Tumour necrosis factor α (cachectin) mimics some of the effects of tumour growth on the disposal of a [^{14}C]lipid load in virgin, lactating and litter-removed rats

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Tumour necrosis factor α (cachectin) was administered to virgin, lactating and litter-removed rats, and subsequent disposal of an oral [^{14}C]triolein (glycerol tri[^{14}C]oleate) load examined. Absorption of the lipid and $^{14}\text{CO}_2$ production were significantly depressed in all three groups. [^{14}C]Lipid accumulation was decreased in carcass, liver and adipose tissue (brown and white) of virgin and litter-removed rats and the mammary gland of lactating rats. The plasma triacylglycerol concentration was increased in all three groups, and lipoprotein lipase activity was decreased in the white adipose tissue of virgin and litter-removed animals and in the mammary gland of lactating animals. Some, but not all, of these effects mimic tumour burden in the same physiological states [Evans & Williamson (1988) *Biochem. J.* 252, 65–72].

INTRODUCTION

Tumour burden has profound effects on lipid metabolism. These include decreases in whole-body lipid oxidation rates (Costa *et al.*, 1976), lipoprotein lipase activity and the rate of lipogenesis in white adipose tissue (Thompson *et al.*, 1981; Lanza-Jacoby *et al.*, 1984), and an increase in plasma triacylglycerols (Lanza-Jacoby *et al.*, 1984). Recently the tissue-specific effects of malignant tumour (Walker-256 carcinosarcoma) burden on mammary-gland and adipose-tissue lipid metabolism during lactation and after premature removal of the pups have been described (Evans & Williamson, 1988). The tumour was found to exert its deleterious effects mainly during periods of rapid transition in metabolism (parturition and establishment of lactation; weaning) (Evans & Williamson, 1988; Williamson *et al.*, 1988). These effects include decreased [^{14}C]lipid accumulation in plasma together with hypertriglyceridaemia, and decreased lipogenesis in white and brown adipose tissue in litter-removed rats, effects not seen during tumour growth in established lactation (Evans & Williamson, 1988). Tumour growth before parturition inhibits lactation *post partum* (Williamson *et al.*, 1988). Some of the effects of tumour burden in rats can be mimicked by macrophage cytokines, notably suppression of lipoprotein lipase activity in adipose tissue by recombinant-derived tumour necrosis factor α (TNF α , cachectin) both *in vitro* (Price *et al.*, 1986) and *in vivo* (Semb *et al.*, 1987), and this had led to the suggestion that it may be the causative agent of tumour cachexia (Beutler & Cerami, 1987). In a human study, half the patients with active cancer had increased serum TNF α (Balkwill *et al.*, 1987). Much of the present information on the effects of TNF α on lipid metabolism comes from studies on cultured cell lines rather than *in vivo* (see Moldawer *et al.*, 1988). In the present study we have investigated the effect of recombinant-derived human TNF α on lipid metabolism *in vivo* during lactation

and on litter removal to examine whether this cytokine can mimic the previously reported effects of tumour growth (Evans & Williamson, 1988). For comparison, effects of TNF α on disposal of a [^{14}C]lipid load in virgin rats were also examined.

MATERIALS AND METHODS

Animals

All animals were fed *ad libitum* on a chow diet consisting (by weight) 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 $22 \pm 2^\circ\text{C}$ with a 12 h-light/12 h-dark cycle (lights on from 07:00 h). Food was weighed daily. Three groups of Wistar rats were studied: virgin females (body wt. 200–250 g), lactating rats with nine or ten pups used 10–12 days *post partum* (body wt. 270–330 g) and lactating rats 10–13 days *post partum* whose litters had been prematurely removed 24 h previously (body wt. 270–330 g). Only lactating rats whose litters were increasing in weight around the average for the colony (1.8 g/day per pup) were used.

Biochemicals

All enzymes and coenzymes were obtained from Boehringer Corp. (London) Ltd., Lewes, Sussex, U.K. Recombinant-derived human TNF α was generously given by BASF/Knoll AG, Ludwigshafen, Germany. [^{14}C]Triolein (glycerol tri[^{14}C]oleate) and glycerol tri[9,10(n)- ^3H]oleate were obtained from Amersham International, Amersham, Bucks., U.K.

Cytokine administration

Experimental animals were injected with 0.6 mg of recombinant-derived human TNF α /kg body wt. in

0.5 ml of phosphate-buffered saline (50 mM-potassium phosphate/0.9% NaCl, pH 8.5) (contamination with endotoxin < 0.137 ng/mg of protein by *Limulus* assay) intravenously through the dorsal tail vein under light diethyl ether anaesthesia; control animals received 0.5 ml of vehicle. All injections were administered between 09:00 and 10:00 h on the day of the experiment, with the animals being killed 6 h later; food was not available during this period.

