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Tissue-specific effects of rapid tumour growth on lipid metabolism in the rat during lactation and on litter removal

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1. The effect of tumour burden on lipid metabolism was examined in virgin, lactating and litter-removed rats. 2. No differences in food intake or plasma insulin concentrations were observed between control animals and those bearing the Walker-256 carcinoma (3–5% of body wt.) in any group studied. 3. In virgin tumour-bearing animals, there was a significant increase in liver mass, blood glucose and lactate, and plasma triacylglycerol; the rate of oxidation of oral [^{14}C]lipid to $^{14}CO_2$ was diminished, and parametrial white adipose tissue accumulated less [^{14}C]lipid compared with pair-fed controls. 4. These findings were accompanied by increased accumulation of lipid in plasma and decreased white-adipose-tissue lipoprotein lipase activity. 5. In lactating animals, tumour burden had little effect on the accompanying hyperphagia or on pup weight gain; tissue lipogenesis was unaffected, as was tissue [^{14}C]lipid accumulation, plasma [triacylglycerol] and white-adipose-tissue and mammary-gland lipoprotein lipase activity. 6. On removal (24 h) of the litter, the presence of the tumour resulted in decreased rates of lipogenesis in the carcass, liver and white and brown adipose tissue, decreased [^{14}C]lipid accumulation in white adipose tissue, but increased accumulation in plasma and liver, increased plasma [triacylglycerol] and decreased lipoprotein lipase activity in white adipose tissue. 7. The rate of triacylglycerol/fatty acid substrate cycling was significantly decreased in white adipose tissue of virgin and litter-removed rats bearing the tumour, but not in lactating animals. 8. These results demonstrate no functional impairment of lactation, despite the presence of tumour, and the relative resistance of the lactating mammary gland to the disturbance of lipid metabolism that occurs in white adipose tissue of non-lactating rats with tumour burden.

INTRODUCTION

Lactation is a normal physiological state characterized by increased food intake, mammary-gland hypertrophy and changes in lipid metabolism in specific tissues (see Williamson, 1980). These changes include increased lipoprotein lipase activity (see Scow & Chernick, 1987), triacylglycerol uptake (Oller do Nascimento & Williamson, 1986; Scow & Chernick, 1987) and lipogenesis (Robinson *et al.*, 1978) in the lactating mammary gland, and decreased lipoprotein lipase activity (Scow & Chernick, 1987), triacylglycerol uptake (Oller do Nascimento & Williamson, 1986) and lipogenesis (Robinson *et al.*, 1978) in white adipose tissue. The net result of these reciprocal alterations in the two tissues is a 'redirection' of lipid precursors (mainly glucose) and triacylglycerols from adipose tissue to mammary gland (Williamson, 1980), and, because the triacylglycerol extracted by the gland is not available for mobilization, there is conservation of dietary lipid (Oller do Nascimento & Williamson, 1986). The mammary gland itself has been likened to a benign tumour, in that it consumes large quantities of substrate with no direct benefit to the lactating mother.

Malignant tumour burden is a pathological state associated with decreased food intake and a considerable demand by the rapidly growing tumour for glucose and amino acids. There is controversy in the literature regarding the effect of tumour growth on lipid metabolism in experimental animals and man. Specifically, some authors report increased whole-body lipid oxidation (Costa *et al.*, 1976; Hansell *et al.*, 1986), others unchanged (Waterhouse & Kemperman, 1971) or decreased rates (Costa *et al.*, 1976). There is agreement that an early event during tumour growth is a loss of lipoprotein lipase activity from white adipose tissue (Thompson *et al.*, 1981; Lanza-Jacoby *et al.*, 1984) and a concomitant increase in plasma triacylglycerol concentration (Lanza-Jacoby *et al.*, 1984); at later stages the rate of lipogenesis in adipose tissue is depressed (Thompson *et al.*, 1981). Thus there are some clear analogies between the changes in lipid metabolism in adipose tissue during lactation and those in the presence of a rapidly growing tumour.

