Effects of hypothyroidism on the metabolism of lipid emulsion models of triacylglycerol-rich lipoproteins in rats

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Methimazole-treated hypothyroid rats were injected intravenously with triacylglycerol/cholesteryl oleate/cholesterol/phospholipid emulsions designed to model the composition of chylomicrons. Compared with controls, hypothyroidism decreased the clearance rates of emulsion cholesteryl oleate. Clearance of emulsion triolein was affected much less and could be accounted for by residual triolein in remnants, suggesting that triacylglycerol lipolysis by lipoprotein lipase was unaffected by hypothyroidism but that clearance of remnants from plasma was decreased. Assays *in vitro* showed increased activities of lipoprotein lipase and hepatic lipase in hypothyroid rats. Emulsions were incubated with post-heparin plasma lipoprotein lipase to prepare remnants *in vitro*. The clearance from plasma of pre-formed remnants was slower after injection into hypothyroid rats than in control rats. Uptake of remnant cholesteryl oleate by the liver was significantly decreased in the hypothyroid rats. Treatment of hypothyroid rats for 7 days with 3,3',5'-tri-iodo-L-thyronine (T_3) reversed the inhibition of hepatic remnant uptake and normalized plasma cholesterol. A thyroid hormone analogue with decreased hypermetabolic side-effects, L-94901, attenuated plasma cholesterol and improved but did not normalize remnant clearance. Emulsions incubated with plasma from hypothyroid rats had a decreased ratio of apolipoprotein E/apolipoprotein C compared with control rats or hypothyroid rats treated with T_3 . The change in the apolipoprotein E/apolipoprotein C ratio probably accounts for the defect in remnant clearance in hypothyroidism.

INTRODUCTION

Cholesterol and lipoprotein metabolism are strongly influenced by thyroid hormone status. In man, hypercholesterolaemia accompanies hypothyroidism, whereas cholesterol concentrations are diminished in hyperthyroidism. A significant proportion of hypercholesterolaemic individuals have raised levels of thyrotropin, indicative of hypothyroidism [1]. Serum low-density lipoprotein (LDL) cholesterol decreases with thyroxine treatment in hypothyroid women [2]. In subclinical hypothyroidism, LDL concentrations are increased, whereas plasma total cholesterol and triacylglycerol (TG) are unaltered [3].

We previously reported that removal of chylomicron remnants was impaired after injection of lymph chylomicrons into hypothyroid hypercholesterolaemic rats [4]. In the current study we have examined the effects of hypothyroidism on remnant metabolism without inducing gross hypercholesterolaemia in the rats. The changes in remnant metabolism have been investigated using lipid emulsion models of chylomicrons which mimic the metabolism of lymph chylomicrons [5]. The changes have been evaluated in terms of changes in lipase activities and apolipoprotein contents. A thyroid hormone analogue, L-94901, shown to lower plasma cholesterol [6], was used to examine the regulatory effects of thyroid hormone on remnant clearance.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing about 200 g were obtained from the Animal Resources Centre (Willetton, Western Australia). Groups of rats were made hypothyroid by treatment for 38 days with methimazole (0.3 g/l) in their drinking water. The drug was omitted from the drinking water of control groups. Where indicated, doses of 3,3',5'-tri-iodo-L-thyronine (T_3) or L-94901

dissolved in 0.15 M-NaCl/0.01 M-NaOH were given by gavage daily for 7 days. Thyroid hormone activity was assessed by measurements of hepatic mitochondrial glycerol-3-phosphate dehydrogenase (G3PDH) [6].

All rats were fed on a regular commercial pelleted diet containing about 5% fat, except in a single study where control and hypothyroid rats were fed on a high-fat diet [7] for 2 days before measurements of the plasma lipids.

Preparation of emulsions

Emulsions were prepared in a manner similar to methods described previously [5]. Triolein, cholesteryl oleate, cholesterol (Nu Chek Prep, Elysian, MN, U.S.A.) and egg yolk phosphatidylcholine (grade I, Lipid Products, South Nutfield, Surrey, England) each greater than 99% pure were dispensed from stock solutions into vials followed by [^{3} H]cholesteryl oleate and [^{14} C]triolein (Amersham, Surry Hills, NSW, Australia). Solvents were then evaporated under a stream of N_2 before overnight desiccation to remove any residue. Mixtures of pure lipids were emulsified by sonication in 150 mM-NaCl/10 mM-Hepes buffer, pH 7.4. The lipids were sonicated in 8.5 ml of this NaCl buffer solution at 55–56 °C (monitored by a thermocouple in the vessel). The atmosphere above the mixture was purged with N_2 to prevent lipid oxidation. Sonication was for 20 min using a 1 cm probe with continuous output of 90–110 W. The density of the crude emulsion was increased to 1.10 g/ml by adding KBr (0.14 g/ml). After placing 4 ml portions at the bottom of two centrifuge tubes, 2.5 ml of NaCl solutions of densities 1.065, 1.020 and 1.006 g/ml were then sequentially layered above. The tubes were centrifuged in a Beckman SW41 rotor for 22 min at 24191 g_{av} at 20 °C. The large coarsely emulsified particles were removed and replaced with a 1.006 g/ml solution. This was followed by a second centrifugation

Abbreviations used: TG, triacylglycerol; LPL, lipoprotein lipase; T_3 , 3,3',5'-tri-iodo-L-thyronine; G3PDH, glycerol-3-phosphate dehydrogenase; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; HDL, high-density lipoprotein.

