Effects of triiodothyronine and propylthiouracil on plasma lipoproteins in male rats

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Abstract Hyperalphalipoproteinemia, characterized by increased plasma concentrations of apoA-I and of HDL lipid and protein, was observed in rats treated with triiodothyronine (T_3) for 7 days. The increase in the plasma HDL apoproteins was general for apoC, apoE plus A-IV, and apoA-I, as determined by isoelectric focusing. Hypotriglyceridemia, characterized by decreased concentrations of VLDL and apoB, was also observed in the hyperthyroid state. Although in the mildly hypothyroid animals (propylthiouracil-treated), hepatic metabolism of free fatty acid is shifted toward esterification to triglyceride and VLDL formation, as we reported previously, plasma HDL and apoA-I concentrations were not different from control plasma values, while the d 1.006–1.063 g/ml (IDL + LDL) lipoprotein fraction tended to be increased. In general, the proportion of apoE in the (IDL + LDL) fraction of the hypothyroid rat was greater than in controls and hyperthyroid animals, while the proportion of apoE tended to be lower in VLDL from both hypo- and hyperthyroid rats than in VLDL from controls. An enhanced release of apoA-I by perfused livers isolated from rats treated with T_3 was also observed; this enhanced output of apoA-I may explain, in part, the hyperalphalipoproteinemia observed in these rats. The depressed net output of apoA-I in vitro by perfused livers from rats treated with propylthiouracil (PTU) was not expressed in a statistically significant diminished plasma concentration of HDL or apoA-I in the intact animals. Treatment with T_3 also resulted in modification of the content of essential fatty acids in various lipid classes. Linoleic acid residues were significantly reduced and arachidonic acid content was increased in plasma phospholipids and esterified cholesterol in T_3-treated rats. However, the relative fatty acid composition of unesterified fatty acids and triglyceride fatty acids was not altered by T_3 treatment. PTU treatment had no effect on fatty acid distribution in any of the plasma lipids. Secretion of biliary lipids was increased in perfused livers from T_3-treated rats, while treatment with PTU did not affect release of lipids in the bile. These observations suggest a regulatory role for thyroid hormones that determine concentration and composition of plasma HDL and other lipoproteins.—**Wilcox, H. G., W. G. Keyes, T. A. Hale, R. Frank, D. W. Morgan, and M. Heimberg.** Effects of triiodothyronine and propylthiouracil on plasma lipoproteins in male rats. *J. Lipid Res.* 1982. **23:** 1159–1166.

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Thyroid hormones appear to be important regulators of lipid metabolism. Hypercholesterolemia or hypocholesterolemia is usually associated with hypothyroidism or hyperthyroidism, respectively (1). Considerable variability has been reported in plasma concentrations of triglyceride, which may be increased, decreased, or normal in hyperthyroidism (2, 3). In hypothyroidism, the plasma triglyceride concentration is usually increased but may be normal (3, 4). We reported previously (5, 6) that perfused livers from rats treated with T_3 esterified less and oxidized more free fatty acid than did livers from euthyroid animals. Treatment with propylthiouracil (PTU) sufficient to lower plasma T_3 levels to 60% of normal, and to induce a mild hypothyroidism, had the opposite effect. One consequence of these divergent pathways of hepatic fatty acid metabolism is the lower secretion rate of VLDL by perfused livers from hyperthyroid rats, and the reverse in hypothyroidism (5, 6).

The influence of thyroid hormones on HDL metabolism has not previously been studied. It has been observed, however, that HDL cholesterol is moderately depressed along with other classes of plasma lipoproteins in hyperthyroidism (7). In hypothyroidism, the concentration of HDL cholesterol is variable, but usually normal or slightly increased (7, 8). Dory and Roheim (9) observed elevated plasma levels of IDL and LDL in the rat made hypothyroid by feeding PTU for 30 days; decreased removal of these apoE-rich lipoprotein fractions from the circulation may have, in part, ac-

Abbreviations: T_3, triiodothyronine; PTU, propylthiouracil; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; TG, triglyceride; PL, phospholipid; C, cholesterol; CE, cholesteryl ester; SDS, sodium dodecyl sulfate; FFA, free fatty acid; RID, radial immunodiffusion; LCAT, lecithin:cholesterol acyltransferase; PAGE, polyacrylamide gel electrophoresis.

