Effects of tri-iodothyronine administration on the disposal of oral [^{14}C]triolein, lipoprotein lipase activity and lipogenesis in the rat during lactation and on removal of the litter

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The effect of tri-iodothyronine (T_3) administration on the utilization of dietary [^{14}C]lipid by the mammary gland and adipose tissue of lactating and litter-removed rats was studied. (1) After an oral load of [^{14}C]triolein, the lactating rats treated with T_3 (50 $\mu\text{g}/100$ g body wt.) over 24 h showed an increase in $^{14}\text{CO}_2$ production and a decrease in the total [^{14}C]lipid transferred through the mammary gland that was paralleled by a decrease in tissue lipoprotein lipase (LPL) activity. (2) T_3 administration decreased plasma prolactin in the lactating rats. Prolactin replacement in T_3 -treated rats restored LPL activity in the mammary gland, but did not increase the amount of dietary [^{14}C]lipid transferred to the milk. (3) Chronic T_3 administration (4 days) to lactating rats did not affect pup growth or the lipogenic rate in

the mammary gland. (4) The administration of T_3 to litter-removed rats inhibited the increase of LPL activity in white adipose tissue and decreased the accumulation of dietary [^{14}C]lipid. This decrease was accompanied by increased $^{14}\text{CO}_2$ production and [^{14}C]lipid accumulation in skeletal muscle and heart. (5) It is concluded that hyperthyroidism depresses LPL activity in mammary gland and white adipose tissue, but not in muscle. The increased accumulation of [^{14}C]lipid in muscle and increased production of $^{14}\text{CO}_2$ in lactating and in litter-removed rats treated with T_3 is in part due to the decreased total LPL in mammary gland and adipose tissue respectively, which are therefore less able to compete with muscle for the available plasma triacylglycerols.

INTRODUCTION

Thyroid hormones have an important role in mammary-gland development and function [1,2]. Tri-iodothyronine (T_3) administration in physiological amounts is able to stimulate prolactin-induced synthesis of milk products and the enzymic activities related with this process in differentiating rat and mouse mammary gland [2–5], although high doses of T_3 inhibit prolactin stimulation of mammary development [6]. The regulatory role of the thyroid hormones in mammary-gland development could be mediated through alterations in the binding of prolactin, and it has been postulated that thyroid hormones modulate the number of prolactin receptors per cell [7].

Lactation induces a state of relative hypothyroidism; lactating rats show decreases in plasma thyroxine (T_4) and T_3 and an elevation of plasma thyrotropin [8–10]. The extra-thyroidal conversion of T_4 into T_3 in lactating rats is lower compared with non-lactating rats [10]. Nevertheless, the mammary gland is responsive to thyroid hormones, nuclear T_3 receptors are present in this tissue, and their number increases during lactation [11]. Chronic administration of thyroid hormones to pregnant rats has shown adverse effects on subsequent maternal behaviour and on lactational performance [12,13]. It has been suggested that thyroid hormones and prolactin have an antagonistic effect at the level of the mammary-gland development [6].

Lipoprotein lipase (LPL) is the enzyme responsible for the fatty acid uptake from chylomicrons or very-low-density lipoproteins by the lactating mammary gland for milk lipid synthesis [14]. LPL activity is increased in the mammary gland during lactation, whereas it decreases in adipose tissue just before parturition and remains low throughout lactation and

increases again on removal of the pups. This pattern results in a specific direction of plasma triacylglycerols to mammary tissue during lactation and for repletion of adipose-tissue depots after removal of the litter [15]. The other major source of milk lipid is synthesis *de novo* (lipogenesis) within the gland from glucose (see [16]). Studies with non-pregnant non-lactating rats have shown that excess thyroid hormones inhibit LPL activity in adipose tissue [17–19], and have variable effects on activity in heart and skeletal muscle [20–22]. In contrast, hyperthyroidism increases hepatic lipogenesis [23–25]. The present work was undertaken to answer two questions: does T_3 effect (a) lipogenesis or triacylglycerol utilization by the lactating mammary gland, or (b) inhibit the ‘switch-on’ of LPL activity in adipose tissue on removal of the pups and cessation of lactation?

EXPERIMENTAL

Animals

Female rats of the Wistar strain were used. The animals were fed on a diet consisting by weight of 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 08:00 to 20:00 h. Lactating rats (280–350 g) with ten pups per litter were used after a lactation period of 10–12 days. A group of lactating rats had their pups removed at different intervals (24 h or 7 days) before the experiment. Food intake of the dams and pup growth rates were determined from day 5 of lactation until the day of the experiment. Hyperthyroidism was achieved by intraperitoneal injection of T_3 (500 $\mu\text{g}/\text{kg}$ body wt.) 24 h and 5 h before killing the rats, or daily

Abbreviations used: T_3 , 3,3',5-tri-L-iodothyronine; LPL, lipoprotein lipase.

