow (1.5 g/100 g body wt., approx. 35 % of that eaten in 2 h *ad libitum*), the ¹⁴CO₂ production was not significantly different from starved values, but the ¹⁴C-lipid accumulation in the carcass increased by 36 % (Table 1). The recovery of absorbed radioactivity (¹⁴CO₂ plus ¹⁴C-lipid in carcass plus ¹⁴C-lipid transferred to pups) in these experiments ranged from 55 to 72 %, and did not differ significantly within each experimental group of rats. The radioactivity not recovered is likely to be present in water-soluble compounds (carbohydrates, amino acids, bicarbonate) (Leyton *et al.*, 1987).

¹⁴C-lipid accumulation in mammary gland

The decreased transfer of ¹⁴C-lipid to the pups in the starved lactating rats (Table 1) suggested that the uptake of the ¹⁴C-lipid by the lactating mammary gland was depressed, because mammary tissue is the major site of ¹⁴C-lipid accumulation in the fed lactating rat (Oller do Nascimento & Williamson, 1986). Starvation did not

Table 3. Effects of starvation and refeeding on lipoprotein lipase activity in mammary gland and white and brown adipose tissue of virgin, lactating and lactating rats with pups removed

For experimental details see the text. The results are means $\pm$ s.e.m. for the numbers of rats shown in parentheses. Values that are significantly different by Student's *t* test from those for the appropriate fed group are shown by ***P* < 0.01, ****P* < 0.001.

Experimental group	Lipoprotein lipase activity (nmol of fatty acid released/min per mg of acetone-dried tissue)					
	Virgin			Lactating		
	White adipose tissue	Brown adipose tissue	White adipose tissue	Brown adipose tissue	Mammary gland	White adipose tissue
Chow-fed	0.49 $\pm$ 0.007 (6)	0.49 $\pm$ 0.08 (6)	0.23 $\pm$ 0.04 (5)	0.33 $\pm$ 0.06 (5)	0.90 $\pm$ 0.15 (5)	1.41 $\pm$ 0.32 (4)
Starved (24 h)	0.13 $\pm$ 0.03** (4)	0.51 $\pm$ 0.07 (4)	0.28 $\pm$ 0.09 (5)	0.38 $\pm$ 0.13 (5)	0.27 $\pm$ 0.05** (5)	0.07 $\pm$ 0.01** (4)
Refed with chow (2 h <i>ad libitum</i>)	0.17 $\pm$ 0.09*** (6)	0.79 $\pm$ 0.03** (6)	0.14 $\pm$ 0.05 (4)	0.25 $\pm$ 0.06 (4)	0.87 $\pm$ 0.09 (4)	0.47 $\pm$ 0.09** (6)
Refed chow (2 h) + mannoheptulose	—	—	0.14 $\pm$ 0.04 (4)	0.23 $\pm$ 0.06 (3)	0.65 $\pm$ 0.11 (4)	0.18 $\pm$ 0.02** (8)
						0.71 $\pm$ 0.12 (4)
						0.13 $\pm$ 0.03** (4)
						0.22 $\pm$ 0.02*** (6)
						0.33 $\pm$ 0.09* (8)
						0.02 $\pm$ 0.01*** (4)
						0.07 $\pm$ 0.01*** (6)
						0.08 $\pm$ 0.03*** (8)

Table 4. Effects of starvation and refeeding on tissue accumulation of 14 C-lipid in white and brown adipose tissue of virgin, lactating and lactating rats with pups removed

For experimental details see the text. The results are means $\pm$ s.e.m. for the numbers of rats shown in parentheses. Values for the chow-fed groups are taken from Oller do Nascimento & Williamson (1986). Values that are significantly different by Student's *t* test from those for the appropriate fed group are shown by **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