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of 20 min at 71 092 g_{av} at 20 °C. The emulsion particles which floated to the surface were analysed for size and lipid composition and injected into rats within 1 day. Oxidation was prevented by storage under argon.

Injection studies

Non-fasted control and hypothyroid rats were anaesthetized with ether and cannulated in the left carotid artery and the left jugular vein as described previously [7]. After surgery the rats were placed in individual cages and allowed to recover fully from the effects of anaesthesia for 2–4 h. The dose of lipid injected was 2–3 mg in a volume of 0.3–0.4 ml. Blood samples of 0.4 ml were then taken at 3, 5, 8, 12, 20, 25 and 30 min through the carotid artery for the removal of a chylomicron-like emulsion, and after 3, 5, 8 and 10 min for remnants. After each withdrawal an equivalent volume of 0.9% NaCl was injected. The plasma collected was measured for radioactivity by taking 150 μ l and adding 5 ml of scintillant. After completion of the clearance study 20 mg of sodium pentobarbitone was injected, a blood sample of 5 ml was taken by heart puncture, and the liver and spleen were removed and washed thoroughly in ice-cold 0.15 M-NaCl. The liver was flushed via the portal vein with 0.15 M-NaCl for studies concluded after 5 min or 10 min, but this step was omitted for studies concluding after 30 min, because residual plasma radioactivity was very low compared with organ counts. The final blood sample was used for the measurement of plasma TG and cholesterol. Lipids of the liver and spleen were extracted with 20 vol. of chloroform/methanol (2:1, v/v) [8] and radioactivity in the purified lipid extract was measured in 10 ml of scintillant.

Preparation of remnants

To obtain lipoprotein lipase (LPL) without contamination with hepatic lipase, control rats were anaesthetized with pentobarbitone and then a functional hepatectomy was achieved by ligating in sequence the coeliac and mesenteric arteries, the cardio-oesophageal junction, the rectum and finally the portal vein through a mid-line abdominal incision. The abdominal wall was sutured, then heparin (500 units/kg body wt.) was injected into a tail vein. After 10 min, blood was collected by cardiac puncture and plasma (LPL plasma) was separated. For control studies, post-heparin plasma was obtained from intact non-hepatectomized rats.

To prepare remnants *in vitro*, BSA (510 mg) was dissolved in 5 ml of 0.16 M-Tris buffer (pH 8.0)/0.55 mM- NaN_3 . LPL plasma (450 μ l) was then mixed with the albumin solution, followed by chylomicron-like emulsion containing 30 mg of TG in a volume of 3 ml. The resulting mixture was incubated at 37 °C for 3 h. To isolate the remnants the density was increased to 1.10 g/ml by dissolving KBr (0.14 g/ml). Then 4 ml portions were placed at the bottom of two centrifuge tubes, and 2.5 ml each of NaCl solutions of densities 1.065, 1.020 and 1.006 g/ml were then sequentially layered above. The tubes were then centrifuged in a Beckman SW41 rotor for 2 h at 197 478 g_{av} . Remnant particles which floated to the surface of the gradient were analysed for lipid compositions and injected into control and hypothyroid rats.

Measurements of LPL and hepatic lipase

Control and hypothyroid rats anaesthetized with pentobarbitone (65 mg/kg body wt.) were injected with heparin (250 units/kg body wt.) into a common iliac vein, and then bled after 4 min by heart puncture. Plasma was collected by low-speed centrifugation and lipase activities were measured immediately. Two methods of assay were used. The first procedure [9] was modified so that the substrate contained 10 μ mol of [^{14}C]triolein

(400 000 d.p.m.) and 200 000 d.p.m. of [9,10- ^3H (N)]oleic acid as internal standard, 59 nmol of phosphatidylcholine, 60 mg of BSA (fatty-acid free), 25 μ mol of $(\text{NH}_4)_2\text{SO}_4$ and 30 mg of gum arabic in 1.0 ml of water. To measure total lipase activity, 25 μ l of rat serum and 275 μ l of 0.15 M-NaCl were mixed with 200 μ l of post-heparin plasma, then 500 μ l of substrate was added and the mixture was incubated for 20 min in a shaking water-bath at 37 °C. The assay was stopped [10] by adding 5 ml of propan-2-ol/hexane/0.5 M- H_2SO_4 (40:10:1, by vol.) followed by 3 ml of hexane and then 2 ml of 0.05 M- H_2SO_4 . The tube contents were mixed and allowed to stand for 5 min, and then after low-speed centrifugation 3 ml of the upper phase was transferred into tubes containing 1.5 ml of 0.1 M-NaOH in ethylene glycol. The tubes were capped, shaken vigorously for 10 min and centrifuged. The upper hexane layer was removed and 1.0 ml was transferred from the lower phase into liquid scintillation vials and counted for radioactivity. To measure hepatic lipase, in a second tube the rat serum and 0.15 M-NaCl were replaced with 300 μ l of 3 M-NaCl to give a final concentration of 1 M. Under these conditions LPL is inactive [11]. The LPL activity was then calculated as the difference between total and hepatic lipase activities.