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(08:00–09:00 h) for 4 days in the group of chronically treated rats. The control rats were injected with the vehicle for T_3 (1 mM NaOH). The last dose of T_3 was given just before the oral load of [$1\text{-}^{14}\text{C}$]triolein or 24 h before for the measurements of lipogenesis in chronically treated rats. Prolactin (sheep) was administered by subcutaneous injection (in 3 doses of 20 i.u. each), the first one 24 h before the experiment and then 8 h and 16 h later. All other experimental procedures were started between 09:00 and 10:00 h, except for those determinations of lipogenesis in which T_3 was given in the same dosage and timing as for the rats loaded with [$1\text{-}^{14}\text{C}$]triolein, where the rate of lipogenesis was measured between 13:30 and 14:30 h (5 h after the last dose of T_3).

Chemicals

[$1\text{-}^{14}\text{C}$]Triolein (glycerol tri[$1\text{-}^{14}\text{C}$]oleate) and $^3\text{H}_2\text{O}$ were obtained from Amersham International, [$9,10\text{-}^3\text{H}$]triolein was obtained from DuPont (Boston, MA, U.S.A.). T_3 , sheep luteotropic hormone (prolactin), glycerol trioleate (99% pure) and lecithin were purchased from Sigma (St Louis, MO, U.S.A.). Rat prolactin radioimmunoassay kit was obtained from IDS Ltd. (Bodon, Tyne and Wear, U.K.) and an insulin radioimmunoassay kit was from CIS (U.K.) Ltd. (High Wycombe, Bucks., U.K.). Other biochemicals were from Boehringer (London, U.K.).

Measurement of [^{14}C]lipid oxidation and tissue [^{14}C]lipid accumulation *in vivo*

The metabolic fate of an oral load of [$1\text{-}^{14}\text{C}$]triolein was determined by the method previously described by Oller do Nascimento and Williamson [26]. After intragastric administration of [$1\text{-}^{14}\text{C}$]triolein (about 0.5 g; 1.6 μCi per rat), the oxidation of the oral lipid load ($^{14}\text{CO}_2$ production) was measured every 1 h for 5 h. After this period (14:00–15:00 h), rats were anaesthetized with pentobarbital (60 mg/kg body wt.) and samples of mammary gland, parametrial adipose tissue, heart and skeletal muscle (soleus) were freeze-clamped for LPL measurement. Arterial blood was collected into a heparinized syringe for hormone (plasma) and metabolite determinations. The whole of the intestinal tract, mammary gland, liver, intrascapular brown adipose tissue and soleus muscle were dissected. The parametrial adipose tissue was removed and then all the remaining macroscopically (non-parametrial) differentiable adipose tissue was pooled. The intestinal tract was homogenized in 3% (w/v) HClO_4 . Unlike the previous study [26] from this laboratory, the skin was separated from the remaining carcass (minus the above tissues), and both were autoclaved and homogenized separately. Litters were killed under diethyl ether anaesthesia and the stomachs were dissected. Milk clots were removed, weighed and mixed. The remainder of the pup was autoclaved and homogenized. Duplicate samples of each tissue and homogenates of intestinal tract, carcass and skin were used for lipid extraction after saponification with 30% (w/v) KOH [27]. The radioactivity in the extracted fatty acids was measured for determination of the [^{14}C]lipid accumulated by the tissues. The [^{14}C]lipid remaining in the intestinal tract was used to determine the absorption of the [$1\text{-}^{14}\text{C}$]triolein.

Measurement of LPL activity

The LPL activity in acetone/ether-dried powders of mammary gland, parametrial adipose tissue, heart and soleus muscle were

measured by the method described by Nilsson-Ehle and Schotz [28], with [$9,10\text{-}^3\text{H}$]triolein as substrate. The activity is expressed as nmol of fatty acid released/min per mg of acetone/ether-dried tissue.

Measurement of lipogenesis *in vivo*

Lipogenesis was measured *in vivo* with $^3\text{H}_2\text{O}$ as described by Robinson et al. [29]. Rats were injected intraperitoneally with 2 mCi (0.2 ml) of $^3\text{H}_2\text{O}$. Mammary gland, liver, parametrial adipose tissue and intrascapular brown adipose tissue were removed 60 min after $^3\text{H}_2\text{O}$ administration and samples were taken for lipid extraction [27].

Metabolites and hormones in blood

Whole-blood glucose [30], D-3-hydroxybutyrate and acetoacetate [31], and plasma triacylglycerol [32] were measured by enzymic methods. Non-esterified fatty acids were determined with a commercial kit (Wako NEFA C test kit; Wako Chemicals, Neuss, Germany).

Plasma T_3 , prolactin and insulin were determined by radioimmunoassay.

RESULTS AND DISCUSSION

LPL activity and [^{14}C]lipid accumulation in mammary gland

The absorption of oral [^{14}C]lipid was approx. 80% of the administered dose in the animals without hormonal treatment. Oral [$1\text{-}^{14}\text{C}$]triolein absorption was lower (23–26%) in the rats treated with T_3 (Table 1), in part due to diarrhoea. In order to correct for differences in absorption between experimental groups, all the results are expressed on the basis of percentage of dose absorbed.