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counted for their observations. A modest elevation of apoA-I was also observed (9). Experimental hyperthyroidism was not studied by these investigators.

Our interest in evaluation of effects of thyroid status on lipid metabolism, through hepatic mechanisms and establishment of a reliable model for such studies, led us to examine the concentrations and composition of plasma lipids, lipoproteins, and apolipoproteins in rats made hyperthyroid with T_3 or hypothyroid with PTU. This was of particular interest since we had previously observed important differences in hepatic free fatty acid metabolism and VLDL formation in livers from such animals. Additionally, we wished to evaluate effects of thyroid status on hepatic output of HDL and biliary lipids. A preliminary report of this work has been presented (10).

METHODS

Male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA), having initial weights of 150–175 g, were housed in individual wire-bottom cages. Lighting was maintained between 0430 and 1630 hr. Animals were made hyperthyroid by subcutaneous injection of T_3 (10 μg/100 g body wt per day) or made hypothyroid by subcutaneous injection of PTU (1 mg/100 g body wt per day). Injections were carried out between 1530 and 1630 hr. In another group, animals were treated similarly, but injections were carried out at 1000–1100 hr. The drugs were dissolved in 0.9% NaCl with 1 N NaOH and the pH was adjusted to 8.5. Control (euthyroid) rats were injected with an equivalent volume of the vehicle at the same pH. All animals were treated with drugs for 7 days and allowed free access to food (powdered Purina Lab Chow) and water. Daily food consumption was measured and was equal among all groups for the duration of treatment (5, 6). The reason for selection of treatment periods and drug dosage was documented in previous publications from our laboratory (5, 6). These treatments produced mild hyperthyroid and hypothyroid states in the rats and did not affect food consumption. Under those experimental conditions (5, 6), identical to those used in the present study, the plasma concentrations of T_3 were 47% higher following administration of T_3, and were 60% lower than the euthyroid rats, treated with PTU, at the time blood samples were taken for analysis (6); plasma thyroxine (T_4) concentrations were 90% lower with T_3 and 75% lower after treatment with PTU, than in the euthyroid animal.

At the end of the treatment period, rats were anesthetized with diethyl ether between 0830 and 1030 hr, 17–24 hr after the last injection of drug. Blood was obtained from the abdominal aorta using EDTA (1 mg/ml blood) as the anticoagulant. There were no discernible differences in the lipid and lipoprotein parameters measured between the morning and the afternoon injection groups, so they were treated as one group. Plasma was collected by low speed centrifugation to sediment the formed elements of the blood. Isolation of lipoproteins was started immediately after collection of blood from individual randomly selected samples.

Perfusions of the isolated rat liver were carried out as reported previously from this laboratory (5, 6). The perfusion medium consisted of Krebs-Henseleit bicarbonate buffer (pH 7.4), 30% washed bovine erythrocytes, glucose (100 mg/dl), and 3% delipidated bovine serum albumin. An oleic acid albumin complex was infused at a rate of 166 μmol of oleic acid per hour (6 g of albumin and 1419 μmol of oleic acid/dl of infusate).

VLDL, IDL + LDL, and HDL were isolated from plasma by ultracentrifugation after sequential density adjustments (11) with solid NaBr (12). VLDL was collected at a density of 1.006 g/ml after 18 hr centrifugation in a Type 40 rotor at 10°C and 39,000 rpm in a Beckman-Spinco ultracentrifuge. The isolated VLDL was recentrifuged as before to free the VLDL from contamination with higher density proteins (principally albumin). IDL + LDL was collected in the density range 1.006–1.063 g/ml following an 18-hr centrifugation and was not recentrifuged. HDL (d 1.063–1.21 g/ml) was isolated and recentrifuged for 24 hr under the same conditions. The lipoproteins were dialyzed at 4°C overnight against 100 volumes of 0.001M EDTA–0.02% NaN_3.

An aliquot of plasma, bile, or isolated lipoprotein was extracted with chloroform–methanol 2:1 (v/v) (13). Individual lipid classes were separated by thin-layer chromatography (14). Analysis of triglyceride (15), cholesterol (16), and phospholipid (17) was carried out on the appropriate silicic acid band. Lipoprotein-protein was measured by the method of Lowry et al. (18) as modified by Markwell et al. (19), a method which uses sodium dodecyl sulfate as the delipidating agent for clearing turbidity. Fatty acyl group analysis was carried out as described previously (14, 20). A Packard-Becker 421 Gas Chromatograph interfaced to a Perkin-Elmer Sigma 10 integrator/computer was used for these analyses.