In the second assay procedure the substrate was a [^{14}C]triolein-labelled chylomicron-like emulsion prepared as described above for the injection studies, diluted with 0.15 M-NaCl to a TG concentration of 1.5 mg/ml. For measurement of total lipase activity 300 μ l of substrate was added to 25 μ l of rat serum and 325 μ l of 0.15 M-NaCl containing 70 mg of BSA. For assay of hepatic lipase activity, 325 μ l of 2 M-NaCl replaced the 0.15 M solution and the rat serum. Then 50 μ l of post-heparin plasma was added and the mixture was incubated in a shaking water-bath for 20 min at 37 °C. The reaction was stopped by adding 3.5 ml of propan-2-ol/hexane/0.5 M- H_2SO_4 (40:10:1, by vol.) followed by 2.1 ml of hexane and then 1.4 ml of 0.05 M- H_2SO_4 . The tubes were capped and shaken for 5 min, then after low-speed centrifugation 2.1 ml of the upper phase was transferred into tubes containing 1.05 ml of 0.1 M-NaOH in ethylene glycol. The tubes were capped, shaken vigorously for 10 min and centrifuged. The upper hexane layer was removed and 0.75 ml from the lower phase was transferred into liquid scintillation vials and counted for radioactivity in 5 ml of scintillant. Recoveries of liberated ^{14}C -labelled fatty acids were corrected by adding an internal standard of [^3H]oleic acid to the post-heparin plasma (60 000 d.p.m./ml). LPL activity was calculated as the difference between total and hepatic lipase activities.

G3PDH assay

Measurement of hepatic mitochondrial G3PDH activity was carried out with a method modified from the described procedure [12]. Liver mitochondrial samples were assayed in 0.45 M-triethanolamine buffer (pH 7.9)/0.45% BSA/1 mM-dihydroxyacetone phosphate/0.2 mM NADH and measured at 340 nm. NADH was omitted from blanks. Fresh dihydroxyacetone phosphate was prepared from dihydroxyacetone phosphate dimethylketal dimonocyclohexylamine salt monohydrate, obtained from Sigma.

Apolipoproteins associated with emulsions after incubation

To prepare very-low-density lipoprotein (VLDL)-free plasma for incubation with emulsions, plasma was obtained by heart puncture, with EDTA as anticoagulant, from control, hypothyroid and T_3 -treated hypothyroid rats. Samples of plasma were centrifuged for 16 h at 159 957 g_{av} at 4 °C in the Beckman SW41 rotor and the VLDL floating in the top 5 mm of the tube was discarded. Emulsions were incubated with VLDL-free plasma for 15 min at 37 °C, with 750 μ g of emulsion TG/ml. The emulsion particles were then separated from the incubation

Table 1. Rat plasma lipids, organ and body weights, and G3PDH

The concentration of methimazole (MMZ) used was 0.3 g/litre in the drinking water. The results are means $\pm$ S.E.M., with numbers of rats in the treatment group given in parentheses. Initially the rats weighed 189 ± 1.2 g ($n = 136$). ND, not determined. Significant differences from controls: * $P < 0.01$, ** $P < 0.001$.

Treatment	Plasma lipids (mg/dl)		Weight (g)			G3PDH activity (units)
	Cholesterol	TG	Body	Liver	Spleen	
None (13)	41 ± 4.3	45 ± 4	263 ± 4	10.8 ± 0.1	0.6 ± 0.00	0.91 ± 0.02
MMZ (13)	$68 \pm 7.6^*$	$82 \pm 5^{**}$	$241 \pm 6^*$	10.3 ± 0.1	$0.4 \pm 0.01^{**}$	$0.52 \pm 0.08^{**}$
MMZ + T ₃ (0.1 mg/kg per day) (5)	37 ± 1.6	$76 \pm 5^{**}$	$223 \pm 5^{**}$	$9.0 \pm 0.2^{**}$	0.7 ± 0.03	$1.92 \pm 0.11^{**}$
MMZ + L-94901 (0.1 mg/kg per day) (4)	$73 \pm 2.8^{**}$	$105 \pm 7^{**}$	$221 \pm 4^{**}$	$8.7 \pm 0.1^{**}$	0.6 ± 0.00	ND
MMZ + L-94901 (1.0 mg/kg per day) (4)	42 ± 1.7	$85 \pm 12^*$	278 ± 5	$7.7 \pm 0.1^{**}$	0.7 ± 0.02	$0.64 \pm 0.07^*$
MMZ + L-94901 (6) (10 mg/kg per day) (6)	51 ± 2.5	$81 \pm 4^{**}$	257 ± 5	$8.5 \pm 0.2^{**}$	0.7 ± 0.02	$1.08 \pm 0.04^*$