T_3 administration did not affect the amount of dietary [^{14}C]lipid accumulated in the mammary gland (Table 1), but decreased the accumulation of radioactive lipid in the milk clot and therefore the total lipid transferred through the mammary gland (mammary gland + milk clot + pups) decreased (28%) significantly. The low transfer of dietary [^{14}C]lipid through the mammary gland was paralleled by a decrease (58%) in LPL activity in this tissue in the T_3 -treated rats (Table 1). Pup growth rate, maternal body weight and food intake were not affected after 24 h of T_3 administration (results not shown).

The lactating rats treated with T_3 showed a decrease (74%) in plasma prolactin [controls 114.4 ± 33.4 ng/ml ($n = 6$); T_3 -treated 30.4 ± 16.0 ng/ml ($n = 6$); $P < 0.001$]. This may be related to the decrease in thyrotropin-releasing hormone (a physiological regulator of prolactin secretion [33]) after T_3 injection [34]. The decreased prolactin concentration in response to T_3 administration could be responsible for the lower [^{14}C]lipid transfer to the milk and the lower tissue LPL [35]. Prolactin administration to lactating rats treated with T_3 reversed the inhibitory effects on mammary-gland LPL activity, but did not restore dietary lipid transfer to the pups via the milk clot (Table 1). Administration of prolactin alone to lactating rats did not improve the already high [^{14}C]lipid transfer through the lactating mammary gland. It has been shown that chronic administration of thyroid hormone decreased the plasma PRL concentration in rats at 21 days of pregnancy, and these animals were unable to have successful lactation, due to a lack of adequate maternal behaviour [13]. The lactating rats treated with T_3 in our study did not show changes in their nursing behaviour or in the weight of the milk clot [controls 4.54 ± 1.06 g ($n = 5$; mean $\pm$ S.D.); T_3 -treated 3.55 ± 1.29 g ($n = 6$)]. The failure of prolactin to restore

Table 1 Effects of T_3 and prolactin (PRL) administration on mammary-gland LPL activity and [^{14}C]lipid accumulation in the mammary gland, milk clot and pup carcass of lactating rats after administration of [$1-^{14}C$]triolein

For experimental details see the text. The results are means $\pm$ S.D. for 6 animals in each group. Values for T_3 and prolactin that are statistically different by Student's t test from the normal control are indicated by * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Experimental group	Absorption of [^{14}C]triolein (%)	Total [^{14}C]lipid accumulation (% of absorbed dose per total tissue)				Mammary-gland LPL activity (nmol/min per mg of powder)
		Mammary gland	Milk clot	Pups	Mammary gland + milk clot + pups	
Lactating	87.6 $\pm$ 7.8	34.9 $\pm$ 5.4	19.9 $\pm$ 9.1	5.4 $\pm$ 2.5	60.6 $\pm$ 10.5	3.55 $\pm$ 0.86
+ T_3	67.4 $\pm$ 12.4**	36.4 $\pm$ 5.4	3.7 $\pm$ 2.7**	3.3 $\pm$ 1.9	43.4 $\pm$ 7.0**	1.49 $\pm$ 0.34***
+ T_3 + PRL	64.7 $\pm$ 6.8***	37.9 $\pm$ 5.8	5.0 $\pm$ 4.4	1.4 $\pm$ 0.7	44.4 $\pm$ 10.4**	2.72 $\pm$ 0.53
+ PRL	87.7 $\pm$ 4.5	57.2 $\pm$ 12.9*	9.5 $\pm$ 9.9	1.3 $\pm$ 0.9	68.0 $\pm$ 9.9	3.15 $\pm$ 0.01

Table 2 Effects of T_3 administration on parametrial-adipose-tissue LPL activity and [^{14}C]lipid accumulation in parametrial adipose tissue, non-parametrial adipose tissue, brown adipose tissue and skin of lactating and litter-removed rats after administration of [$1-^{14}C$]triolein

For experimental details see the text. The results are means $\pm$ S.D. for 6 animals in each group. Values for T_3 -treated rats that are statistically different by Student's t test from the respective control are indicated by * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Experimental group	Total [¹⁴ C]lipid accumulation (% absorbed dose per total tissue)				Parametrial-adipose-tissue LPL activity (nmol/min per mg of powder)
	White adipose tissue		Brown adipose tissue	Skin	
	Parametrial	Non-parametrial			
Lactating	0.04 ± 0.01	0.17 ± 0.03	0.04 ± 0.01	1.81 ± 0.43	0.60 ± 0.46
+ T ₃	0.07 ± 0.02**	0.29 ± 0.09*	0.13 ± 0.04	1.40 ± 0.33	0.51 ± 0.32
Litter removed (24 h)	2.53 ± 0.74	10.5 ± 3.9	0.96 ± 0.30	9.32 ± 4.08	2.95 ± 1.23
+ T ₃	1.00 ± 0.55***	4.63 ± 0.94***	1.54 ± 0.29	3.70 ± 0.65***	1.17 ± 1.03*
Litter removed (7 days)	1.19 ± 0.69	3.30 ± 2.19	1.50 ± 0.23	5.54 ± 2.77	2.13 ± 1.45
+ T ₃	0.17 ± 0.07***	0.81 ± 0.34*	1.68 ± 0.28	3.07 ± 0.83***	0.68 ± 0.28*