In the present paper we have examined whether the presence of a rapidly growing tumour, the transplantable Walker-256 carcinosarcoma, affects lipid metabolism (lipogenesis, triacylglycerol uptake) *in vivo* in the lactating mammary gland of rat or the restoration of lipid deposition (triacylglycerol uptake, lipogenesis, and rate of the triacylglycerol/fatty acid cycle) which occurs on cessation of lactation in adipose tissue (Agius *et al.*, 1979; Oller do Nascimento & Williamson, 1986; Hansson *et al.*, 1987). In addition, for the purposes of comparison, the effects of the tumour growth on lipid metabolism in virgin rats were also examined.

EXPERIMENTAL

Rats

All rats were fed *ad libitum*, except where stated, on a chow diet consisting of 52% carbohydrate, 21% protein and 4% fat (the residue was non-digestible material;

Special Diet Services, Witham, Essex, U.K.) with free access to drinking water, and were maintained at an ambient temperature of 22±2 °C with a 12 h-light/12 h-dark cycle (lights on from 07:00 h). Three groups of Wistar rats were studied. Group 1 were virgin females (body wt. 200–250 g), Group 2 were lactating rats (body wt. 250–350 g) with eight to eleven pups used 10–13 days *post partum*, and Group 3 were lactating rats whose litters had been prematurely removed for 24 h 10–13 days *post partum* (body wt. 250–350 g).

Biochemicals

All enzymes and coenzymes were from Boehringer Corp. (London) Ltd., Lewes, Sussex, U.K.

Radioactive compounds

^3H_2O, [1-^{14}C]triolein (glycerol tri[1-^{14}C]oleate) and glycerol tri[9,10(n)-^{3}H]oleate were obtained from Amersham International, Amersham, Bucks., U.K., and [^{14}C]glycerol was from New England Nuclear.

Tumour implantation

A Walker-256 carcinoma cell suspension (approx. 2×10^7 cells in 0.5 ml) was injected subcutaneously on the left flank of the rats, and the control rats were sham-injected with 0.5 ml of 0.9% (w/v) NaCl under light diethyl ether anaesthesia. Groups 2 and 3 were inoculated 2–3 days after parturition. The Walker-256 carcinoma is a rapidly growing tumour with a volume doubling time of 0.86 days (Herzfeld & Greengard, 1972); the amount implanted ensured that the tumour mass was 3–5% of carcass weight at the time of the experiments. We are grateful to Dr. Marion Stubbs, St. George's Hospital, London, for a supply of the tumour. Food intake, maternal body weight and litter weight were measured daily. Virgin control rats were 'pair fed' with the previous 24 h measured food consumption of their tumour-bearing matched partners, and experiments on this group were, therefore, performed after a 24 h delay. All experiments were commenced between 09:00 and 10:00 h, 9–11 days after tumour implantation.

Measurement of lipogenesis

Lipogenic rate *in vivo* was determined by using ^3H_2O as previously described (Robinson *et al.*, 1978). At 1 h before being killed, animals were injected with 0.3 ml (3.0 mCi) of ^3H_2O intraperitoneally; 5 min before death the animals were anaesthetized with pentobarbital (60 mg/kg body wt.); at 60 min after injection, aortic blood was collected with a heparinized syringe for plasma and blood samples, and duplicate samples were taken of liver, parametrial adipose tissue, inguinal mammary gland (*post-partum* animals), tumour and interscapular brown adipose tissue (single sample). Tissues were saponified and fatty acids extracted by the method of Stansbie *et al.* (1976).

Measurement of lipid oxidation and tissue lipid accumulation

The metabolic fate of an orally administered [^{14}C]lipid load was examined as described by Oller do Nascimento & Williamson (1986). About 0.7 g (0.33 μCi) per rat of [1-^{14}]triolein was given enterally by gastric intubation, without anaesthetic but with minimal stress to the animal. Expired CO_2 was then collected hourly for 5 h by absorption in Lumasorb (May and Baker, Dagenham, Essex, U.K.), and the rate of $^{14}CO_2$ production was estimated by counting radioactivity in a sample of the Lumasorb. After the 5 h collection period, the animal was killed; blood was collected as above, the gastrointestinal tract was homogenized in 150 ml of 3% (w/v) $HClO_4$, and the same tissues as for the measurements of lipogenesis were removed. Samples of tissues, intestinal tract and plasma were saponified and the lipid was extracted (Stansbie *et al.*, 1976). The amount of lipid extracted was determined gravimetrically. The extracted fatty acids were dissolved in 10 ml of scintillation fluid (Optiphase 'Safe'; LKB) for measurement of radioactivity and hence determination of [^{14}C]lipid accumulation. Triolein absorption was calculated by subtracting total gastrointestinal radioactivity from that administered.