Measurement of lipid oxidation and tissue lipid accumulation

The metabolic fate of orally administered [^{14}C]-triolein was measured as described by Oller do Nascimento & Williamson (1986). The dose administered was 700 mg of [^{14}C]-triolein, containing about 0.33 μCi . After collection of expired CO_2 for 5 h the animal was killed (6 h after $\text{TNF}\alpha$ administration), aortic blood collected with a heparinized syringe for plasma and blood samples, and the gastrointestinal tract was removed and homogenized in 150 ml of 3% (w/v) HClO_4 . Duplicate samples of liver, parametrial adipose tissue, inguinal mammary gland (animals *post partum*) and interscapular brown adipose tissue (single sample) were removed. Carcasses, after removal of liver, mammary gland and heart, were autoclaved, blended, and triplicate samples taken; all tissues were saponified and fatty acids extracted by the method of Stansbie *et al.* (1976).

Lipoprotein lipase

Parametrial-adipose-tissue, mammary-gland and heart lipoprotein lipase activities were determined on acetone/diethyl ether-dried powders of the tissues as described previously (Evans & Williamson, 1988).

Plasma triacylglycerols

Plasma triacylglycerol was measured by the method of Eggstein & Kreutz (1966).

RESULTS AND DISCUSSION

The effect of $\text{TNF}\alpha$ on exogenous [^{14}C]lipid absorption and disposal is shown in Table 1. Absorption was calculated by subtracting radioactivity remaining in the gastrointestinal tract from that administered, and a significant impairment was observed in $\text{TNF}\alpha$ -treated rats from all three study groups (virgin, lactating and litter-removed) to less than half their respective control values (Table 1). Consequently, subsequent lipid disposal was calculated as a percentage of absorbed lipid (Oller do Nascimento & Williamson, 1986). Profound gastrointestinal-tract dysfunction, with even macroscopic haemorrhagic necrosis of the small bowel, has been reported in virgin animals in response to high doses of $\text{TNF}\alpha$ (Tracey *et al.*, 1986). This effect may account for many of the metabolic derangements noted in previously reported studies, as recorded food intake or even gastric gavage may be an unreliable index of metabolic substrate availability to the animal *in vivo*. In our experiments (0.6 mg of $\text{TNF}\alpha$ /kg body wt.) the bowel appeared macroscopically normal and no rats died (see also Sun & Hsueh, 1988).

Whole-body lipid oxidation rate, as estimated by $^{14}\text{CO}_2$ production, was significantly diminished (about 90%) in all groups (Table 1), including lactating rats, which have low oxidation rates (Oller do Nascimento &

Table 1. Effect of $\text{TNF}\alpha$ on the absorption and metabolic fate of orally administered [^{14}C]triolein in virgin, lactating and litter-removed rats

State of rats		Absorption of [¹⁴ C] lipid administered dose/5 h)	¹⁴ CO ₂ production (% of absorbed dose/h)	Tissue [¹⁴ C]lipid accumulation (% of absorbed dose/5 h per g)					
				Carcass	Liver	Mammary gland	Parametrial adipose tissue	Brown adipose tissue	Plasma
Virgin									
Control	(7)	70.0 ± 2.85	7.47 ± 1.18	0.114 ± 0.013	0.540 ± 0.044	—	0.350 ± 0.070	8.23 ± 1.02	0.049 ± 0.006
TNFα	(4)	23.5 ± 2.30	0.280 ± 0.095**	0.044 ± 0.006**	0.308 ± 0.105*	—	0.080 ± 0.036*	0.998 ± 0.245***	0.090 ± 0.031
Lactating									
Control	(6)	84.6 ± 2.80	3.02 ± 0.82	0.050 ± 0.015	0.273 ± 0.054	2.32 ± 0.253	0.057 ± 0.028	0.766 ± 0.629	0.050 ± 0.015
TNFα	(4)	26.7 ± 1.74***	0.148 ± 0.032*	0.034 ± 0.015	0.133 ± 0.031	0.221 ± 0.055***	0.030 ± 0.011	0.086 ± 0.028	0.167 ± 0.061
Litter-removed									
Control	(5)	74.5 ± 2.88	4.17 ± 0.33	0.192 ± 0.011	0.624 ± 0.043	0.100 ± 0.017	2.42 ± 0.35	8.10 ± 2.28	0.104 ± 0.019
TNFα	(5)	35.4 ± 11.4**	0.525 ± 0.290***	0.116 ± 0.031*	0.308 ± 0.115*	0.034 ± 0.009**	0.402 ± 0.195**	2.20 ± 1.15*	0.182 ± 0.053

$^{14}\text{CO}_2$ production was calculated between 2 and 5 h of the experiment after triolein administration. For further details, see the Materials and methods section. The results are mean values $\pm$ S.E.M., with the numbers of observations in parentheses. $\text{TNF}\alpha$ -administered values that are significantly different by Student's *t* test from their control values are indicated by: * $P > 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 2. Effect of TNF α on plasma triacylglycerol concentration and tissue lipoprotein lipase activity in virgin, lactating and litter-removed rats

For details see the Materials and methods section. The results are mean values $\pm$ S.E.M., with the numbers of observations in parentheses. Values that are significantly different by Student's *t* test from control values are indicated by: **P* < 0.05; ****P* < 0.001.