the dietary [^{14}C]lipid transfer from the mammary gland to the pups in T_3 -treated rats suggests that there is an impairment of the passage of newly labelled lipid through the secretory cell. It must be emphasized that only a small proportion of the total milk clot lipid was from the exogenous [^{14}C]lipid (controls 7%; T_3 -treated 1%). The explanation for this is two-fold: (a) the mammary gland has a high content of preformed lipid, and (b) some of the milk clot may have been transferred before the start of the experiment. It is unlikely that the decreased absorption of dietary [^{14}C]lipid in the T_3 -treated group (Table 1) played a role in the decreased transfer because injection of 3H -labelled chylomicrons to control and T_3 -treated rats showed a similar decrease in total mammary-gland [3H]lipid accumulation (at 15 min) in the latter group (results not shown).

Adipose tissue

Lipid deposition is depressed in white adipose tissue of the rat during lactation ([35,36]; Table 2). Administration of T_3 to lactating rats increased [^{14}C]lipid accumulation (75%) in the adipose tissue, although this increase was small in quantitative terms compared with that (70-fold) which occurred in control rats after litter removal (Table 2). In these animals, T_3 administration decreased dietary [^{14}C]lipid accumulation by the adipose tissue (60% and 86% in 24 h- or 7 days-litter-removed rats respectively), and this was associated with a decrease in LPL activity in parametrial adipose tissue (70% in both groups) (Table 2). It has been shown that thyroid hormones control LPL

activity in adipose tissue of male rats. Hypothyroidism has been associated with a marked rise of LPL in adipose tissue [17,18,20]. The addition of T_3 to cultured adipocytes of normal male rats decreases LPL activity, and the synthesis of the enzyme [17]. Similarly, administration of T_3 depresses the raised activity of LPL in adipose tissue of food-restricted rats [19].

Dietary [^{14}C]lipid accumulation in the skin changed in parallel with the adipose tissue in all the groups studied (Table 2). The present work indicates that the skin is an important site of [^{14}C]lipid accumulation in the litter-removed rat, and, as this tissue responds to lactation and to T_3 in the same way as white adipose tissue, it is likely that the [^{14}C]lipid is extracted by adipocytes associated with the epidermis. T_3 administration had no effect on the increased accumulation of [^{14}C]lipid in the interscapular brown adipose tissue of the litter-removed rats (Table 2), which is in agreement with the absence of an effect of the hormone on the same depot of rats fed *ad libitum* or food-restricted rats [19].

$^{14}CO_2$ production and [^{14}C]lipid accumulation in liver, skeletal muscle and heart

The dietary lipid is conserved during lactation to provide milk fat or in the immediate period after weaning to replenish depleted adipose-tissue stores, and therefore less dietary triacylglycerols are available for oxidation [26]. The present measurements of $^{14}CO_2$ production confirm the previous finding [26] in that there were no differences in the oxidation of dietary [^{14}C]lipid between

Table 3 Effects of T_3 administration on $^{14}CO_2$ production, muscle and heart LPL activity and [^{14}C]lipid accumulation in liver, muscle and heart of lactating and litter-removed rats after intragastric administration of [^{14}C]triolein

For experimental details see the text. The results are means $\pm$ S.D. for 6 animals in each group. Values for T_3 that are statistically different by Student's *t* test from the respective control are indicated by * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Experimental group	CO_2 production (% of administered dose)	Tissue [^{14}C]lipid accumulation (% of absorbed dose)				Lipoprotein lipase activity (nmol/min per mg of powder)	
		Carcass	Skeletal muscle	Heart	Liver	Heart	Soleus
Lactating	7.9 $\pm$ 2.8	3.6 $\pm$ 1.3	1.42 $\pm$ 0.21	0.03 $\pm$ 0.009	10.0 $\pm$ 1.4	1.35 $\pm$ 0.67	0.14 $\pm$ 0.07
+ T_3	12.0 $\pm$ 2.1*	5.1 $\pm$ 0.8*	3.12 $\pm$ 0.76***	0.04 $\pm$ 0.013	14.5 $\pm$ 5.6	1.20 $\pm$ 0.39	0.07 $\pm$ 0.03
Litter removed (24 h)	12.1 $\pm$ 3.4	24.2 $\pm$ 3.6	5.40 $\pm$ 0.41	0.15 $\pm$ 0.50	28.0 $\pm$ 5.4	1.05 $\pm$ 0.35	0.15 $\pm$ 0.07
+ T_3	20.1 $\pm$ 4.9*	22.4 $\pm$ 2.9	8.17 $\pm$ 1.93**	0.26 $\pm$ 0.080*	23.1 $\pm$ 5.2	1.05 $\pm$ 0.33	0.11 $\pm$ 0.03
Litter removed (7 days)	32.9 $\pm$ 8.5	19.7 $\pm$ 2.6	4.96 $\pm$ 1.32	0.09 $\pm$ 0.029	19.3 $\pm$ 5.8	1.96 $\pm$ 0.77	0.14 $\pm$ 0.09
+ T_3	34.4 $\pm$ 4.5	23.2 $\pm$ 3.3	10.12 $\pm$ 2.25***	0.13 $\pm$ 0.057	13.3 $\pm$ 3.6	2.38 $\pm$ 1.23	0.14 $\pm$ 0.09

lactating and 24 h-litter-removed rats, and after 7 days of litter removal $^{14}CO_2$ production was 3 times higher compared with the lactating or the 24 h-litter-removed group (Table 3). The administration of T_3 to lactating or 24 h-litter-removed rats increased $^{14}CO_2$ production (by 50 and 67% respectively), but did not increase further the high $^{14}CO_2$ production in 7 days-litter-removed group (Table 3).