Experimental group	Tissue 14 C-lipid accumulation (% of absorbed dose/g of tissue)					
	Virgin			Lactating		
	White adipose	Brown adipose	White adipose	Brown adipose	White adipose	Brown adipose
Chow-fed	0.41 $\pm$ 0.10 (6)	5.50 $\pm$ 0.60 (6)	0.17 $\pm$ 0.06 (5)	1.02 $\pm$ 0.57 (5)	1.57 $\pm$ 0.16 (4)	3.98 $\pm$ 0.63 (4)
Starved (24 h)	0.23 $\pm$ 0.03 (4)*	8.00 $\pm$ 2.00 (4)	0.05 $\pm$ 0.01 (5)*	1.09 $\pm$ 0.65 (5)	0.18 $\pm$ 0.06 (4)***	1.38 $\pm$ 0.27 (4)**
Refed chow (2 h <i>ad libitum</i>)	0.21 $\pm$ 0.06 (4)	20.3 $\pm$ 2.6 (4)***	0.16 $\pm$ 0.04 (4)	1.87 $\pm$ 0.54 (4)	1.59 $\pm$ 0.33 (6)	7.95 $\pm$ 1.56 (6)
Refed chow (1.5 g/100 g body wt.)	—	—	—	—	0.73 $\pm$ 0.21 (4)**	4.60 $\pm$ 0.46 (4)
Refed chow (2 h) + mannoheptulose	—	—	0.06 $\pm$ 0.01 (4)	0.84 $\pm$ 0.32 (4)	0.29 $\pm$ 0.05 (8)***	5.50 $\pm$ 1.59 (8)

Table 5. Effects of starvation and refeeding on the concentration of blood glucose and plasma insulin of virgin, lactating and lactating rats with pups removed

For experimental details see the text. The results are means $\pm$ S.E.M. for the numbers of rats shown in parentheses. Values that are significantly different by Student's *t* test from those for the appropriate fed group are shown by **P* < 0.05, ***P* < 0.01, ****P* < 0.005.

Experimental group	Virgin		Lactating		Pups removed for 24 h	
	Glucose (μ mol/ml)	Insulin (μ units/ml)	Glucose (μ mol/ml)	Insulin (μ units/ml)	Glucose (μ mol/ml)	Insulin (μ units/ml)
Chow-fed	8.10 $\pm$ 0.23 (6)	40.4 $\pm$ 8.3 (6)	5.10 $\pm$ 0.66 (6)	23.4 $\pm$ 7.1 (5)	7.33 $\pm$ 0.58 (4)	122 $\pm$ 15.1 (4)
Starved (24 h)	4.54 $\pm$ 0.32*** (5)	9.9 $\pm$ 5.3* (5)	5.84 $\pm$ 0.66 (5)	7.1 $\pm$ 2.5* (5)	5.13 $\pm$ 0.19** (4)	5.0 $\pm$ 1.8** (4)
Refed with chow (2 h) <i>ad libitum</i>	6.10 $\pm$ 0.39 (4)	65.2 $\pm$ 10.8 (6)	5.84 $\pm$ 0.27 (5)	25.9 $\pm$ 8.7 (5)	6.93 $\pm$ 0.13 (6)	121.5 $\pm$ 28.1 (6)
Refed with chow (1.5 g/100 g body wt.)	—	—	—	—	6.88 $\pm$ 0.23 (4)	99.5 $\pm$ 7.0 (4)
Refed with chow (2 h) + mannoheptulose	—	—	8.2 $\pm$ 1.0* (3)	11.5 $\pm$ 1.2 (3)	10.8 $\pm$ 1.5 (7)	48.0 $\pm$ 11.3** (7)

significantly change the ¹⁴C-lipid accumulation in mammary gland when expressed per g wet wt. of tissue (Table 2), but the mammary-gland mass decreased on starvation, and therefore the total accumulation of ¹⁴C-lipid in mammary tissue decreased by 58 % (Table 2). Refeeding the starved lactating rats *ad libitum* [2 h; 6.3 $\pm$ 0.85 (*n* = 3) g/100 g body wt.] restored the ¹⁴C-lipid accumulation in the total mammary tissue to a value not significantly different from that for fed lactating rats (Table 2). Removal of pups decreased the accumulation of ¹⁴C-lipid in mammary tissue by an order of magnitude (Table 2; Oller do Nascimento & Williamson, 1986), and starvation or feeding had no significant effect on ¹⁴C-lipid accumulation (Table 2).

A major determinant of the ¹⁴C-lipid accumulation in mammary gland is the activity of lipoprotein lipase (see Scow & Chernick, 1987), which decreased by 70 % in mammary tissue from starved lactating rats and was restored to fed values on refeeding *ad libitum* (Table 3).