State of rats		Plasma triacylglycerol concentration (mg/100 ml)	Lipoprotein lipase activity (nmol of fatty acid released/min per mg of acetone-dried tissue)		
			Parametrial adipose tissue	Mammary gland	Heart
Virgin					
Control	(7)	51.1 $\pm$ 9.64	1.12 $\pm$ 0.119	—	0.903 $\pm$ 0.115 (4)
TNF α	(4)	107.3 $\pm$ 23.6*	0.548 $\pm$ 0.184*	—	0.658 $\pm$ 0.114
Lactating					
Control	(5)	67.0 $\pm$ 14.6	—	0.774 $\pm$ 0.071	0.320 $\pm$ 0.025
TNF α	(4)	508.2 $\pm$ 149.4*	—	0.329 $\pm$ 0.072*	0.402 $\pm$ 0.052
Litter-removed					
Control	(5)	173.6 $\pm$ 36.5	1.52 $\pm$ 0.237	0.052 $\pm$ 0.016	0.356 $\pm$ 0.083
TNF α	(9)	535.7 $\pm$ 87.6*	0.487 $\pm$ 0.092***	0.065 $\pm$ 0.014	0.397 $\pm$ 0.036

Williamson, 1986; Evans & Williamson, 1988). This decrease in lipid oxidation may represent an energy-conservation mechanism, as in the normal lactating rat (Oller do Nascimento & Williamson, 1986), or may simply indicate unavailability of the lipid substrate to oxidation sites. The $^{14}\text{CO}_2$ production rate was analysed for each rat over the 5 h collection period and was found to be linear between 2 and 5 h in each case ($r > 0.9$), indicating that the rate of appearance of [^{14}C]lipid in the circulation, even when absorption is diminished by the cytokine, is presumably constant.

Accumulation of the [^{14}C]triolein was significantly decreased in a variety of tissues in virgin and litter-removed animals after TNF α administration (Table 1); thus carcass, liver and white and brown adipose tissue all accumulated less radioactivity than their respective controls. Mammary-gland accumulation was low 24 h after litter removal, but was depressed still further in the TNF α -treated group (Table 1). [^{14}C]Lipid tended to increase in plasma in all groups treated with TNF α , but this was not significant (Table 1). In lactating animals, accumulation in tissues other than mammary gland is diminished as a physiological mechanism of 're-direction' of energetic substrates to the active mammary gland (Oller do Nascimento & Williamson, 1986), and this was confirmed in the present experiments. The high accumulation in the lactating mammary gland was decreased (90%) after TNF α administration (Table 1). This indicates that the effect of TNF α is not confined to adipose tissue and may be contrasted with the situation with tumour burden, where there is no effect on mammary-gland lipid accumulation at stages of tumour growth when accumulation in other tissues was significantly inhibited (Evans & Williamson, 1988).

Administration of TNF α was found to cause hypertriglyceridaemia in virgin, lactating and litter-removed animals (Table 2). Hyperlipaemia has been noted previously in tumour-bearing animals (Lanza-Jacoby *et al.*, 1984; Evans & Williamson, 1988). A possible mechanism in tumour-bearing rats is the suppression of adipose-tissue lipoprotein lipase activity (Thompson *et al.*, 1981;

Lanza-Jacoby *et al.*, 1984; Evans & Williamson, 1988), and this was observed in the parametrial adipose tissue of virgin and litter-removed rats treated with TNF α (Table 2); this activity is physiologically low in the adipose-tissue activity is significantly depressed (Evans & 1987) and therefore was not measured. However, lipoprotein lipase activity was decreased in lactating mammary gland, a novel finding indicating a relatively non-tissue-specific effect of the cytokine (Table 2). Tumour burden has been shown not to suppress lipoprotein lipase activity in lactating mammary gland at a time when adipose tissue activity is significantly depressed (Evans & Williamson, 1988), suggesting that TNF α has additional effects not seen during tumour growth (Evans & Williamson, 1988). However, some tissue specificity of TNF α action appears to exist, as lipoprotein lipase activity in heart was not suppressed by this cytokine in any experimental group (Table 2). It has recently been reported that TNF α has no short-term effect on the activity of lipoprotein lipase in the perfused heart (Hülsmann *et al.*, 1988).

Thus TNF α has a wide range of actions on lipid metabolism, including decreased lipid absorption and decreased lipid assimilation by lactating mammary gland, effects not observed in response to tumour growth in rats (Evans & Williamson, 1988). The decreased absorption may be due to a delay in gastric emptying (Patton *et al.*, 1987). The hyperlipaemia occurring in response to TNF α administration (despite a decrease in lipid absorption) in the present experiments may be explained by the decrease in lipoprotein lipase activity in mammary gland (lactating rats) or adipose tissue (virgin and litter-removed rats); however, a contribution from increased lipolysis in adipose tissue in these states cannot be ruled out. Stimulation of lipolysis by TNF α *in vitro* has been demonstrated (Patton *et al.*, 1986; Kawakami *et al.*, 1987; but see Price *et al.*, 1986; Rofe *et al.*, 1987).

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