Chylomicron triacylglycerol uptake by skeletal muscle accounts for a significant portion of the triacylglycerol removed from plasma [37,38]. This uptake is most evident in the high-oxidative-type muscle fibres typical of the soleus [22,37]. The dietary [^{14}C]lipid accumulation in the carcass (mainly skeletal muscle and bone) in the litter-removed rats was 7 times higher in comparison with the lactating group (Table 3). Although the carcass had skin and all visible adipose tissue removed, the increased accumulation of [^{14}C]lipid on cessation of lactation might still be due to an increase in intermuscular adipose tissue. However, this would seem unlikely, because in T_3 -treated rats adipose-tissue accumulation of [^{14}C]lipid decreased appreciably (Table 2), whereas there was no change in the carcass (Table 3). Total skeletal-muscle lipid accumulation was calculated by using the value for [^{14}C]lipid accumulation in the soleus muscle and assuming that this muscle is representative of the total muscle mass. After removal of the litter (24 h or 7 days), the [^{14}C]lipid accumulation increased significantly in the heart and in the skeletal muscle (5- and 3-fold respectively) (Table 3). These results are in agreement with the changes in lipid content described in the muscle of weaned rats [39]. T_3 administration increased the dietary [^{14}C]lipid accumulation in the skeletal muscle of lactating and litter-removed rats and in the heart of the 24 h-litter-removed rats (Table 3). This value is an underestimate, because some of the fatty acids taken up by the high-oxidative red fibres are likely to have been oxidized.

Hyperthyroidism increases triacylglycerol uptake by the skeletal muscle, and this accounts for part of the enhanced plasma triacylglycerol turnover found in hyperthyroid male rats [22]. It has been assumed that the presence of substantial LPL activity in muscle is related to the uptake of plasma triacylglycerol fatty acids by the tissue [37]. The effect of thyroid hormones on the control of LPL in skeletal muscle and heart is controversial. Hypothyroidism in rats is accompanied by an increase [20] or no change in heart LPL activity [22]. Hyperthyroidism has been associated with an increase in rat heart LPL activity [19,21,22],

whereas in the skeletal muscle the enzyme activity is decreased [22]. In the present study there were no changes in the heart or soleus LPL activity 24 h after T_3 administration in the lactating or litter-removed rats (Table 3). The increased lipid uptake by the skeletal muscle of T_3 -treated rats (Table 3) could be related to alterations in the blood flow after T_3 treatment and/or the more effective capture of NEFA released from chylomicrons or very-low-density lipoproteins.

Blood and plasma metabolites and insulin

Lactating T_3 -treated rats showed a tendency to hypoglycaemia, significant hypertriglyceridaemia and increased plasma non-esterified fatty acids, but no change in blood ketone-body concentrations. T_3 administration to the litter-removed group did not cause any changes in blood or plasma metabolites (Table 4). The plasma insulin tended to be lower in the lactating T_3 -treated rats, but this difference did not reach statistical significance ($P < 0.062$; Table 4). Recent work from this laboratory [40,41] has implicated insulin in the regulation of mammary-gland LPL, and therefore the combination of lower plasma insulin and prolactin after treatment of lactating rats with T_3 may be responsible for the observed decrease in LPL (Table 1). In confirmation of previous studies [35,42,43], plasma insulin increased 3-fold on removal of the litter for 24 h, but it was not significantly decreased by T_3 treatment. This finding suggests that the action of T_3 to decrease adipose-tissue LPL activity (Table 2) does not involve changes in plasma insulin. It is of interest in terms of substrate supply that the plasma non-esterified fatty acids in the control and T_3 -treated groups decreased in parallel on weaning (Table 4).

Chronic administration of T_3

In rats on the normal rat laboratory diet (high in carbohydrate and low in fat), all the lactose and casein, and 50% of the milk lipid, are synthesized in the mammary gland. The remainder of the milk lipid is derived from triacylglycerols and non-esterified fatty acids taken up from the blood and which originate from the diet or are mobilized from adipose tissue [36]. In the present study, the decrease in the dietary lipid uptake by the mammary gland in the rats after 24 h of T_3 administration was not correlated with an impairment of the pup weight gain. To study if the

Table 4 Effects of T_3 administration on blood glucose and ketone bodies, and plasma non-esterified fatty acids, triacylglycerols and insulin, in lactating and litter-removed rats

For experimental details see the text. The results are means $\pm$ S.D. for 6 animals in each group. Values for T_3 that are statistically different by Student's *t* test from the respective control are indicated by * $P < 0.05$, ** $P < 0.01$.