Lipoproteins were delipidated for electrophoretic analysis with chloroform–methanol 2:1 (v/v) (21). Isoelectric focusing of the apolipoproteins was carried out in 16.0 × 0.7 cm tubes using 50 μg HDL protein and 50–100 μg of VLDL or IDL + LDL protein essentially as described by Gidez, Swaney, and Murnane (22). Ampholines (LKB pH 4–6 and 3.5–10) were combined in

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the ratio of 5:3 to provide a wide pH range, but with an expanded pH 4–6 region. Gels prepared with both ammonium persulfate and riboflavin as the catalyst for polymerization were consistently more uniform. Electrophoresis was carried out for 16 hr at 400 volts and 5°C. The extruded gels were stained at 65°C for 45–60 min in a solution of 0.15% Coomassie Blue R250, 30% methanol, 12.5% trichloracetic acid, and 4% sulfosalicylic acid. Destaining was carried out by diffusion in 10% ethyl acetate, 7% ethanol, and 5% acetic acid for 48–72 hr and gels were stored in 5% methanol–7% acetic acid (23). Gels were scanned at 550 nm using a Gilford 2520 gel scanner; peak areas were estimated by triangulation and later by use of a Sigma 10 integrator (Perkin-Elmer). The areas of the individual polymorphic forms of apoA-I, apoE (also containing apoA-IV), and apoC were combined and are reported as groups of isoforms.

Plasma apoA-I levels were estimated by the technique of radial immunodiffusion (RID). Rat apoA-I, used as the antigen standard and for antibody production, was obtained from lyophilized rat plasma HDL (d 1.063–1.21 g/ml) delipidated with ethanol–ether 3:2 (v/v) (24). The delipidated HDL was dissolved in 0.2 M Tris–6 M urea buffer (pH 8.0) and passed through two Sephacryl S 200 (Pharmacia) columns in tandem (2.6 × 100 cm) at a rate of 10 ml/hr, using the same buffer. Recently, we obtained even more satisfactory separation using Sephacryl 200 and 300 columns in tandem. The eluted apoA-I peak was dialyzed against 4 M urea and further purified using preparative flatbed isoelectric focusing (LKB Multiphor 2100) on Sephadex-IEF in the pH range 4–6 (Ampholines LKB). The final preparation of apoA-I was judged to be pure by analytic isoelectric focusing (Fig. 1) and SDS-PAGE (not shown).

Antiserum to rat apoA-I was produced in New Zealand white male rabbits by intradermal injections at multiple sites on the back. One mg of apoA-I in 0.9% NaCl mixed with complete Freund's adjuvant 1:1 (v/v) was used for injection. A booster injection was given 2 weeks later and the antisera was harvested after another 2 weeks. The antiserum was monovalent as determined by immunodiffusion (Ouchterlony); a single line of identity between whole rat serum, apoA-I, and rat HDL was observed. RID plates were prepared with 1% agarose–5% T-10 dextran (25) in 0.05 M barbital buffer, pH 8.6 (0.02% NaN_3–0.001 M EDTA). Five-microliter samples of plasma diluted 1:10 with 0.1 M sodium decyl sulfate were placed in 4 mm wide × 2 mm deep wells in gel adhering to Gelbond Film (Marine Colloids, Rockland, ME). Plates (8.5 × 9.5 cm) contained 25 evenly spaced wells. Linearity of apoA-I concentration was obtained by plotting the square of the precipitin ring diameter against standard apoA-I concentrations (50 to 350 ng) in 0.5% bovine serum albumin.

ApoB[3] and additional estimates of apoA-I[4] were determined in plasma and perfusate samples by radioimmunoassay procedures developed in our laboratory using Triton X-100 to dissociate the apoprotein from the native lipoproteins.

Statistical significance was calculated using the unpaired Student's t test. The value $2P < 0.05$ was accepted as an indication of statistical significance.