mixture by centrifugation in a Beckman SW28 rotor for 17 h at $96764 g_{av.}$. The emulsion particles that floated at the surface were delipidated [13], and the associated apolipoproteins were separated by isoelectric focusing on polyacrylamide gels as previously described [14]. After staining with a mixture of Coomassie G250 (0.04 %) and R250 (0.01 %) in 3.5 % HClO₄, the bands were scanned with a Bio-Rad model 620 densitometer at a wavelength of 600 nm.

Chemical analysis

TG was extracted from 0.5 ml of plasma in 20 ml of chloroform and 3 g of TG purifier (Sigma). The chloroform phase was then assayed for TG by the chromotropic acid method [15]. Total cholesterol was assayed by the *o*-phthalaldehyde procedure [16] in 0.1 ml of plasma after saponification. The extracted lipids from remnants and emulsions were separated by t.l.c. chromatography on 0.2 mm layers of silica gel (DC-Alufolien Kieselgel 60; E. Merck, Darmstadt, Germany) in the solvent system light petroleum (b.p. 40–60 °C)/diethyl ether/formic acid (90:10:1, by vol.). The TG, cholesteryl ester and cholesterol bands were scraped from the plate for assay. Phospholipid was measured directly in the emulsion and remnant particles [17].

Statistical analysis

Data were evaluated by analysis of variance. Homogeneity of group variances was ascertained by the Bartlett test. Differences between individual means were established by the Newman-Keuls procedure. The *t* test for independent means was used where appropriate. Probability values of < 0.05 were accepted as significant.

Table 2. Comparison of chylomicron-like emulsions with their remnants prepared *in vitro*

Results are means $\pm$ S.E.M. for numbers of observations in parentheses. * $P < 0.001$ by Student's *t* test.

	Lipid composition (% by wt.)			
	Triolein	Cholesteryl oleate	Cholesterol	Phospholipid
Emulsions (8)	82.0 ± 1.5	2.4 ± 0.5	0.9 ± 0.2	14.7 ± 1.8
Remnants (10)	$43.0 \pm 4.3^*$	$28.3 \pm 3.2^*$	$8.7 \pm 1.5^*$	20.0 ± 1.9

RESULTS

Plasma removal of TG/phospholipid emulsions

Treatment with methimazole caused significant increases in both plasma total cholesterol and plasma TG compared with control euthyroid rats (Table 1). Treatment with T₃ reversed the change in cholesterol but not that in TG. Changes in the thyroid status of the rats were supported by the measurements of G3PDH, with results confirming previous findings [6]. Body weight was decreased in hypothyroid rats and decreased further in rats treated with T₃, but in rats treated with higher doses of L-94901 body weights were similar to those of controls.

The compositions of the lipid emulsions injected into the rats are given in Table 2. The emulsions were designed to represent the lipid compositions of small chylomicrons or large VLDL particles. The apparent particle diameter was 80 nm, calculated as described by Miller & Small [18]. The pattern of removal of emulsion particles injected into rats fed on a regular diet is shown in Fig. 1. To calculate these curves, the plasma volumes were estimated by the Wang & Hegsted procedure [19].

Fig. 1 shows that the removal rate of cholesteryl oleate was

Fig. 1. Effect of hypothyroidism on the removal from plasma of radioactive triolein (▲, △) and cholesteryl oleate (●, ○) after injection of lipid emulsions

Each point is the mean of at least 8 experiments and the bars represent S.E.M. ●, ▲, Hypothyroid rats; ○, △, control rats.

Table 3. Plasma removal and organ uptake of injected emulsions

Chylomicron-like lipid emulsions were injected into unanaesthetized rats. The fractional clearance rates were calculated from the exponential curves fitted to the plasma radioactivity data for the period 3–12 min after injection of emulsion. Spleen uptake was measured to exclude significant uptake by reticulo-endothelial cells. Results are means $\pm$ S.E.M. for the numbers of observations in parentheses. ND, not determined. Significant differences from controls: * $P < 0.01$, ** $P < 0.001$. Significant differences from 30 min study: † $P < 0.05$, †† $P < 0.001$. The concentration of methimazole (MMZ) used was 0.3 g/litre in the drinking water.