Experimental group	Glucose (μ mol/ml)	Ketone bodies (μ mol/ml)	Non-esterified fatty acids (μ -equiv./ml)	Triacylglycerol (μ mol/ml)	Insulin (μ units/ml)
Lactating	4.27 $\pm$ 1.59	0.28 $\pm$ 0.08	0.74 $\pm$ 0.15	1.01 $\pm$ 0.5	32.4 $\pm$ 6.9
+ T_3	3.13 $\pm$ 0.84	0.30 $\pm$ 0.13	1.05 $\pm$ 0.11**	2.08 $\pm$ 0.79*	22.0 $\pm$ 10.1
Litter removed (24 h)	4.81 $\pm$ 1.62	0.27 $\pm$ 0.10	0.57 $\pm$ 0.09	1.71 $\pm$ 0.78	99.0 $\pm$ 36.2
+ T_3	4.23 $\pm$ 1.92	0.23 $\pm$ 0.05	0.78 $\pm$ 0.26	2.58 $\pm$ 0.99	75.5 $\pm$ 15.3
Litter removed (7 days)	6.01 $\pm$ 0.92	0.24 $\pm$ 0.07	0.18 $\pm$ 0.02	0.65 $\pm$ 0.11	—
+ T_3	6.48 $\pm$ 0.87	0.20 $\pm$ 0.05	0.38 $\pm$ 0.10**	0.93 $\pm$ 0.46	—

Table 5 Effects of chronic administration of T_3 during lactation on maternal body weight, food intake and pup weight

For experimental details see the text. The results are means $\pm$ S.D. for 6 animals in each group. Values for T_3 that are statistically different by Student's *t* test from the control are indicated by ** $P < 0.01$.

Experimental group	Maternal body wt. (g)		Food intake (g/100 g body wt. per day)		Pup wt. (g/pup)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Lactating	298.1 $\pm$ 11.5	313.6 $\pm$ 13.7	17.4 $\pm$ 2.1	19.6 $\pm$ 2.2	12.7 $\pm$ 1.9	19.4 $\pm$ 2.5
+ T_3	287.8 $\pm$ 29.4	275.6 $\pm$ 21.7**	17.4 $\pm$ 3.3	21.5 $\pm$ 4.7	12.9 $\pm$ 2.7	18.2 $\pm$ 3.5

Table 6 Effect of chronic administration of T_3 on the rate of lipogenesis in lactating and litter-removed rats

For experimental details see the text. The results are means $\pm$ S.D. for 6 animals in each group. Values for T_3 that are statistically different by Student's *t* test from the respective control are indicated by * $P < 0.05$.

Experimental group	Rate of fatty acid synthesis (μ mol of 3H_2O incorporated/h per g of tissue)			
	Mammary gland	White adipose tissue	Liver	Brown adipose tissue
Lactating	124.6 $\pm$ 17.6	2.3 $\pm$ 0.8	34.3 $\pm$ 18.8	15.8 $\pm$ 4.6
+ T_3	115.9 $\pm$ 34.2	2.3 $\pm$ 0.7	52.1 $\pm$ 15.3	19.6 $\pm$ 6.4
Litter removed (24 h)	3.3 $\pm$ 0.7	10.0 $\pm$ 3.6	41.0 $\pm$ 8.0	120.7 $\pm$ 32.2
+ T_3	5.6 $\pm$ 3.2	5.3 $\pm$ 2.7*	36.0 $\pm$ 17.7	81.9 $\pm$ 34.3

chronic administration of T_3 during lactation had an effect on pup growth rate, lactating rats were injected for 4 days with the hormone, and pup weight gain, maternal body weight and food intake were determined.

Chronic administration of T_3 to lactating rats did not affect the pup weight gain (Table 5) or weight [control 4.76 ± 1.56 g ($n = 6$; $\pm$ S.D.); T_3 -treated 4.16 ± 1.49 g ($n = 6$)] or lipid content [control $25.2 \pm 1.4\%$ ($n = 6$); T_3 -treated $23.3 \pm 2.4\%$ ($n = 6$)] of the milk clot, despite a decrease in maternal body weight (Table 5). After 4 days of T_3 treatment the lactating rats lost on average 12 g, while in the same period the control group gained 15 g with no difference in the food intake (Table 5). The decrease in maternal body weight during chronic T_3 treatment may be due to a decrease in body fat content and/or an increase in energy expenditure described in rats chronically treated with T_3 [44].

Administration of T_3 for 4 days to lactating rats decreased the liver wet weight (15.5 ± 0.9 g in lactating rats, versus 11.3 ± 1.0 g in lactating plus T_3 group; $P < 0.001$); this decrease may be related to the loss of liver glycogen after thyroid-hormone treatment [23].