Blood metabolites

Whole-blood glucose was determined by the method of Slein (1963) and lactate by the method of Hohorst (1963). Plasma triacylglycerol was measured by the method of Eggstein & Kreutz (1966).

Substrate cycling

The rate of triacylglycerol/fatty acid substrate cycling was measured *in vivo* in parametrial adipose tissue as described by Hansson *et al.* (1987), by using 0.6 ml (6 mCi) of ^3H_2O (injected intraperitoneally) and correcting for varying recovery by adding [^{14}C]glycerol but omitting the freeze-clamp technique as originally described.

Lipoprotein lipase

Parametrial-adipose-tissue and mammary-gland lipoprotein lipase activities were estimated by a modification of the technique of Nilsson-Ehle & Ekman (1977). Tissue samples were dried to a powder with acetone/ether, and then resolubilized and used in an assay system containing [^{3}H]triolein as substrate; [^{3}H]fatty acids released after a 60 min incubation period were extracted and determined by the method of Nilsson-Ehle & Schotz (1976). Lipoprotein lipase activity is expressed as nmol of fatty acid released/min per mg of acetone-dried powder.

Insulin

Plasma insulin was determined by radioimmunoassay with a rat insulin standard (Albano *et al.*, 1972).

RESULTS AND DISCUSSION

Effects of tumour growth on tissue mass and fat content

Since hypophagia is known to have profound effects on insulin status and lipid metabolism, especially during lactation (Robinson *et al.*, 1978; Wilde & Kuhn, 1979; Munday & Williamson, 1983; Jones *et al.*, 1984), and many tumours, including the Walker-256 carcinoma, are anorexigenic in their late stages of growth, experiments were planned to occur at a relatively early stage of tumour growth. Thus duration of tumour burden was 9–11 days, after which time the tumour mass was about 10 g (approx. 3–5% of carcass wt.) and no hypophagia was detectable; all groups showed the food intake appropriate to their physiological state (Table 1). No detectable differences in plasma insulin were found between any of the corresponding control and tumour-bearing groups (results not shown).

Table 1. Food intake, tumour mass, duration of tumour growth and lactation, and body fat content during tumour burden in fed virgin, lactating and litter-removed rats

Carcass mass is body mass minus tumour and gastrointestinal-tract mass in g. Food intake of the litter-removed group is for the last 24 h. For further details see the Experimental section. The results are mean values ± S.E.M. with the numbers of observations in parentheses. Values that are significantly different by Student's *t* test from those of the appropriate control group are indicated by: $*P < 0.05$; $***P < 0.001$.

	State of rats					
	Virgin		Lactating		Litter-removed	
	Control (15)	Tumour-bearing (15)	Control (9)	Tumour-bearing (12)	Control (12)	Tumour-bearing (11)
Tumour present (days)	–	10.5±1.3	–	10.4±1.7	–	9.8±1.4
Food intake (g/day)	22.2±0.51	22.2±0.51	47.0±3.2	49.6±2.4	31.2±2.7	30.7±2.3
Lactation (days)	–	–	9.8±0.9	11.4±0.4	9.8±0.6	11.3±0.4
Tissue mass (g)						
Carcass	167±7.8	171±4.5	191±7.8	188±5.7	196±9.1	194±8.2
Liver	8.5±0.3	10.6±0.3***	15.5±1.01	14.8±0.55	14.9±0.9	14.4±0.5
Mammary gland	–	–	20.2±2.2	19.3±1.9	23.4±1.9	27.4±2.4
Fat content (%)						
Carcass	9.89±0.62	8.26±0.64*	9.96±0.80	8.14±0.72	8.90±0.62	8.81±0.78
Liver	6.53±0.49	5.69±0.43	7.30±0.85	6.18±0.55	7.21±0.46	6.84±0.40
Mammary gland	–	–	18.7±1.87	13.3±0.97*	17.3±1.63	14.8±1.80
Parametrial adipose tissue	78.6±3.0	72.4±3.3	83.6±2.5	80.3±4.1	83.4±0.67	82.2±2.1

