Nascent hepatic lipoproteins in hypothyroid rats[1]

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Abstract Cholesterol-feeding of hypothyroid Long-Evans rats results in a marked hypercholesterolemia and hepatic secretion of cholesteryl ester and apoE-rich VLDL and LDL which accumulate in the serum compartment (*J. Lipid Res.* 1981 **22:** 971–989). The present study segregates the effects of hypothyroidism from the combined effects reported above. Hypothyroidism alone does not result in the secretion of cholesteryl ester-rich lipoproteins by the liver which, in contrast, contains depressed quantities (30%) of triglyceride-rich (71% of lipid mass) VLDL and low levels of LDL which is also triglyceride-rich when compared to the nascent lipoproteins of euthyroid rat livers. The nascent lipoproteins from the hepatic Golgi cisternae and secretory vesicles of hypothyroid rats all had pre-β or slow pre-β migration on agarose gel electrophoresis and were very dissimilar in lipid and apoprotein composition from the β-migrating LDL that accumulates in the sera of these animals and contains 13.8% triglyceride, 51.3% cholesteryl ester, and has an apoB/apoE ratio of 32.7. We conclude that the serum LDL in hypothryoid rats, which contains only apoB_{100}, is not secreted directly by the liver but represents a normal catabolite of triglyceride-rich VLDL that may accumulate due to reduced receptor-mediated clearance rather than an overproduction of its immediate precursor, hepatic VLDL.—**Dolphin, P. J., and S. J. Forsyth.** Nascent hepatic lipoproteins in hypothyroid rats. *J. Lipid Res.* 1983. **24:** 541–551.

Supplementary key words propylthiouracil • hepatic Golgi and secretory vesicle lipoproteins • cholesterol • triacylglycerol secretion • apolipoproteins A-I, B_{100}, B_{48}, and E • LDL

There are presently many animal models of experimental atherosclerosis (1–7). Most require dietary-induced hypercholesterolemia and some, particularly the rat and the dog, require the simultaneous induction of hypothyroidism (8). Despite species-specific aberrations in the plasma lipoprotein profiles, certain common features have emerged. These include marked elevations in serum apoE and cholesterol, transported primarily as LDL and as a β-migrating very low density lipoprotein (β-VLDL), and the presence of an α-migrating lipoprotein, HDLc (4, 8). We have shown (9) that the hepatic secretory vesicles and Golgi cisternae of hypothyroid, cholesterol-fed rats contain cholesteryl ester and apoE-rich VLDL and LDL that are similar to their serum counterparts. Perfused livers from these animals secrete cholesteryl ester-rich lipoproteins that contain radiolabeled protein precursors (10). Furthermore, isolated hepatocytes maintain their ability to secrete cholesteryl ester-rich lipoproteins that are both spherical and discoidal (11) when incubated in vitro in a lipid-deficient medium (12). Guo et al. (13) have recently made similar observations using perfused livers from cholesterol-fed guinea pigs. Thus it appears that the liver of these animals responds to cholesterol feeding and the subsequent accumulation of chylomicron remnants by synthesizing, de novo, cholesteryl ester and apoE-rich lipoproteins that significantly contribute to the observed hypercholesterolemia. Our previous studies utilized the hypothyroid cholesterol-fed rat with the rationale that hypothyroidism promoted the effects of cholesterol feeding by increasing intestinal cholesterol absorption (14) and reducing apoB,E receptor-mediated clearance of the cholesteryl ester-rich plasma lipoproteins (15). Thyroid disfunction, however, is known to result in secondary hyperlipidemia that is characterized in man by elevations in LDL and, on occasions, VLDL (16), and will exacerbate hypercholesterolemia in Type III patients (17). Dory and Roheim (18) have also reported elevations in serum apoB, apoE, and total cholesterol associated with increased levels of IDL and LDL in hypothyroid rats. Euthyroid rats have very low levels of plasma LDL. The origin(s) and reasons for the abnormal accumulation of this lipoprotein species in hypothyroid rat plasma are presently unknown and it is possible that the secretion of cholesteryl ester-rich LDL by livers from hypothyroid, cholesterol-fed rats reflects the hypothyroid rather than the cholesterol-fed state. Thus there is a need to distinguish between the degree to which each of these factors influences the

Abbreviations: PTU, propylthiouracil; VLDL, very low density lipoproteins; β-VLDL, beta-migrating very low density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; HDLc, cholesterol-induced lipoprotein particles that are rich in cholesterol and contain apoE; apo (as a prefix), defines a lipid-free protein (apolipoprotein) that is normally associated with lipid to form a lipoprotein; T_4, thyroxine; LCAT, lecithin:cholesterol acyltransferase; TCA, trichloroacetic acid.