The decrease in LPL activity in the mammary gland after T_3 treatment (Table 1) decreases the uptake of exogenous lipid (chylomicrons and very-low-density lipoproteins) by the gland. This decrease in the lipid uptake by the mammary gland in the T_3 -treated rats might be expected to decrease pup weight gain, if it is not compensated by an increased rate of lipogenesis and/or extraction of plasma non-esterified fatty acids; the latter were increased by 42% in the T_3 -treated lactating rats (Table 4). It has been shown that T_3 treatment during pregnancy increases fatty acid synthesis in the mammary gland in the immediate period

before parturition [13]. We have therefore measured the rate of fatty acid synthesis in the lactating rats after 4 days of T_3 administration.

Chronic administration of T_3 for 4 days (last dose 24 h before measurements) did not alter the rate of fatty acid synthesis in the mammary gland (Table 6). It was considered possible that any effect of T_3 on mammary-gland lipogenesis was transitory and reversed when the concentration of the hormone in plasma decreased [5 h after administration the plasma T_3 was: control 0.6 ± 0.01 nmol/l; T_3 -treated > 8.2 nmol/l ($n = 6$)]. The measurements of mammary-gland lipogenesis were therefore repeated in rats dosed with T_3 24 and 5 h before the administration of 3H_2O . Again there was no significant difference between the control (75.7 ± 30.7 μ mol of 3H_2O incorporated/h per g of tissue; $n = 6$; $\pm$ S.D.) and T_3 -treated rats (83.0 ± 50.9 μ mol of 3H_2O incorporated/h per g of tissue; $n = 6$). The lower values and greater variability for the control rats in this experiment compared with those in Table 6 is a consequence of the measurements being made at the nadir (14:00 h) of the diurnal cycle for lipogenesis in mammary tissue [45].

It is generally agreed that thyroid hormone stimulates hepatic lipogenesis in the non-lactating rat [23–25]. In white adipose tissue the effect of T_3 is uncertain; it has been reported that thyroid hormone treatment decreases [25,46] or increases [23] the synthesis of fatty acids in this tissue. In the present study, chronic administration of T_3 to lactating or 24 h-litter-removed rats did not modify the rate of lipogenesis in liver, white or brown adipose tissue, except that there was significant decrease in the rate in white adipose tissue of litter-removed rats (Table 6). A possible reason for the failure of T_3 to stimulate hepatic lipogenesis in the present study is that the rate of this process is already high in lactating and litter-removed rats. Support for this view is that T_3 did not stimulate hepatic lipogenesis in food-restricted rats [19], which also have increased rates.

Concluding remarks

The present results on the inhibitory effects of T_3 on the 'switch-on' of LPL activity in adipose tissue of 24 h-litter-removed lactating rats (Table 2) extend the previous findings of a similar action of T_3 on LPL activity of adipose tissue in food-restricted rats *in vivo* [19]. The present evidence with isolated adipocytes suggests a direct effect of the hormone to decrease LPL activity, mass and synthesis [17]. The decrease of LPL activity in mammary gland of T_3 -treated rats (Table 1) is more likely to be due to the associated decrease in plasma prolactin and insulin than a direct effect of T_3 . The mechanism for the decreased transfer of [^{14}C]lipid to the milk clot on administration of T_3 is not clear, particularly as it was not reversed by prolactin repletion.

The absence of an inhibitory effect of T_3 on mammary-gland lipogenesis (Table 6) indicates that the effects of the hormone on lipid metabolism are limited to the pathway of exogenous lipid uptake. Despite the decrease in mammary-gland LPL activity in T_3 -treated lactating rats, there was no change in milk-clot lipid content, milk-clot weight or pup weight gain. This suggests that synthesis *de novo* of fatty acids within the gland, together with the increased availability of plasma non-esterified fatty acids, are able to support milk lipid production when LPL activity is decreased.

Finally, the present results underline the importance of the high total tissue LPL activity in mammary gland or white adipose tissue in effectively competing with skeletal muscle for the available plasma triacylglycerols in lactating or 24 h-litter-removed rats. When total tissue LPL in mammary gland or

adipose tissue was decreased by T_3 treatment, the production of $^{14}CO_2$ and skeletal-muscle content of [^{14}C]lipid increased without any apparent change in muscle LPL activity. Clearly, measurements of LPL activity expressed per g fresh wt. or per mg of acetone-dried powder do not necessarily give a complete or valid picture of inter-tissue competition for plasma triacylglycerols.