Presence of the tumour in the virgin rats was associated with a significant increase in liver mass (absolute and as a percentage of carcass wt.) compared with control rats, but not with a change in carcass mass (body wt. − tumour wt.). This increase has been noted previously (Ekman *et al.*, 1982; Lanza-Jacoby *et al.*, 1982); the percentage fat content of liver was not increased by the presence of tumour in any group (Table 1). The significant decrease (15%) in carcass fat in the virgin rats agrees with other published work in rats (Kralovic *et al.*, 1977; Mider *et al.*, 1949) and mice (Costa & Holland, 1962; Thompson *et al.*, 1981), although hypophagia may have occurred in some of these studies. There was no significant decrease in carcass fat content in the lactating or litter-removed groups bearing the tumour (Table 1). The tumour itself was found to comprise less than 4% of fat.

Effects of tumour growth on lactational performance

Tumour burden in the lactating group was observed to have no demonstrable effect on the process of lactation, as evidenced by the maintenance of mammary-gland mass (Table 1) and mean daily pup weight gain [1.51±0.08 g/day ($n = 12$) for tumour-bearing against 1.37±0.13 g/day ($n = 8$) for control animals]. Pup growth is a sensitive indicator of lactational efficiency, and is highly dependent on maternal food intake (Williamson *et al.*, 1984). The presence of the tumour did not prevent lactation-induced hyperphagia or the decrease in food intake on removal of the litter. It should, however, be noted in this context that, although in this study tumour implantation was performed 2–3 days post-parturition, if the tumour is implanted immediately *pre partum*, the tumour appears to grow faster, and to decrease maternal food intake and pup growth significantly by the same time interval (about 9 days) (D. H. Williamson, R. D. Evans & S. Wood, unpublished work).

Blood metabolites

Most tumours are considered to rely mainly on glycolysis for energy production, and therefore consume glucose and produce lactate. This may lead to glucose mobilization, increased Cori-cycle activity and enhanced gluconeogenesis in the host (Gold, 1974; Holroyde *et al.*, 1975; Singh *et al.*, 1978, 1980; Waterhouse *et al.*, 1979). Blood glucose was significantly increased in the tumour-bearing virgin rats and tended to be increased in the tumour-bearing lactating rats (Table 2); thus in no group did the presence of the tumour result in hypoglycaemia. As might be expected, blood lactate was increased in the virgin and lactating rats bearing the tumour (Table 2). It should be noted that lactate can act as substrate for the lactating mammary gland of the rat (Katz *et al.*, 1974; Williamson, 1980).

Tissue lipogenesis *in vivo*

Rates of lipogenesis in several tissues of the virgin animal showed no change in response to the presence of tumour (Table 3). Thus, although there was a tendency to decreased ^3H incorporation into saponified lipid in carcass and parametrial adipose tissue, this did not achieve statistical significance. This finding is in agreement with Lanza-Jacoby *et al.* (1984), who demonstrated that a decrease in the activities of lipogenic enzymes in liver and adipose tissue in rats bearing a mammary adenocarcinoma (AC 33) was a relatively late event during tumour growth. Similarly, in mice bearing a preputial-gland tumour (ESR-586) the decrease in tissue lipogenesis was correlated with increase in tumour mass (Thompson *et al.*, 1981). In the lactating group, tissue lipogenic rates were also unaffected by the presence of the tumour (Table 3), and in particular there was no decrease in the high rate of mammary-gland lipogenesis. Although there was a tendency in the tumour-bearing

Table 2. Effects of tumour burden on blood metabolite concentrations in fed virgin, lactating and litter-removed rats

All groups of rats were fed *ad libitum*, except virgin control animals, which were 'pair-fed' according to the food intake of their tumour-bearing matched partners. For further details see the Experimental section. The results are mean values ± S.E.M., expressed as μmol/ml of blood, with the numbers of observations in parentheses. Values that are significantly different by Student's *t* test from control values are indicated by: $*P < 0.05$; $**P < 0.01$.