Treatment	Removal from plasma (fractional clearance rate; min ⁻¹)		Organ uptake of lipid radioactivities (% of injected dose)			
			Triolein		Cholesteryl oleate	
	Triolein	Cholesteryl oleate	Liver	Spleen	Liver	Spleen
30 min studies						
None (5)	0.257 $\pm$ 0.007	0.212 $\pm$ 0.017	16.3 $\pm$ 1.6	0.2 $\pm$ 0.02	69.2 $\pm$ 2.5	0.5 $\pm$ 0.04
MMZ (8)	0.261 $\pm$ 0.016	0.084 $\pm$ 0.018**	20.0 $\pm$ 1.7	0.4 $\pm$ 0.15	64.2 $\pm$ 6.1	0.7 $\pm$ 0.13
5 min studies						
None (6)	ND	ND	21.8 $\pm$ 1.7†	0.7 $\pm$ 0.06	53.4 $\pm$ 1.9††	1.0 $\pm$ 0.06
MMZ (15)	ND	ND	17.9 $\pm$ 0.2*	0.4 $\pm$ 0.01	44.9 $\pm$ 1.0**	0.6 $\pm$ 0.01
MMZ-T ₃ (0.1 mg/kg per day) (5)	ND	ND	24.4 $\pm$ 3.4	0.9 $\pm$ 0.09	53.8 $\pm$ 5.9	1.1 $\pm$ 0.11

much slower in methimazole-treated rats than in controls, but triolein clearance was little affected. The fractional clearance rates calculated for both labels for the initial rapid phase of clearance are listed in Table 3, confirming the slowing of cholesteryl oleate removal.

The pattern of emulsion clearance was consistent with a defect in remnant clearance, after normal depletion of emulsion triolein by the action of LPL. Remnants contain about 20% of the original TG, sufficient to account for the small impairment of triolein clearance apparent up to 8 min in Fig. 1. It was expected that liver uptake of emulsion cholesteryl oleate would be diminished because of its slow plasma clearance, but Table 3 shows that the recovery of cholesteryl oleate in the liver after 30 min was similar to that in euthyroid controls. Measurements after 30 min could have been too late to show a change. Uptake by the spleen was always very small (Table 3).

Sampling of the hepatic uptake of emulsion 5 min after injection showed decreased recoveries of both cholesteryl oleate and triolein in the hypothyroid rats. The lowered uptake of cholesteryl oleate was consistent with its decreased plasma clearance (Fig. 1) and, as discussed above, residual triolein in remnants can account for the decreased triolein uptake at 5 min. Compared with recoveries measured 30 min after emulsion injection, there was a little more triolein and less cholesteryl oleate. Treatment of hypothyroid rats with T₃ restored the liver uptake of cholesteryl oleate back to euthyroid values, and also increased the hepatic recovery of triolein. Recovery of triolein in the livers of control rats at 5 min was 21.8% of the dose, compared with 16.3% after 30 min (Table 3), possibly due to triolein hydrolysis and re-utilization of fatty acids or oxidation. Confirmation of the defect in remnant uptake by the liver was sought by injecting pre-formed remnants prepared by incubation with LPL plasma *in vitro*.

Clearance of emulsion remnants

Table 2 compares the compositions of the remnants prepared *in vitro* with the precursor emulsions. Emulsion triolein was decreased to about half of initial values, whereas contents of cholesteryl oleate and cholesterol had increased approx. 10-fold. The ratio of cholesteryl oleate/triolein increased from 0.03 to 0.66, indicating a 22-fold decrease in triolein content. In control studies the remnants prepared with post-heparin plasma from

hepatectomized rats were compared with remnants prepared with post-heparin plasma from intact rats. After injection into euthyroid rats the plasma clearance and liver uptakes were not significantly different.

Table 4 shows that methimazole treatment significantly decreased the plasma fractional clearance rate and the hepatic uptake of remnants compared with euthyroid controls. Treatment with T₃ considerably enhanced remnant clearance and liver uptake, to values exceeding those in control rats. In other experiments (results not shown) rats were injected with emulsions rich in cholesterol, shown previously to resemble remnants in their metabolism [20,21], and hepatic uptake of emulsion radioactive lipid was again significantly decreased in hypothyroid rats compared with euthyroid controls.

Effect of L-94901

As shown in Table 1, and confirming the findings of Underwood *et al.* [6], L-94901 decreased the plasma cholesterol of hypothyroid rats to control values (daily dose of 1.0 mg/kg body wt.). Plasma TG was unaffected. A daily dose of 0.1 mg/kg was ineffective. Measured values of G3PDH activity were less than in euthyroid controls at an L-94901 dose of 1.0 mg/kg, and only slightly more than in controls with a daily dose of 10 mg/kg.

The decrease in the clearance of remnants produced by methimazole was reversed and even accelerated by treatment with T₃, but L-94901 was much less effective in restoring either remnant clearance from plasma or uptake by the liver (Table 4). At a daily dose of 1 mg/kg body wt., liver uptake was greater than in untreated rats (48.3% compared with 29.6% of dose, $P < 0.005$) but remained lower than in euthyroid controls even at a dose of 10 mg/kg, and the apparently improved fractional clearance rates were not statistically significant. The same doses of L-94901 lowered the plasma cholesterol to euthyroid values (Table 1).