[1] A portion of this work was presented in poster form at the 6th International Symposium on Atherosclerosis, Berlin, West Germany, in June 1982, and a further portion appears in abstract form in *Arteriosclerosis.* 1982. **2:** 438A.

genesis of hypercholesterolemia. The status of hepatic triacylglycerol secretion in hypothyroidism is presently confused. Hypertriglyceridemia has been noted in human hypothyroid patients (16). In the rat, hypothyroidism results in decreased hepatic fatty acid oxidation (19) which favors VLDL triacylglycerol synthesis. Studies using isolated perfused rat livers have reported unchanged (20) or increased triacylglycerol secretion (21) and our own studies with isolated hepatocytes from hypothyroid rats (12) showed no significant decrease in triacylglycerol secretion when compared to euthyroid cells. Dory, Krause, and Roheim (22), in contrast, showed a pronounced decrease in VLDL secretion and triacylglycerol turnover in hypothyroid rats in vivo. The purpose of this study was to evaluate the role of the hypothyroid rat liver in the synthesis of cholesteryl ester-rich LDL and/or elevated levels of triacylglycerol-rich VLDL. As the lipoproteins present within the hepatic Golgi cisternae and secretory vesicles have been shown to be the immediate precursors of the plasma lipoproteins in vivo (23), we chose to determine the amount and composition of the lipoproteins present within the hepatic subcellular fractions of livers from hypothyroid rats and thereby delineate the nature and probable in vivo level of secretion of the nascent hepatic lipoproteins in experimental hypothyroidism. This approach avoided the complications inherent in in vitro studies in which high levels of lipogenic substrates are required for cell or tissue maintenance. The results obtained are consistent with the hypothesis that the liver of hypothyroid rats secretes decreased quantities of very triacylglycerol-rich VLDL. The plasma LDL that accumulates in these animals is not a direct secretory product of the liver and is most probably derived from the catabolism of plasma VLDL.

MATERIALS AND METHODS

Animals

Male Long-Evans rats (Canadian Breeding Farms, St. Constant, Quebec) weighing 200–250 g were maintained on commercial Purina rat chow. Blood samples, taken from overnight (18 hr) fasted animals, were obtained from the tail vein of all Long-Evans rats prior to the induction of hypothyroidism caused by administration of 0.1% (w/v) PTU dissolved in the drinking water for a period of 24 or 44 days. The mean weight of the animals on day zero, prior to entering the 24-day PTU regimen, was 224 ± 7 g and their mean weight after 24 days was 229 ± 4 g. Thus no significant weight gain occurred during reduction of thyroid function by PTU. Similarly, animals entering the 44-day regimen had a mean starting weight of 203 ± 3 g and a mean weight of 207 ± 3 g after 44 days. The animals were fasted overnight on the 23rd or 43rd day and were killed the following morning between 10:00 and 12:00 AM. Blood samples were obtained by cardiac puncture and the serum was prepared for T_4, apolipoprotein, and lipid quantitation. The livers were then perfused, removed, and weighed, and the hepatic Golgi cisternae and secretory vesicles were prepared (9). After isolation of the nascent hepatic VLDL from the lysed secretory vesicles and Golgi cisternae, the pelleted membrane fractions were resuspended in 10% (w/v) TCA, re-sedimented by centrifugation at 2000 *g* for 15 min, and delipidated with 10 ml of chloroform–methanol 2:1 (v/v) at −20°C. The resulting membrane proteins were then solubilized in 1 M NaOH and quantitated by the method of Lowry et al. (24). Analysis of the apoB components within the VLDL, IDL, and LDL serum fractions of hypothyroid cholesterol-fed and hypothyroid rats was performed after isolation of the lipoproteins from animals maintained on the high cholesterol thrombogenic diet for 44 days as previously described (9), or after 24 days of PTU treatment.

Lipoprotein isolation

Lipoproteins were isolated by preparative ultracentrifugation according to the method of Havel, Eder, and Bragdon (25), modified as previously described (10). VLDL (d < 1.006 g/ml) and LDL (d > 1.006–d < 1.063 g/ml) were isolated sequentially by recentrifugation of the serum or hepatic subcellular lysate after addition of solid NaBr. An IDL (d > 1.006–d < 1.019 g/ml) fraction was not isolated due to the very low levels of apolipoprotein present within the hepatic subcellular fractions from hypothyroid animals.