State of rats	Blood metabolite concn. (μmol/ml)	
	Glucose	Lactate
Virgin		
Control (8)	7.46±0.35	3.07±0.21
Tumour-bearing (8)	8.81±0.43*	4.83±0.76*
Lactating		
Control (5)	4.99±0.33	2.30±0.19
Tumour-bearing (6)	6.24±0.58	3.52±0.29**
Litter-removed		
Control (6)	6.11±0.41	2.64±0.46
Tumour-bearing (6)	5.96±0.41	3.38±0.27

rats towards depression of brown-adipose-tissue lipogenesis during lactation, this was not significant (Table 3). However, on removal of the litter, the rate of lipogenesis in white adipose tissue did not increase in the tumour-bearing group, as it did in the control group (Table 3; Agius *et al.*, 1979; Vernon & Flint, 1983). Similarly, brown-adipose-tissue lipogenesis was also only about 25% of control values in the litter-removed tumour-bearing rats (Table 3). Interestingly, the expected increase in hepatic lipogenesis on removal of the litter (Agius *et al.*, 1979) did not occur in the tumour-bearing rats; this depression of liver lipogenesis was due entirely to decreased synthesis of saponified lipids, the non-saponified component being unaffected (results not shown). These decreased rates of lipogenesis in white and brown adipose tissue, liver and carcass on removal of the litter (Table 3) were not due to differences in food intake in the previous 24 h (Table 1) nor in plasma insulin [control rats 101.0±32.9 μunits/ml ($n = 6$); tumour-bearing rats 89.8±58.2 μunits/ml ($n = 6$); difference not significant].

These results indicate that, under the present experimental conditions, the presence of the tumour only has a significant inhibitory effect on tissue lipogenesis during the period of rapid restoration of the process in white and brown adipose tissue and liver immediately after the removal of the litter.

The measured lipogenic rate of the tumour itself increased almost 2-fold during lactation (Table 3; $P < 0.05$), but decreased again after removal of the litter. The rates of lipogenesis in the tumour were low compared with host tissues, which suggests that the Walker-256 carcinoma may not synthesize much of the lipid it requires for growth. This finding is in agreement with measurements of lipogenesis in the preputial-gland tumour ESR-586 in mice (Thompson *et al.*, 1981).

Absorption, oxidation and tissue accumulation of an oral lipid load

Administration of [1-^{14}C]triolein by intragastric intubation revealed no abnormality of fat absorption over a 5 h period between any tumour-bearing group and its respective control; however, absorption within the virgin group was significantly less than in the lactating group (Table 4; Oller do Nascimento & Williamson, 1986), and, 24 h after weaning, the tumour-bearing group had returned to virgin values, but its control group had not. The reasons for this are not clear, although this observation provides further support for the view that tumour burden does not inhibit lactation-induced events, in this case enhanced gastrointestinal absorption. The rate of lipid oxidation, as estimated by $^{14}CO_2$ excretion, was significantly depressed in the virgin tumour-bearing animal compared with its pair-fed control (Table 4). However, this difference was not present during lactation

Table 3. Effects of tumour burden on tissue lipogenesis in fed virgin, lactating and litter-removed rats

All groups of rats were fed *ad libitum*, except virgin control animals, which were 'pair-fed' according to the food intake of their tumour-bearing matched partners. For further details see the Experimental section. The results are mean values ± S.E.M., expressed as μmol of ^3H_2O incorporated into saponified lipid/h per g wet wt. of tissue. Values that are significantly different by Student's *t* test from those of the control group are indicated by: $*P < 0.05$; $***P < 0.001$. No significant difference was observed between virgin tumour-bearing and control groups by the paired *t* test.

State of rats	^3H_2O incorporation into saponified lipid (μmol of ^3H_2O/h per g wet wt. of tissue)					
	Carcass	Liver	Mammary gland	Parametrial adipose tissue	Brown adipose tissue	Tumour
Virgin						
Control (6)	6.39±0.62	21.0±5.42	–	17.4±4.76	222.3±49.1	–
Tumour-bearing (6)	5.18±0.63	24.8±3.65	–	9.28±2.77	231.0±31.6	2.17±0.48
Lactating						
Control (8)	6.07±0.76	22.0±3.17	112.8±24.4	2.91±0.96	33.9±24.1	–
Tumour-bearing (5)	5.70±0.90	17.2±2.64	99.8±13.2	2.22±0.54	5.66±1.06	3.77±0.61
Litter-removed						
Control (6)	4.03±0.73	34.9±4.67	2.94±0.36	8.77±2.69	89.4±13.7	–
Tumour-bearing (6)	2.33±0.24*	18.3±4.14*	2.12±0.66	2.12±0.22*	21.9±2.78***	2.48±0.45