Effects of fasting and a high-fat diet on plasma lipids

Emulsion clearance was studied in absorptive animals to simulate the physiological post-prandial state. To establish how absorptive load might affect remnant clearance by changes in pool sizes of plasma lipids, we also measured plasma lipids in fasted rats, and in groups of rats fed on a high-fat diet.

Table 4. Plasma clearance and organ uptake of injected remnants

Remnants were prepared from chylomicron-like emulsions by incubation *in vitro* with LPL plasma as described in the Materials and methods section, then injected into unanaesthetized recipient rats. The fractional clearance rates were calculated from the exponential curves fitted to the 3–10 min interval of the plasma radioactivity data. Organ radioactivity was measured at the completion of the experiment, 10 min after injection. The results are means $\pm$ S.E.M. for the numbers of rats indicated in parentheses. Significant differences from controls: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; significant differences from methimazole (MMZ) treatment alone, † $P < 0.005$.

Treatment	Fractional clearance rate (min ⁻¹)	Organ uptake (% of injected dose)	
		Liver	Spleen
None (13)	0.075 $\pm$ 0.009	60.0 $\pm$ 5.3	2.7 $\pm$ 0.2
MMZ (13)	0.025 $\pm$ 0.014*	29.6 $\pm$ 4.5***	2.0 $\pm$ 0.2
MMZ + T ₃ (0.1 mg/kg per day) (5)	0.163 $\pm$ 0.011***	83.3 $\pm$ 2.6***	1.8 $\pm$ 0.2
MMZ + L-94901 (0.1 mg/kg per day) (4)	0.042 $\pm$ 0.013	38.9 $\pm$ 7.8*	4.9 $\pm$ 0.8
MMZ + L-94901 (1.0 mg/kg per day) (4)	0.046 $\pm$ 0.009*	48.3 $\pm$ 1.5†	2.8 $\pm$ 0.2
MMZ + L-94901 (10 mg/kg per day) (6)	0.038 $\pm$ 0.009*	38.9 $\pm$ 3.6**	2.0 $\pm$ 0.2

Table 5 shows that all groups of hypothyroid rats, whether fasted, non-fasted or fed on high-fat or control diets, showed increased plasma cholesterol compared with euthyroid controls. Only the non-fasted rats fed on the control diet showed an increase in plasma TG due to hypothyroidism. In both fasted or non-fasted rats, plasma cholesterol and TG were increased in rats fed on the high-fat diet. Except for the control rats fed on the control diet, plasma TG was increased in non-fasted compared with fasted rats. Plasma cholesterol was significantly higher in overnight-fasted rats compared with the appropriate control

group in all cases, except for hypothyroid rats fed on the high-fat diet, where the difference was not statistically significant.

Observations of the patterns obtained after density-gradient ultracentrifugation of prestained plasma [22] and by non-denaturing electrophoresis on 4–30 % (w/v) acrylamide gels [23] showed an increase in high-density lipoprotein (HDL) in methimazole treated rats, with the appearance of HDL₁. Also, LDL was increased and VLDL was decreased. These findings were consistent with previous reports [24,25].

Activities of LPL and hepatic lipase. Our studies of emulsion clearance indicated that lipolysis was probably unimpaired in hypothyroid rats. Assays *in vitro* of lipolysis in post-heparin plasma were made with two types of emulsion substrate in the absence or presence of high NaCl concentrations, the latter inhibiting the activity of LPL [11] and therefore allowing the determination of LPL and hepatic lipase activities. Table 6 shows

Table 5. Plasma cholesterol and TG in fasted and non-fasted control and hypothyroid rats fed on a high-fat diet for 2 days

Rats were fed on either the usual laboratory ration or a high-fat diet for 2 days before bleeding between 9:00 and 10:00 h; rats were either fasted overnight, or unfasted. Results are means $\pm$ S.E.M., for numbers of observations in parentheses. *The hypothyroid group is significantly different from controls in its own treatment block ($P < 0.05$). †The high fat group has higher cholesterol and TG than its corresponding control diet group ($P < 0.05$). ‡The non-fasted cholesterol is significantly lower than its corresponding fasted group ($P < 0.05$). §Excepting control rats on the control diet, non-fasted TG is significantly higher than in the corresponding fasted group ($P < 0.05$).