The decrease (58 %) in ¹⁴C-lipid accumulation in lactating mammary gland in response to starvation (24 h) is less than the decrease (> 95 %) that occurs in lipogenesis (Munday & Williamson, 1981; Jones *et al.*, 1984; Bussmann *et al.*, 1984) or lactose synthesis (Carrick & Kuhn, 1978; Bussmann *et al.*, 1984). The lipid contained in milk can come from different sources: (a) hydrolysis by lipoprotein lipase of chylomicrons (derived from dietary fat) or very-low-density lipoproteins (secreted by the liver) and uptake of the resultant fatty acids; (b) uptake of fatty acids released from adipose tissue; (c) synthesis *de novo* (lipogenesis) of fatty acids within the gland. The endogenously synthesized fatty acids are mainly of medium chain length (Grigor & Warren, 1980), whereas the exogenous fatty acids are long-chain. Maintenance of lipoprotein lipase activity in the mammary gland during short-term starvation (up to 24 h) means that long-chain fatty acids can still be provided for milk lipid from hepatic very-low-density lipoproteins, although synthesis *de novo* of fatty acids from glucose and lactate has virtually ceased (Williamson *et al.*, 1984). Evidence for this view is that diet-restricted

(25 g/day) lactating rats which eat all their food within 6 h produce milk of unchanged lipid content (in the post-absorptive period) but decreased medium-chain fatty acid content compared with rats fed *ad libitum* (Grigor & Thompson, 1987).

¹⁴C-lipid accumulation in brown and white adipose tissue

Starvation decreased ¹⁴C-lipid accumulation in white adipose tissue of virgin rats (44 %), lactating rats (70 %) and lactating rats with pups removed (89 %) (Table 4), the absolute changes being 8–10-fold greater in the latter group. Refeeding (2 h) did not restore the ¹⁴C-lipid accumulation in white adipose tissue of starved virgin rats, whereas the values for the refed lactating rats and lactating rats with pups removed were not significantly different from the corresponding fed groups (Table 4). ¹⁴C-lipid accumulation in brown adipose tissue of virgin or lactating rats was not altered by starvation, but there was a 65 % decrease in the brown adipose tissue of the lactating rats with pups removed (Table 4). Refeeding caused an approx. 4-fold increase in ¹⁴C-lipid accumulation in brown adipose tissue of the virgin rats (compared with fed rats) and restored the accumulation in the lactating rats with pups removed (Table 4). Refeeding a restricted amount of chow (1.5 g/100 g body wt.) to starved lactating rats with pups removed partially restored the accumulation of ¹⁴C-lipid in white adipose tissue, and completely restored it in brown adipose tissue (Table 4).

Cryer *et al.* (1974) have demonstrated in male rats that there is a good correlation between the activity of lipoprotein lipase in the epididymal fat-body and the uptake of intravenously administered chylomicron triacylglycerols labelled in the fatty acid moiety with ¹⁴C. In the present experiments, starvation decreased lipoprotein lipase activity in white adipose tissue of virgin rats and lactating rats with pups removed, but had no effect on the low activity of lactating rats (Table 3). The activity of the enzyme was decreased by starvation in the brown adipose tissue of the lactating rat with the pups removed, but was unchanged in the virgin and lactating rats (Table 3). Refeeding chow did not restore the lipoprotein lipase

activity in white adipose tissue of virgin rats, and only partially restored the activity in lactating rats with pups removed (Table 3). Administration of oral glucose to starved male rats caused a substantial restoration of adipose-tissue lipoprotein lipase activity within 3 h (Cryer *et al.*, 1974). Activity of lipoprotein lipase in brown adipose tissue of refed virgin rats was increased above the values for fed controls, whereas the activity in lactating rats and the lactating rats with pups removed was not increased (Table 3). In white adipose tissue the changes in lipoprotein lipase activity on starvation and refeeding tended to parallel the accumulation of ^{14}C -lipid (Tables 3 and 4), whereas this was not true for brown adipose tissue (see also Oller do Nascimento & Williamson, 1986).

Role of insulin in ¹⁴C-lipid accumulation

Several lines of evidence implicate the involvement of insulin in the maintenance of lipoprotein lipase activity in adipose tissue. It has been shown that activity of the enzyme increases in white adipose tissue taken from starved rats when incubated in the presence of insulin (Salamon & Robinson, 1966; Wing *et al.*, 1966). Furthermore, the increase in adipose-tissue lipoprotein lipase activity on refeeding starved male rats with amounts of glucose, fructose or sucrose of equal energy content correlated well with the increase in plasma insulin (Cryer *et al.*, 1974).