RESULTS

The concentrations of plasma HDL (d 1.063–1.210 g/ml) protein, phospholipid, and cholesterol were increased by treatment with T_3, while treatment with PTU did not affect the plasma concentration of these components (**Table 1**). Hyperthyroid animals had lower concentrations of protein and cholesterol in the VLDL (d < 1.006 g/ml) than did euthyroid control animals. The levels of VLDL phospholipid and triglyceride tended to be lower in hyperthyroid rats, but did not quite reach ($2P < 0.07$) the assigned value for statistical significance. The concentrations of cholesterol and triglyceride in the VLDL were lower in PTU-treated animals than in controls. Although the levels of protein and phospholipid in VLDL tended to be lower in the PTU group, they did not reach statistical significance. The concentrations of protein, phospholipid, and cholesterol in the plasma IDL + LDL fraction (d 1.006–1.063 g/ml) of hyperthyroid animals did not differ from controls. Phospholipid and cholesterol of the d 1.006–1.063 g/ml lipoprotein fraction in PTU-treated animals were higher than in euthyroid animals, while protein tended to be higher but did not reach statistical significance.

The plasma concentration of apoA-I also increased in T_3-treated animals in comparison to euthyroid controls (**Table 2**), complementing the increase in levels of HDL protein and lipids. Treatment with PTU under our experimental conditions, however, did not alter the plasma apoA-I level significantly. The apoB content was diminished in plasma of hyperthyroid animals and unchanged in rats treated with PTU in comparison to euthyroid controls. These differences must reflect the changes in the VLDL and LDL lipids and protein, since these lipoprotein classes constitute the major carriers of apoB.

The isoelectric focusing patterns of the HDL apoproteins are shown in **Fig. 1**. Estimation of the relative distribution of the grouped apoC, apoE, and apoA-I isoforms is presented in **Table 3** for all plasma lipopro-

[3] Hale, T., H. G. Wilcox, and M. Heimberg. Unpublished results.
[4] Frank, R., H. G. Wilcox, T. Hale, and M. Heimberg. Unpublished results.

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TABLE 1. Effects of treatment with triiodothyronine or propylthiouracil on the composition of rat plasma lipoproteins[a]

Lipoprotein Class	Thyroid Status	Protein	Phospholipid	Cholesterol[b]	Triglyceride
		mg/dl		*μmol/dl*	
VLDL	Euthyroid	6.8 ± 0.9 (11)[c]	8.8 ± 1.4 (10)	11.1 ± 2.2 (10)[c,d]	35.3 ± 5.1 (10)[c]
d < 1.006	T_3	4.3 ± 0.5 (11)[c]	5.5 ± 0.9 (9)	4.1 ± 0.8 (10)[c]	23.7 ± 3.1 (10)
	PTU	4.8 ± 0.7 (11)	6.5 ± 1.2 (11)	6.2 ± 0.8 (10)[d]	21.3 ± 3.9 (10)[c]
IDL + LDL	Euthyroid	15.8 ± 1.6 (11)	13.6 ± 1.1 (11)[c]	42.1 ± 4.0 (11)[c]	
d 1.006–1.063	T_3	15.3 ± 2.1 (11)	17.0 ± 1.9 (10)	46.9 ± 6.8 (10)	
	PTU	19.0 ± 1.6 (10)	18.1 ± 1.7 (9)[c]	58.0 ± 3.7 (10)[c]	
HDL	Euthyroid	60.3 ± 3.5 (11)[c]	38.6 ± 2.8 (10)[c]	74.4 ± 6.9 (11)[c]	
d 1.063–1.210	T_3	87.0 ± 2.8 (11)[c,d]	68.0 ± 5.6 (11)[c,d]	100.3 ± 6.1 (10)[c,d]	
	PTU	57.2 ± 4.0 (11)[d]	40.4 ± 2.6 (10)[d]	68.2 ± 9.1 (11)[d]	

[a] All data are means ± SEM. Figures in parentheses indicate the number of observations.
[b] Represents the sum of separate analyses for free and esterified cholesterol.
[c,d] Values within a particular lipoprotein class with the same superscript are statistically different ($2P < 0.05$) from one another.
Euthyroid, untreated animals; T_3, T_3-treated (hyperthyroid) animals; PTU, PTU-treated (hypothyroid) animals.

tein classes. The percentage distribution of apoproteins in HDL did not differ between euthyroid controls and T_3-treated animals. However, there was a lower percentage of apoA-I and a higher percentage of apoE in HDL obtained from plasma of PTU-treated animals compared to control or T_3-treated animals. An increased percentage of apoE in the IDL + LDL fraction in the PTU-treated rat was also suggested. The percentage of apoA-I in the IDL + LDL was relatively small and did not appear to be different among the groups. ApoE appears to make up a higher proportion of the apoprotein of the VLDL in euthyroid controls than in either PTU- or T_3-treated animals.