	Plasma lipids (mg/dl)	
	Cholesterol	TG
Fasted rats:		
Control diet		
Control (5)	80.5 $\pm$ 5.2	43.7 $\pm$ 9.1
Hypothyroid (5)	100.8 $\pm$ 6.3*	31.8 $\pm$ 1.7
High-fat diet		
Control (8)	102.5 $\pm$ 2.8†	60.5 $\pm$ 8.1
Hypothyroid (10)	135.0 $\pm$ 6.4*†	51.3 $\pm$ 3.9†
Non fasted rats:		
Control diet		
Control (13)	41.2 $\pm$ 4.3‡	45.4 $\pm$ 3.9
Hypothyroid (13)	68.0 $\pm$ 7.6*‡	81.5 $\pm$ 5.2*§
High-fat diet		
Control (10)	88.8 $\pm$ 3.5†‡	154.3 $\pm$ 37.5†§
Hypothyroid (10)	126.6 $\pm$ 6.8*†	123.8 $\pm$ 15.1†§

Table 6. Activities of LPL and hepatic lipase in control and hypothyroid rats

Rats were injected with heparin (250 units/kg body wt.) and plasma was obtained 4 min later. Two types of substrate were used, either a gum-arabic-stabilized triolein emulsion or a chylomicron-like emulsion as used in the injection studies. Inhibition of LPL by a high NaCl concentration was used to differentiate the two lipase activities. Results are means $\pm$ S.E.M.; there were five rats in each group. *Activity in the hypothyroid group is significantly increased compared with the corresponding controls ($P < 0.05$); †Significant increase compared with the gum-arabic emulsion ($P < 0.05$); ‡significant decrease compared with the gum arabic emulsion ($P < 0.05$).

	Lipase activity (μ mol of fatty acid/h per ml of plasma)	
	LPL	Hepatic lipase
Gum arabic emulsion		
Control rats	10.9 $\pm$ 0.6	19.2 $\pm$ 0.2
Hypothyroid rats	11.6 $\pm$ 0.3	29.9 $\pm$ 0.8*
Chylomicron-like emulsion		
Control rats	18.3 $\pm$ 3.2†	0.9 $\pm$ 0.2‡
Hypothyroid rats	28.6 $\pm$ 1.8*†	1.7 $\pm$ 0.2*‡

Fig. 2. Effect of thyroid status on the association of apolipoproteins with incubated emulsion

Emulsions similar in lipid composition to lymph chylomicrons were incubated with VLDL-free plasma, and then the associated apolipoproteins were separated by iso-electric focusing on polyacrylamide gels. After delipidation, approx. 100 µg of protein was applied to gels for focusing as described in the text. The separated bands were stained with a mixture of Coomassie G250 (0.04 %) and R250 (0.01 %) in 3.5 % HClO₄. Lane 1, incubation with plasma from control euthyroid rats; lane 2, incubation with plasma from hypothyroid rats; lane 3, incubation with plasma from hypothyroid rats treated with T₃. E and C indicate apolipoproteins E and C respectively.

that lipase activity was increased in hypothyroid rats compared with euthyroid controls. Total lipolysis in hypothyroid rats was greater by 35 % and 58 % with the gum arabic and chylomicron-like emulsions respectively. Activity attributed to LPL was higher with the chylomicron-like emulsion, whereas hepatic lipase was much more active against the gum-arabic-stabilized emulsion.

Incubation studies with emulsions

Fig. 2 shows the apolipoproteins associated with emulsions after incubation with the VLDL-free fraction of plasma. After incubation with the VLDL-free plasma from hypothyroid rats there was relatively more apolipoprotein C and relatively less apolipoprotein E than after incubation with the VLDL-free plasma from control rats or rats treated with T₃. By integration of densitometric scans, the apolipoprotein E/apolipoprotein C ratio was 0.33 in hypothyroid rats compared with 0.64 in control rats and 0.81 in T₃-treated hypothyroid rats. Also apparent from Fig. 2 is a change in the distribution of the isoforms of apolipoprotein E when emulsions were incubated with plasma from hypothyroid rats. The E₄ isoform was decreased relatively more than the E₃ isoform, so that the E₄/E₃ ratio was lower in hypothyroid rats compared with controls.

DISCUSSION

The possibility that remnants may contribute to accelerated atherogenesis in hypothyroidism was raised by previous studies [4,26] describing the effects of hypothyroidism on the catabolism of chylomicrons in rats. In contrast with the present study and that of Floren & Nilsson [27], rats in previous studies [4,26] were fed on an atherogenic diet. An atherogenic diet greatly expands the plasma cholesterol pool, complicating the differentiation of the roles of hypothyroidism and hypercholesterolaemia on remnant clearance. As shown in Table 1, in the present study hypercholesterolaemia was minimal, although plasma cholesterol

was significantly higher than in euthyroid controls. Expansion of the plasma cholesterol pool by 1.6-fold (Table 1) was associated with a decrease of emulsion cholesteryl oleate clearance by 2.5-fold (Table 3) and of remnant clearance by 3-fold (Table 3), suggesting that the clearance defect was not due simply to an expansion in the whole plasma cholesterol pool size. However, it cannot be excluded that the plasma remnant pool size was not expanded by 2.5–3-fold, because remnants cannot be measured in plasma. Our findings are therefore consistent with expansion of the size of the plasma remnant pool or with a specific defect in remnant clearance that has little effect on other pools of cholesterol in plasma.