Table 4. Effects of tumour burden on the absorption and metabolic fate of orally administered [1-^{14}C]triolein in fed virgin, lactating and litter-removed rats

All groups of rats were fed *ad libitum*, except virgin control animals, which were 'pair-fed' according to the food intake of their tumour-bearing matched partners. $^{14}CO_2$ production was calculated between hours 2 and 5 of the experiment after triolein administration, corresponding to linearity of $^{14}CO_2$ excretion. For further details see the Experimental section. The results are mean values ± S.E.M., with the numbers of observations in parentheses. Tumour-bearing values that are significantly different by Student's *t* test from their control values (all groups) are indicated by: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. Those that are significantly different by paired *t* test from control values (virgin group) are indicated by: $\dagger P < 0.05$; $\dagger\dagger P < 0.001$.

State of rats	Absorption of [^{14}C]lipid (% of 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	Tumour
Virgin									
Control (9)	69.2 ± 2.5	7.52 ± 0.92	0.119 ± 0.011	0.520 ± 0.038	–	0.398 ± 0.076	7.48 ± 0.92	0.046 ± 0.005	–
Tumour-bearing (9)	54.6 ± 8.0	3.83 ± 0.95*†	0.122 ± 0.024	0.705 ± 0.110	–	0.103 ± 0.017**††	6.81 ± 1.27	0.155 ± 0.038*†	0.074 ± 0.016
Lactating									
Control (4)	86.6 ± 2.4	1.96 ± 0.56	0.067 ± 0.016	0.289 ± 0.070	2.39 ± 0.390	0.065 ± 0.040	1.07 ± 0.950	0.047 ± 0.023	–
Tumour-bearing (6)	82.7 ± 3.1	2.01 ± 0.43	0.059 ± 0.007	0.257 ± 0.036	2.09 ± 0.410	0.029 ± 0.006	0.192 ± 0.030	0.033 ± 0.007	0.033 ± 0.006
Litter-removed									
Control (6)	75.4 ± 5.6	2.67 ± 0.16	0.270 ± 0.015	0.579 ± 0.038	0.209 ± 0.060	2.63 ± 0.34	4.53 ± 0.37	0.095 ± 0.007	–
Tumour-bearing (5)	55.6 ± 8.3	2.61 ± 0.57	0.160 ± 0.060	0.800 ± 0.090*	0.175 ± 0.030	0.956 ± 0.415*	4.06 ± 1.77	0.436 ± 0.070***	0.115 ± 0.010

Table 5. Effects of tumour burden on plasma triacylglycerol concentration and tissue lipoprotein lipase activity in fed virgin, lactating and litter-removed rats

For full details see the Experimental section. The results are mean values ± 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: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

State of rats	Plasma triacylglycerol concn. (mg/100 ml)	Lipoprotein lipase activity (nmol of fatty acid released/min per mg of acetone-dried tissue)	
		Parametrial adipose tissue	Mammary gland
Virgin			
Control	54.0 ± 5.4 (14)	0.742 ± 0.108 (6)	–
Tumour-bearing	143.4 ± 21.5 (15)***	0.384 ± 0.048 (7)**	–
Lactating			
Control	72.6 ± 17.4 (4)	0.232 ± 0.095 (5)	0.507 ± 0.115 (5)
Tumour-bearing	63.8 ± 7.9 (6)	0.235 ± 0.092 (7)	0.561 ± 0.122 (7)
Litter-removed			
Control	171.1 ± 21.8 (12)	0.743 ± 0.160 (11)	0.069 ± 0.030 (11)
Tumour-bearing	329.7 ± 46.5 (11)**	0.225 ± 0.062 (9)*	0.020 ± 0.006 (9)

or after litter removal, when both groups have a low rate of exogenous lipid oxidation (Table 4; Oller do Nascimento & Williamson, 1986); more than 48 h after litter removal is required to achieve rates of lipid oxidation seen in virgin rats (Oller do Nascimento & Williamson, 1986). Whether depression of lipid oxidation seen during lactation and weaning was principally lactation-induced or tumour-induced is uncertain; however, the evidence presented for functional integrity of lactation in the tumour-bearing group suggests it may be the former.