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REFERENCES

- Vonderhaar, B. K. and Greco, A. E. (1979) *Endocrinology* (Baltimore) **104**, 409–418
- Bhattacharjee, M. and Vonderhaar, B. K. (1984) *Endocrinology* (Baltimore) **115**, 1070–1077
- Terada, N. and Oka, T. (1982) *FEBS Lett.* **149**, 101–104
- Despande, N. and Mitchell, I. (1980) *Horm. Metab. Res.* **12**, 333–339
- Despande, N. and Mitchell, I. (1981) *Horm. Metab. Res.* **13**, 572–574
- Mittra, I. (1974) *Nature* (London) **248**, 525–526
- Hayden, T. J., Bonney, R. C. and Forsyth, I. A. (1979) *J. Endocrinol.* **80**, 259–269
- Fukuda, H., Ohshima, K., Mori, M., Kobayashi, L. and Greer, M. A. (1980) *Endocrinology* (Baltimore) **107**, 1711–1716
- Lorscheider, F. L. and Mears, G. J. (1973) *J. Endocrinol.* **58**, 393–403
- Kahl, S., Capuco, A. V. and Bitman, J. (1987) *Proc. Soc. Exp. Biol. Med.* **184**, 144–150
- Hayden, T. J. and Forsyth, I. A. (1977) *J. Endocrinol.* **75**, 38P–39P
- Ming-Hsiung, L. and Anderson, R. R. (1975) *Horm. Res.* **6**, 85–94
- Rosato, R. R., Gimenez, M. S. and Jahn, G. A. (1992) *Acta Endocrinol. (Copenhagen)* **127**, 547–554
- Zinder, O., Mendelson, C. P., Blanchette-Mackie, J. and Scow, R. O. (1976) *Biochim. Biophys. Acta* **431**, 526–537
- Hamosh, M., Clary, T. R., Chernick, S. S. and Scow, R. O. (1970) *Biochim. Biophys. Acta* **210**, 473–482
- Williamson, D. H. and Da Costa, T. H. M. (1993) in *Physiologic Basis of Perinatal Care* (Medina, J. M. and Quero, J., eds.), pp. 63–77, Ergon, Salamanca
- Saffari, B., Ong, J. M. and Kern, P. A. (1992) *J. Lipid Res.* **33**, 241–249
- Gavin, L. A., McMahon, F. and Moeller, M. (1985) *Diabetes* **34**, 1266–1271
- Cruz, M. L. and Williamson, D. H. (1993) *Biochim. Biophys. Acta* **1168**, 205–212
- Hansson, P., Nordin, G. and Nilsson-Ehle, P. (1983) *Biochim. Biophys. Acta* **753**, 364–371
- Wilson, D. E., Jubiz, W., Flowers, C. M., Carlile, S. I. and Adolf, A. M. (1977) *Atherosclerosis* **26**, 353–362
- Kaciuba-Usilko, H., Dudley, G. A. and Terjung, R. L. (1980) *Am. J. Physiol.* **238**, E518–E523
- Diamant, S., Gorin, E. and Shafir, E. (1972) *Eur. J. Biochem.* **26**, 553–559
- Sugden, M. C., Steare, S. E., Watts, D. I. and Palmer, N. T. (1983) *Biochem. J.* **210**, 937–944
- Blennemann, B., Moon, Y. K. and Freahe, H. C. (1992) *Endocrinology* (Baltimore) **130**, 637–643
- Oller do Nascimento, C. M. and Williamson, D. H. (1986) *Biochem. J.* **239**, 233–236
- Stansbie, D., Brownsey, R. W., Crettaz, M. and Denton, R. M. (1976) *Biochem. J.* **160**, 413–416
- Nilsson-Ehle, P. and Schotz, M. C. (1976) *J. Lipid Res.* **17**, 536–541
- Robinson, A. M., Girard, J. R. and Williamson, D. H. (1978) *Biochem. J.* **176**, 343–346
- Slein, M. W. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 117–123, Verlag Chemie, New York
- Williamson, D. H., Mellanby, J. M. and Krebs, H. A. (1962) *Biochem. J.* **82**, 90–96
- Eggstein, M. and Kreutz, F. H. (1966) *Klin. Wochenschr.* **44**, 262–267
- Leong, D. A., Frawley, L. S. and Neill, J. D. (1983) *Annu. Rev. Physiol.* **45**, 109–127
- Rondeel, J. M. M., de Greef, W. J. and Visser, T. J. (1992) *Horm. Metab. Res.* **24**, 1–4
- Oller do Nascimento, C. M. and Williamson, D. H. (1989) *Biochem. J.* **258**, 273–278
- Williamson, D. H. (1980) *FEBS Lett.* **117**, K93–K105
- Tan, M. H., Sata, T. and Havel, R. J. (1977) *J. Lipid Res.* **18**, 363–370
- Linder, C., Chernick, S. S., Fleck, R. T. and Scow, R. O. (1976) *Am. J. Physiol.* **231**, 860–864
- Del Prado, M., Ramos, R., Hernandez-Montes, H. and Villalpando, S. (1993) *J. Lipid Res.* **34**, 1115–1120
- Da Costa, T. H. M. and Williamson, D. H. (1993) *Biochem. J.* **290**, 557–561

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- 41 Da Costa, T. H. M. and Williamson, D. H. (1994) *Biochem. J.* **300**, 257–262
- 42 Agius, L., Robinson, A. M., Girard, J. R. and Williamson, D. H. (1979) *Biochem. J.* **180**, 689–692
- 43 Flint, D. J. (1982) *J. Endocrinol.* **93**, 279–285
- 44 Oppenheimer, J. H., Schwartz, H. L., Lane, J. T. and Thompson, M. P. (1991) *J. Clin. Invest.* **87**, 125–132
- 45 Munday, M. R. and Williamson, D. H. (1983) *Biochem. J.* **214**, 183–187
- 46 Llobera, M., Muniesa, A. and Herrera, E. (1979) *Horm. Metab. Res.* **11**, 628–634
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