To characterize further the lipid abnormalities induced by treatment with T_3 or PTU, we examined the fatty acyl group composition of plasma esterified and unesterified fatty acids (**Table 4**). The relative distribution of fatty acid residues in plasma triglyceride was not altered by the thyroid status of the animal. However, there was a significantly higher proportion of 18:1 in the plasma FFA in hyperthyroid rats than in controls. Compensatory decreases occurred in the other fatty acid percentages, but were of insufficient magnitude to be statistically significant. The relative percent of linoleic acid was reduced and that of arachidonic acid was increased in cholesteryl esters and phospholipids in plasma of T_3-treated animals. PTU treatment and the resultant mild hypothyroid state did not seem to alter the relative

TABLE 2. Effects of treatment with triiodothyronine or propylthiouracil on concentrations of rat plasma lipoprotein apoproteins[a]

	Apolipoprotein	
Thyroid Status	ApoA-I	ApoB
	mg/dl	
A. Euthyroid	41.4 ± 2.0 (24)[b]	19.7 ± 1.8 (17)[b]
T_3	58.7 ± 2.2 (31)[b,c]	11.5 ± 0.6 (19)[b,c]
PTU	35.8 ± 1.9 (21)[c]	24.5 ± 2.0 (18)[c]
B. Euthyroid	49.7 ± 2.6 (8)[b]	30.1 ± 2.7 (8)[b]
T_3	87.3 ± 7.6 (8)[b,c]	14.3 ± 1.1 (8)[b,c]
PTU	47.2 ± 4.2 (8)[c]	28.2 ± 2.8 (8)[c]

[a] Data presented in panel A were determined by radial immunodiffusion. All data are means ± SEM. Figures in parentheses indicate the number of observations. Data presented in panel B represent an additional eight animals for which both plasma apoA-I and apoB were estimated by radioimmunoassay.
[b,c] Values within a specific apoprotein class having the same superscript are statistically different ($2P < 0.05$) from one another.

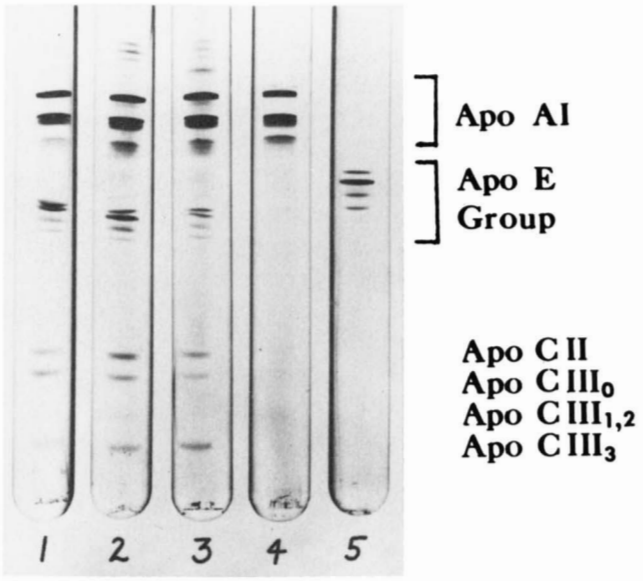

Fig. 1. Effects of treatment with triiodothyronine or propylthiouracil on isoelectric focusing patterns of rat plasma lipoprotein apoproteins. Tube 1 depicts plasma HDL apoproteins from a PTU-treated rat. Tube 2 shows HDL apoproteins from an untreated euthyroid rat. Tube 3 presents HDL apoproteins from a T_3-treated rat. Tube 4 depicts purified apoA-I from rat plasma HDL, and tube 5 shows the purified apoE from plasma VLDL.