In the fed state the plasma insulin in the lactating group was approx. 50 % lower than in the virgin group and 80 % lower than in the lactating group with the pups removed (Table 5). It is noteworthy that the plasma insulin for the lactating rats with pups removed was 3-fold higher than in the virgin group, but the blood glucose was similar (Table 5). This pattern of relative hypoinsulinaemia during lactation, followed by high concentrations of the hormone on removal of the pups, confirms previous results from this and other laboratories (Kuhn, 1977; Robinson *et al.*, 1978; Agius *et al.*, 1979; Flint *et al.*, 1979; Burnol *et al.*, 1983).

Starvation resulted in the expected decrease in plasma insulin to a low value in all groups, but blood glucose only decreased in the virgin group and lactating rats with pups removed (Table 5). Refeeding *ad libitum* restored the blood glucose and plasma insulin to the fed values in all groups of rats (Table 5). If all the experimental groups are included, there is a good correlation between the plasma insulin and the ^{14}C -lipid accumulation in white adipose tissue (correlation coefficient = 0.85; $n = 19$; $P < 0.001$).

To examine the role of insulin in the restoration of ^{14}C -lipid accumulation on refeeding, plasma insulin was decreased by administration of mannoheptulose (Simon & Kraicer, 1966). In both lactating rats and lactating rats with pups removed, mannoheptulose caused hyperglycaemia and decreased the plasma insulin by 56 % and 60 % respectively. Refeeding mannoheptulose-treated lactating rats did not decrease $^{14}\text{CO}_2$ production or increase the amount of ^{14}C -lipid accumulated in the carcass (Table 1). The amount of ^{14}C -lipid accumulated in the total lactating mammary gland was, however, restored to fed values (Table 2), and there was a significant increase (74 %; $P < 0.05$) in the amount of ^{14}C -lipid transferred to the pups (Table 1). In agreement with this finding, insulin deficiency did not prevent the increase in lipoprotein lipase activity in mammary gland

Table 6. Total tissue activity of lipoprotein lipase and the total tissue accumulation of ^{14}C -lipid in white and brown adipose tissue and mammary gland of lactating rats and lactating rats with pups removed

The results for tissue accumulation of ^{14}C -lipid (% of absorbed dose) from Tables 2 and 4 and for lipoprotein lipase activity (nmol of fatty acid released/min) from Table 3 have been recalculated on a total-tissue basis. The mammary-gland mass was obtained by dissection and weighing; the white-adipose-tissue mass was calculated from the carcass fat content, assuming that white adipose tissue contains 80 % lipid; and the brown-adipose-tissue mass was calculated by assuming the value of 0.67 % of body wt. (Smith & Roberts, 1964). The results are mean values for four to seven rats and, for adipose tissue, have been corrected to a carcass wt. of 250 g. The values for mammary-gland ^{14}C -lipid accumulation include the amount transferred to the pups.

Experimental group	Lactating						Pups removed (24 h)					
	Mammary gland		White adipose tissue		Brown adipose tissue		Mammary gland		White adipose tissue		Brown adipose tissue	
	Lipo-protein lipase	^{14}C -lipid	Lipo-protein lipase	^{14}C -lipid	Lipo-protein lipase	^{14}C -lipid	Lipo-protein lipase	^{14}C -lipid	Lipo-protein lipase	^{14}C -lipid	Lipo-protein lipase	^{14}C -lipid
Chow-fed	3383	34.7	491	2.3	52	1.7	1189	3.5	2060	21	111	6.6
Starved (24 h)	842	14.5	130	0.7	59	1.8	113	3.1	102	3.5	20	2.3
Refed chow	3841	39.7	170	2.1	39	3.1	308	2.2	691	31	34	13.3
(2 h <i>ad libitum</i>)												
Refed chow (2 h + mannoheptulose	2372	38.2	145	0.8	36	1.4	340	2.3	265	5.6	52	9.1

on refeeding (Table 3). Similarly, refeeding mannoheptulose-treated lactating rats with their pups removed did not decrease $^{14}\text{CO}_2$ production or increase the amount of lipid in the carcass (Table 1). In this group of rats, however, the expected increase in ^{14}C -lipid accumulation in white adipose tissue on refeeding did not occur (Table 4), and there was only a relatively small increase in lipoprotein lipase activity in this tissue (Table 3). Mannoheptulose treatment did not prevent the restoration of ^{14}C -lipid accumulation in brown adipose tissue of refeed lactating rats with pups removed (Table 4).