In the virgin group, carcass, liver and interscapular brown-adipose-tissue [^{14}C]lipid accumulation was unchanged by the presence of the tumour (Table 4). Two tissues did, however, show significant changes: parametrial adipose tissue from tumour-bearing virgin rats accumulated less lipid than did control tissue, whereas there was increased lipid present in plasma from tumour-bearing animals. Both these changes may be taken as indicators of a functional impairment of lipoprotein lipase activity. Such an impairment would explain the increased accumulation of [^{14}C]lipid in the plasma and the decreased excretion of $^{14}CO_2$ (Table 4).

The conservation of dietary lipid (decreased oxidation to $^{14}CO_2$) and increased uptake by mammary gland was not altered by the presence of the tumour during lactation (Table 4). In lactation, accumulation of the oral [^{14}C]-lipid in white adipose tissue is low (Oller do Nascimento & Williamson, 1986), and this was not significantly changed in the tumour-bearing rats (Table 4). Thus the rapidly growing tumour does not appear to alter the direction of available triacylglycerol to the lactating mammary gland.

In contrast, when the litter was removed there was only a partial restoration of [^{14}C]lipid accumulation in parametrial adipose tissue in the tumour-bearing group (Table 4) (36% of controls). Furthermore, the defect in clearance of plasma lipid seen in the tumour-bearing virgin rats was again revealed by a large [^{14}C]lipid accumulation in this compartment (Table 4). These changes were accompanied by hypertriglyceridaemia and a decreased activity of lipoprotein lipase in adipose tissue (Table 5).

It is also noteworthy that the lipid accumulation by the tumour itself is quantitatively less than that in any host tissue studied (Table 4), lending support to the data of Baker *et al.* (1977), who found tumour tissue to be only poorly competitive with the host for lipid substrate.

Consequently, lipoprotein lipase activity was assayed in parametrial adipose tissue and mammary gland (Table 5). The results indicate a significant depression, by nearly 50%, of adipose tissue activity in the tumour-bearing virgin animal (in agreement with the depressed lipid uptake); this difference was lost during lactation (where the activity in the controls is considerably lower), but re-appeared on removal of the litter, when adipose-tissue lipoprotein lipase activity in tumour-bearing rats was depressed to 30% of control values (Table 5). Mammary-gland lipoprotein lipase activity was not altered by the tumour during lactation; as expected, the activity of this enzyme in mammary tissue decreased in both groups on litter removal (Scow & Chernick, 1987). These changes in lipoprotein lipase activities were paralleled by corresponding alterations in [^{14}C]lipid accumulation in tissues (Table 4) and in plasma triacylglycerol concentration (Table 5).

Triacylglycerol/fatty acid substrate cycling

Substrate or 'futile' cycling is energetically expensive and may be altered during tumour burden; an increased rate may partly explain the increased metabolic rate and energy loss seen in some tumour studies, whereas a decreased rate could indicate intact host compensatory mechanisms. Triacylglycerol/fatty acid substrate cycling in white adipose tissue is known to decrease during lactation (Hansson *et al.*, 1987); one possible reason for this change is maternal energy conservation during a period of increased demand. The rate of this cycle has not been rigorously examined during tumour burden, although Ookhtens *et al.* (1986) have measured the esterification and incorporation of labelled non-esterified fatty acid into acylglycerols of white adipose tissue, using a direct tracer injection technique, and found an inhibition of the rate in mice bearing the Ehrlich ascites carcinoma. They interpreted this as a decreased 'futile

Table 6. Effects of tumour burden on synthesis of triacylglycerol glycerol and triacylglycerol fatty acid, and triacylglycerol/fatty acid substrate cycling in parametrial adipose tissue *in vivo* in fed virgin, lactating and litter-removed rats

All groups of rats were fed *ad libitum*. The triacylglycerol/fatty acid cycling was calculated as described by Hansson *et al.* (1987). For further details see the Experimental section. The results are mean values ± S.E.M., with the numbers of observations in parentheses. Tumour-bearing values that are significantly different by Student's *t* test from control values are indicated: $*P < 0.05$.