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TABLE 3. Effects of treatment with triiodothyronine or propylthiouracil on the apoprotein composition of plasma lipoproteins[a]

Lipoprotein Class	Thyroid Status	ApoA-I	ApoE[b]	ApoC[c]
			% of total	
VLDL	Euthyroid (3)		58 ± 4	42 ± 3
d < 1.006 g/ml	T_3 (5)		43 ± 5	57 ± 5
	PTU (2)		40, 48	53, 59
IDL + LDL	Euthyroid (2)	10, 18	46, 52	36, 40
d 1.006–1.063 g/ml	T_3 (2)	18, 19	45, 59	23, 37
	PTU (2)	14, 18	58, 60	23, 27
HDL	Euthyroid (8)	72 ± 1[d]	19 ± 2[d]	9 ± 1
d 1.063–1.210 g/ml	T_3 (8)	77 ± 2[e]	15 ± 2[e]	8 ± 1
	PTU (9)	65 ± 1[d,e]	26 ± 1[d,e]	10 ± 1

[a] All data are means ± SEM. Figures in parentheses indicate the number of observations. Percentages were obtained from scans of isoelectric focusing gels.
[b] ApoE also reflects the presence of apoA-IV which focuses in the same region as the apoE isoforms.
[c] ApoC refers to the sum of the percentages of all the polymorphic forms of apoC-III and apoC-II as shown in Fig. 1.
[d,e] Values within a specific apoprotein class having the same superscript are statistically different from one another ($2P < 0.05$).

distribution of fatty acyl residues in plasma phospholipid and cholesteryl esters in comparison to that of the euthyroid animal.

The net output of apoA-I by the perfused liver was stimulated by hyperthyroidism and diminished by hypothyroidism. Treatment with T_3 increased the net output of apoA-I (25.4 ± 1.7 vs. 45.4 ± 5.6 μg/g liver per 4 hr for the euthyroid and hyperthyroid animals, respectively). Treatment with PTU reduced the net output of apoA-I to 9.0 ± 1.3 μg/g per 4 hr. In all cases, near linearity of apoA-I output over the 4-hr period was observed. ApoA-I accumulating during the second 2-hr period was about 90% of that of the first 2-hr period of perfusion. Furthermore, all three means were significantly different from each other ($2P < 0.05$). ApoA-I secretion by the liver probably is a good indicator for hepatic output of nascent HDL; this apoprotein is not a protein constituent of hepatic VLDL, the predominant lipoprotein secreted by the liver. Unfortunately, the condition of the perfusions did not allow us to examine the density fraction of the perfusion medium that could be properly identified as HDL. This resulted primarily from the use of bovine serum albumin contaminated with bovine apoA-I in the perfusion medium (6). The d 1.063–1.210 g/ml fraction isolated from the perfusion medium, although containing protein and

TABLE 4. Effect of treatment with triiodothyronine or propylthiouracil on fatty acyl group composition of plasma lipids[a]

Thyroid Status	Plasma Lipids	Fatty Acyl Group 16:0	18:0	18:1	18:2	20:4
				% of total		
Euthyroid (10)	FFA	31.8 ± 2.4	10.7 ± 0.4	11.9 ± 1.5[b]	10.0 ± 1.0	10.0 ± 1.0
	TG	32.4 ± 0.9	3.4 ± 0.2	38.2 ± 2.2	17.0 ± 1.8	1.1 ± 0.2
	PL	29.4 ± 2.3	19.8 ± 0.7	6.1 ± 0.2	26.9 ± 0.4[b]	9.8 ± 0.6[b]
	CE	11.3 ± 0.3	<1%	11.9 ± 1.3	32.8 ± 0.6[d]	33.7 ± 0.9[d]
T_3-treated (10)	FFA	29.4 ± 1.7	9.1 ± 0.6	17.9 ± 1.5[b,c]	12.9 ± 0.9	10.3 ± 1.4
	TG	30.0 ± 1.9	3.6 ± 0.3	36.3 ± 1.4	18.5 ± 1.7	<1%
	PL	26.9 ± 0.7	23.9 ± 1.1	6.1 ± 0.2	22.6 ± 0.9[b,c]	13.7 ± 0.5[b,c]
	CE	8.2 ± 0.4	<1%	10.9 ± 0.4	25.6 ± 1.2[d,e]	45.4 ± 1.8[d,e]
PTU-treated (10)	FFA	28.5 ± 1.9	12.1 ± 0.5	11.4 ± 0.5[c]	8.1 ± 0.5	12.1 ± 0.7
	TG	28.1 ± 0.7	3.9 ± 0.3	34.0 ± 1.6	22.0 ± 0.9	1.2 ± 0.2
	PL	29.6 ± 0.7	19.6 ± 1.3	5.9 ± 0.3	27.8 ± 0.9[c]	8.8 ± 0.7[c]
	CE	12.2 ± 0.4	<1%	12.7 ± 0.7	33.7 ± 1.0[c]	29.3 ± 1.5[c]