Floren & Nilsson [27] found that the plasma clearance of chylomicron remnants was similar in hypothyroid and control rats, in apparent conflict with our findings. In their experiments the remnants were prepared from chylomicrons obtained from euthyroid lymph-cannulated donors by lipolysis in post-heparin plasma from euthyroid rats, then injected into normal-fed hypothyroid rats, or exposed to hepatocyte monolayers. Therefore the chylomicrons and remnants would have had the normal complement of apolipoproteins, although this was not reported [27]. The design of some of our experiments overcomes the difficulty of injecting apolipoproteins from euthyroid donors. Emulsion models of chylomicrons employed in the present study contained no apolipoproteins. For their metabolism the injected emulsions acquired apolipoproteins from the recipient animal. However, this cannot explain the conflict between our findings with remnants (Table 4) and Floren & Nilsson's data from two rats [27], because in both designs remnants were prepared in post-heparin plasma from normal rats. There are several other differences in experimental design between the two studies, and further work is necessary to resolve this and other conflicts, including the effects of T₃, which we found to stimulate remnant clearance, whereas Floren & Nilsson [27] reported an inhibitory effect.

The lowered uptake of remnants that we found in hypothyroid rats was accompanied by a decreased apolipoprotein E/apolipoprotein C ratio (Fig. 2), which has previously been shown to inhibit remnant uptake by the liver [28,29]. The decreased proportion of apolipoprotein E associated with the emulsion particles contrasts with the increased plasma concentration of total apolipoprotein E in hypothyroid rats [25,30], possibly because the different apolipoprotein E isoforms differ in their affinities for the emulsion. Fig. 2 shows that the E₄ isoform is decreased relatively more than the E₃ isoform. Changes in apolipoprotein E isoforms were previously observed in perfusates of livers from hypothyroid rats [31].

Lipoprotein changes in the plasma of rats with experimental hypothyroidism include changes in the VLDL, LDL and HDL fractions. In hypothyroid rats receptor-mediated clearance of LDL is decreased [32–34]. Our findings now show that clearance of remnants is also impaired in hypothyroid rats. The increased plasma cholesterol in hypothyroid rats compared with euthyroid controls (Table 5) is consistent with a defect in the metabolism of cholesterol-containing lipoproteins. The ligand for removal of remnants is apolipoprotein E, which binds to the LDL receptor and also to a separate currently unidentified remnant receptor. Therefore the defect in remnant clearance that we have observed could be attributed to decreased uptake by LDL receptors, but a defect in uptake by putative remnant receptors cannot be excluded. As reviewed by Heimberg *et al.* [35], thyroid hormone decreases plasma LDL content, probably by facilitation of receptor-mediated uptake as demonstrated by clearance studies [32] and in primary cultures of rat hepatocytes [36]. Our data (Table 4) now show that remnant catabolism also is increased by T₃.

Chylomicron clearance in human patients with hypothyroidism was studied by Abrams *et al.* [37]. In hypothyroid patients without hypertriglyceridaemia, chylomicron clearance was considered to be normal. Nevertheless, the patients showed a significant increase in non-chylomicron TG in post-prandial plasma, which can now be explained by impaired removal of chylomicron remnants. Demonstration of a defect in remnant clearance cannot be adequate without measurement of the fate of chylomicron cholesteryl esters. As Fig. 1 shows, cholesteryl ester clearance can be severely impaired with minimal changes in TG clearance.

Hypothyroidism interfered with remnant clearance, but TG lipolysis by LPL, the first step in emulsion metabolism, appeared to be unimpaired. The data of Table 5, where none except the non-fasted rats fed on the control diet showed an increase in plasma TG, suggest that lipolysis was normal in hypothyroidism. Measurements in post-heparin plasma from methimazole-treated hypothyroid rats showed that LPL and hepatic lipase were both increased (Table 6). These findings contrast with measurements reported after propylthiouracil treatment [38] and in hypothyroid patients [37], but are consistent with our previous measurements of LPL activity in adipose tissue [4], a principal site of LPL [39].

The finding that plasma cholesterol was significantly higher in overnight-fasted rats than in non-fasting controls (Table 5) appears surprising, but is confirmatory of long-standing observations by Bragdon *et al.* [40] in Osborne-Mendel and Sprague-Dawley rats fasted for 24–72 h. They showed that the increase was due to an increase in HDL. In a separate experiment we have repeated the result shown in Table 5 (T. G. Redgrave & C. L. Elsegood, unpublished work), again showing significantly higher plasma cholesterol in overnight-fasted euthyroid rats. The effect may vary between rat strains, because fasting for 24 h did not affect plasma cholesterol in studies by Sure *et al.* [41].

Our findings confirmed the cholesterol-lowering effect of the T_3 analogue L-94901 in methimazole-treated rats [6]. Compared with T_3 , L-94901 was less effective in correcting the defect in remnant clearance, although some improvement was found, suggesting that cholesterol lowering by L-94901 may partly depend on improved remnant clearance.

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