Concluding remarks

These experiments on starvation and refeeding indicate the importance of total tissue lipoprotein lipase activity in regulating the disposal of dietary lipid (Table 6). Calculations show that in the fed rat the white-adipose-tissue mass is potentially the major site of triacylglycerol removal, but that on starvation with the decrease in adipose-tissue activity the skeletal muscle becomes more important (Tan *et al.*, 1977). In contrast, in the fed lactating rat the major site of lipoprotein lipase activity, and hence triacylglycerol removal, is the lactating gland (Table 6), and in this case the triacylglycerol cannot be returned to the plasma as non-esterified fatty acids for oxidation. In starvation, the lactating mammary gland becomes less important, and presumably skeletal muscle takes up more of the available triacylglycerol. On removal of the pups, the total tissue activity of lipoprotein lipase in white adipose tissue is increased considerably compared with the lactating mammary gland (Table 6), and therefore fat deposition is the predominant fate of the available triacylglycerol in the fed state. Despite its considerably smaller total mass (about 10%) and apparent lower total lipoprotein lipase activity, brown adipose tissue makes a similar contribution to the accumulation of ^{14}C -lipid as white adipose tissue in fed lactating rats (Table 6). On removal of the pups, the contribution of total brown adipose tissue in the fed state is about 30% that of white adipose tissue (Table 6). Although these calculations are based on a number of assumptions, and do not take into account other factors such as blood flow and different kinetic properties of lipoprotein lipase in the various tissues, it is likely that the overall conclusions are valid. The changes in $^{14}\text{CO}_2$ production, which represent oxidation of [^{14}C]triacylglycerol in skeletal muscle and brown adipose tissue or ^{14}C -chylomicron remnants and ^{14}C -labelled non-esterified fatty acids in liver, are in agreement with the view that there is competition between skeletal muscle, adipose tissue and lactating mammary gland for available triacylglycerol, and that the total tissue activity of lipoprotein lipase is a key factor in determining the fate of the triacylglycerols.

Restoration of ^{14}C -lipid accumulation in adipose tissue of lactating rats with pups removed on refeeding after a period of starvation is dependent on the plasma insulin (Table 5), and it seems likely that the higher accumulation of ^{14}C -lipid compared with the virgin rats is due to relative hyperinsulinaemia (Table 5) rather than the decrease in plasma prolactin (see Scow & Chernick, 1987). There was no significant difference in food intake on refeeding between the two groups (virgin rats 2.68 ± 0.35 g/100 g body wt.; lactating rats with pups removed 4.11 ± 0.72 g/100 g body wt., $n = 6$). In contrast,

^{14}C -lipid accumulation in the lactating mammary gland appears to be less dependent on the plasma insulin concentration, because it was restored to control values in refeed mannoheptulose-treated lactating rats with low plasma insulin (Table 3) and despite relative hypophagia (lactating rats 6.3 ± 0.49 g/100 g body wt., $n = 3$; lactating rats mannoheptulose-treated: 3.22 ± 0.33 g/100 g body wt., $n = 4$; $P < 0.05$). It is noteworthy that plasma prolactin does not decrease in the starved lactating rats when pups are present (Robinson *et al.*, 1978).

The apparent lack of dependence of ^{14}C -lipid accumulation in mammary gland on the plasma insulin concentration means that triacylglycerol can still be extracted by the gland when the plasma insulin decreases in the post-absorptive state (Jones *et al.*, 1984). This suggests that lipoprotein lipase activity in mammary gland is regulated differently from that in adipose tissue or even skeletal muscle (Borensztajn, 1987). The present experiments do not, however, rule out the possibility that lipoprotein lipase activity in the gland is maintained by lower plasma insulin concentrations than for the enzyme in white adipose tissue, because the gland appears to be highly sensitive to the plasma insulin concentration (Mercer & Williamson, 1986; Page & Kuhn, 1986). It is certain, however, that the accumulation of ^{14}C -lipid in the gland is less affected by the withdrawal of food and lowering of plasma insulin than is lipogenesis (Williamson *et al.*, 1984) or lactose synthesis (Carrick & Kuhn, 1978).

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