[a] All data are means ± SEM. Figures in parentheses indicate the number of observations. Percentage sums may differ from 100% due to omission of several fatty acid residues (16:1 and those with chain length greater than 20 carbon atoms).
[b,c,d,e] Values with the same superscript in the same column differ statistically from one another ($2P < 0.05\%$).

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TABLE 5. Effects of treatment with triiodothyronine or propylthiouracil on biliary lipid secretion by perfused livers

Thyroid Status	Cholesterol		Phospholipid	
	μmol/ml bile	*μmol/g per 4 hr*	*μmol/ml bile*	*μmol/g per 4 hr*
Euthyroid (6)	0.090 ± 0.020	0.019 ± 0.004[a]	0.360 ± 0.070	0.077 ± 0.011
T_3-treated (9)	0.140 ± 0.023	0.049 ± 0.010[a,b]	0.425 ± 0.058[a]	0.118 ± 0.016[a]
PTU-treated (9)	0.093 ± 0.010	0.013 ± 0.002[b]	0.256 ± 0.042[a]	0.036 ± 0.009[a]

Livers were perfused as described in the text. Data are means ± SEM. Figures in parentheses are the number of observations.

[a,b] Values in the same column with the same superscript differ statistically from one another ($2P < 0.05$).

rich in lipid, did not contain apoA-I as estimated by RIA. All the apoA-I found in whole perfusates could be accounted for in the d > 1.210 g/ml infranatant solution. It is probable that the lack of apoA-I in the HDL density fraction resulted from the displacement of rat apoA-I from the lipoprotein by the more plentiful and immunochemically distinct bovine apoA-I. That this could occur was demonstrated by examining rat HDL reisolated by ultracentrifugation after incubation with the bovine serum albumin. The reisolated rat HDL no longer reacted with antisera to rat apoA-I by radioimmunoassay and the reactive apoprotein was recoverable entirely in the d > 1.210 g/ml fraction.

We reported previously (6) that the volume of bile secreted by the perfused liver was increased in hyperthyroidism and reduced in hypothyroidism. **Table 5** presents data which also indicate that the secretion of biliary cholesterol by perfused livers from rats treated with T_3 was increased; phospholipid secretion tended also to increase, but failed to reach levels of statistical significance. Biliary secretion of cholesterol and phospholipid were not influenced by the mild hypothyroidism induced by treatment with PTU under conditions of these experiments.

DISCUSSION

The decreased concentrations of plasma VLDL that we and others observed in hyperthyroid animals and patients may have been predicted, in part, by our previous observations of the reduced net rate of VLDL secretion by livers from T_3-treated rats (6); the decreased levels of plasma VLDL may, in part, also have resulted from an enhanced rate of clearance of plasma triglyceride (26). Treatment with PTU under our experimental conditions increased hepatic esterification of fatty acids and secretion of VLDL and decreased fatty acid oxidation by the isolated perfused liver (5, 6). In the present study, however, the plasma concentration of VLDL triglyceride was lower in PTU-treated rats than in controls. Since the plasma FFA concentration is diminished in hypothyroidism (3), this factor per se would tend to reduce the hepatic output of VLDL in vivo. The IDL, partially metabolized VLDL and chylomicrons, and LDL have been shown to be increased in hypothyroidism (9), perhaps due to decreased peripheral catabolism. A similar trend for IDL + LDL was suggested in the present study with less severely hypothyroid animals.

A most interesting observation to us among those reported here was the increase in HDL protein and lipid in the T_3-treated rats, accompanied by the decrease in plasma VLDL. Studies on concentration of plasma HDL in thyroid pathology are sparse. Concentrations of HDL cholesterol are generally lower in hyperthyroid patients than in euthyroid controls (7, 8), while administration of supraphysiologic doses of L-thyroxine to normal male volunteers was found to be without significant effect (7).