State of rats	Rates (μmol/h per g wet wt.)		
	Triacylglycerol glycerol	Triacylglycerol fatty acid	Triacylglycerol/ fatty acid cycling
Virgin			
Control (6)	0.817 ± 0.171	0.109 ± 0.063	2.34 ± 0.47
Tumour-bearing (7)	0.377 ± 0.088*	0.018 ± 0.005	1.11 ± 0.26*
Lactating			
Control (3)	0.135 ± 0.083	0.046 ± 0.021	0.375 ± 0.249
Tumour-bearing (4)	0.131 ± 0.068	0.019 ± 0.007	0.375 ± 0.204
Litter-removed			
Control (6)	1.49 ± 0.24	0.558 ± 0.269	3.90 ± 0.55
Tumour-bearing (6)	0.626 ± 0.130*	0.068 ± 0.025	1.81 ± 0.37*

cycle' of non-esterified fatty acid esterification and hydrolysis. Our data on fatty acid cycling (Table 6), obtained by the method of Hansson *et al.* (1987), indicate a significant decrease in this rate in white adipose tissue during tumour burden in virgin animals by more than 50% (in agreement with the findings of Ookhtens *et al.*, 1986); however, during lactation, the rate is depressed to an equal extent in tumour-bearing and control groups. On removal of the litter, restoration above the rate in the control group occurred (Table 6; Hansson *et al.*, 1987) and, although an increase was seen in white adipose tissue from rats with tumour burden, this was significantly less (46%) than its appropriate control and still less than the virgin control rate (Table 6). The significance of this phenomenon is uncertain, but could represent a host energy-conservation mechanism, functional during tumour burden.

Concluding remarks

The present work confirms and extends the findings of Thompson *et al.* (1981) and Lanza-Jacoby *et al.* (1984), that an early event during tumour growth in non-lactating rats is loss of lipoprotein lipase activity from white adipose tissue and that this occurs despite the same food intake in tumour-bearing and control animals. This loss of enzyme activity is paralleled by decreased accumulation in adipose tissue of oral [^{14}C]lipid and increased plasma triacylglycerol. One novel finding is that the presence of the tumour suppresses the increase in lipid deposition in white adipose tissue which occurs within 24h of premature cessation of lactation. Again, this appears to be due to loss of lipoprotein lipase activity and results in hypertriglyceridaemia. The other finding of interest is that during lactation there is no significant disturbance of lipid metabolism (lipogenesis or [^{14}C]lipid accumulation) in the lactating mammary gland in the tumour-bearing rats, and the lactational performance, at least in terms of pup weight gain, is unimpaired.

A key question is how the changes in adipose-tissue metabolism are brought about. Previous workers have reported a decrease in plasma insulin with increase of tumour mass (Lanza-Jacoby *et al.*, 1984; Singh *et al.*, 1981), and this could be responsible for the decrease in lipoprotein lipase activity in white adipose tissue (Cryer *et al.*, 1976). However, in the present experiments the plasma insulin was not lower in the tumour-bearing rats on removal of the litter. An alternative explanation is that the tumour or its presence results in the production of a factor which inhibits lipoprotein lipase synthesis in adipose tissue, but not in mammary gland. There is a precedent for this view. Tumour growth is associated with increased numbers of macrophages at the tumour site and two peptides produced by macrophages, interleukin 1 (Dinarello, 1984; Beutler & Cerami, 1985) and cachectin (tumour necrosis factor-α) (Beutler & Cerami, 1986), have both been shown to suppress lipoprotein lipase synthesis in differentiating adipocytes in culture (Price *et al.*, 1986*a*,*b*,*c*; Kawakami *et al.*, 1987). Recombinant tumour necrosis factor-α has been shown to inhibit adipose-tissue lipoprotein lipase synthesis and activity *in vivo* (Semb *et al.*, 1987). Furthermore, interleukin-1 has been shown to be cytotoxic to pancreatic-islet β-cells and to suppress insulin responses *in vitro* (Bendtzen *et al.*, 1986). To what extent these or other cytokines are responsible for the effects observed here remains to be investigated.

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