Interestingly, it was reported in 1957 that the administration of triiodothyroacetic acid to euthyroid patients with coronary disease increased α-lipoprotein cholesterol and decreased β-lipoprotein cholesterol (27). No other data have been reported suggesting a hyperalphalipoproteinemic effect of thyroid hormone in man. Treatment of rats with thyroxine (28) or with T_3 (29) was reported to have little effect on lipoprotein levels but reduced cholesterol content in all plasma lipoprotein classes (30). Treatment of rats for 7 days with L-thyroxine (30 μg/d per 100 g) was reported to increase HDL phospholipid by 12% although HDL cholesterol was not changed, while VLDL and LDL protein and lipids were reduced by 20–48% (31).

Many of the apolipoproteins function as regulators of various metabolic processes (32). We reported previously that livers from hypothyroid rats secreted VLDL particles containing higher percentages of apoE and lower percentages of apoC than did the nascent VLDL from livers of hyperthyroid animals (6). The differences were not expressed in the plasma VLDL of hypothyroid rats (Table 3). The increased proportion of apoE in VLDL newly secreted by livers from PTU-treated animals (6) may not be maintained while recirculating in

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the plasma in vivo, during which time apoE exchange reactions involving IDL and HDL occur. Increases in the proportion of apoE in VLDL and LDL of hypothyroid patients (33) and in the triglyceride-rich lipoproteins of PTU-fed hypothyroid rats have been reported by other workers (9).

The principal apoprotein associated with HDL in the rat is apoA-I. This apoprotein probably is derived from the nascent HDL secreted by the liver (34) and from chylomicrons originating in the gastrointestinal tract (35).

We have shown in this study that the net secretion of apoA-I is increased in livers from T_3-treated animals compared to euthyroid controls, which may provide a partial explanation for the higher concentration of plasma apoA-I and HDL in vivo. Hepatic apoA-I biosynthesis may be stimulated in the animals treated with T_3 and diminished in those treated with PTU. However, the increased net secretion of apoA-I observed with the recycling liver perfusion system might also result from reduced hepatic uptake and metabolism of the secreted nascent lipoprotein. In preliminary studies, we observed that HDL and plasma apoA-I levels tended to peak after 5–7 days of treatment with 10 μg T_3/day per 100 g body weight, but then tended to return slowly toward control levels with continued treatment for an additional 7 days. An early and acute effect of T_3 (and perhaps of T_4) administration may be an appreciable increase in plasma HDL concentration. With longer exposure to high levels of thyroid hormone, however, this acute rise may be normalized and even diminished as a result of enhanced catabolism and/or diminished hepatic apoA-I otuput. These considerations are being investigated in our laboratory presently. The depressed net output of apoA-I by perfused livers from rats treated with PTU was not expressed in a diminished plasma concentration of HDL in the animal. The explanation for this observation remains to be determined.

The increased concentration of plasma HDL seen in T_3-treated rats is of interest in relationship to the current theory that an important role of HDL, in concert with LCAT, is in the transport of cholesterol from peripheral tissues to the liver for subsequent excretion (36). Although HDL cholesterol has been suggested as the preferred source of biliary cholesterol (37) and bile acid (38), this has not yet been unequivocally established. Increased biliary excretion of cholesterol by perfused livers from T_3-treated rats was observed in our present studies, in support of observations of others (39–41). Not all patients with hyperthyroid disease have enhanced biliary cholesterol excretion, however, and enhanced excretion has been reported in some hypothyroid patients (42).

The influence of the hyperthyroid state on metabolism of essential fatty acids in rats (43) and in patients has been observed previously. We confirmed the major findings of these studies in the hyperthyroid rat (44, 45). One of the principal effects of thyroid hormone on essential fatty acid metabolism may be enhanced conversion of 18:2 to 20:4 (43). It is unlikely that dietary 18:2 was limited in the hypothyroid animal studied, for neither plasma triglyceride nor FFA contained a lower proportion of 18:2. The mild hypothyroidism resulting from PTU treatment of the rat was not accompanied by the alterations in essential fatty acid metabolism described previously in patients (44).

The data reported in this manuscript clearly demonstrate that alterations in thyroid status can change lipoprotein apoprotein concentration and/or distribution. These alterations may be a consequence of or the reason for the altered lipid metabolism.

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