Analytical methods

Apolipoproteins B, E, and A-I were determined by electroimmunoassay as described elsewhere (9, 12). Individual lipids were separated and quantitated by the gas–liquid chromatographic total lipid profiling technique of Kuksis et al. (26) using a Hewlett-Packard 5840A automated gas chromatograph. Agarose gel electrophoresis of sera and isolated lipoprotein fractions was performed according to Maguire and Breckenridge (27) and the dried electropherograms were stained with 0.2% (w/v) Sudan Black. Sodium dodecylsulfate–3% polyacrylamide gel electrophoresis to resolve the B_{100} and B_{48} components of apoB was performed according to Kane, Hardman, and Paulus (28). Serum T_4 levels were determined by radioimmunoassay (BIO-RIA, Montreal, Quebec) and were found to be 7.4 ± 0.2 μg/dl (n = 18) for euthyroid rats prior to PTU administration and 0.6 ± 0.1 μg/dl (n = 11) after 24 or 44 days

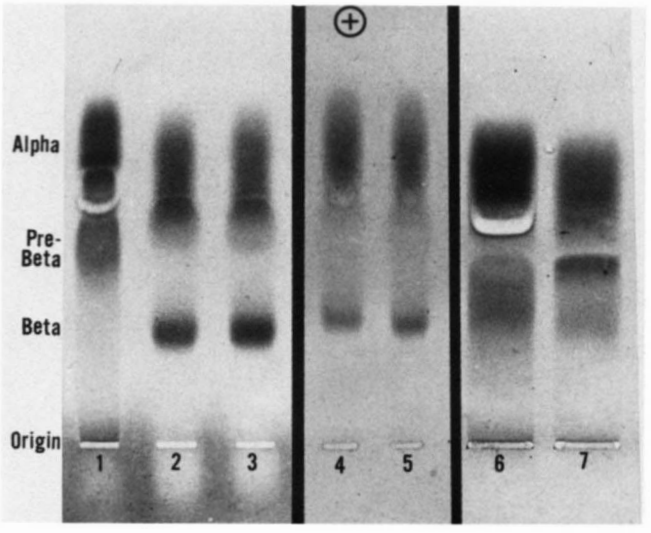

Fig. 1. Agarose gel electrophoresis of fasted sera from euthyroid and hypothyroid Long-Evans rats. Lane 1, euthyroid serum; lanes 2 and 3, sera from hypothyroid rats treated with 0.1% propylthiouracil (PTU) for 24 days, or (lanes 4 and 5) for 44 days. Note the reduction in pre-β lipoproteins after 24 days, the virtual absence at 44 days, and the appearance of a strong β-band in all hypothyroid sera. Pooled sera from rats maintained on PTU for 24 days followed by removal of the drug for 6 days (lane 6) or for 15 days (lane 7). Note the gradual diminution of the β-band and the reappearance of the pre-β band with the return of active serum thyroid hormone. Gels were stained with Sudan Black.

of PTU treatment. Lipoprotein particle diameters were measured after negative staining and visualization in a Philips 200 electron microscope (9). A minimum of 200 particles was measured for each determination. Alternatively, lipoprotein particle diameters were calculated from the lipid compositional analysis using the method of Shen, Scanu, and Kezdy (29) with appropriate compensations for differences in the mean molecular weights of the various lipids between the rat and human lipoproteins. Estimates of the significance of differences between data points were obtained using the Student's *t* test.

RESULTS

Our previous studies on lipoprotein biogenesis by the livers of hypothyroid cholesterol-fed animals utilized rats of the Long-Evans (hooded) strain exclusively (9, 10) and a time frame of 44 days of cholesterol feeding. Recent studies on the nature of the serum lipoproteins in hypothyroid rats used the Sprague-Dawley (albino) strain (18). Studies by Jeffery and Redgrave (30) have shown significant differences between these strains of rats in their response to cholesterol feeding and their respective abilities to clear chylomicron remnants. The data reported here indicate that Long-Evans rats respond to hypothyroidism in a qualitatively similar fashion to Sprague-Dawley rats; however significant differences exist between the strains in the quantitative nature of the response despite the dramatic reduction in plasma T_4 levels induced by PTU in both strains.

Serum lipoprotein, lipid, and apolipoprotein analysis

Fig. 1 shows that the induction of hypothyroidism in male Long-Evans rats over a period of 24 days results in the appearance of a strong β-migrating lipoprotein and a marked reduction in the pre-β-band. Continuation of the treatment for 44 days causes a further reduction in the pre-β-lipoproteins and the continued predominance of the β-lipoproteins. Removal of the PTU after 24 days results in the gradual reduction of the serum β-lipoproteins and the return of the pre-β-lipoproteins over a 15-day period. These gross changes in the serum lipoprotein profile upon the induction of hypothyroidism are reflected in alterations in the total serum lipid values as shown in **Table 1.** Serum triglycerides are significantly depressed after 24 days and reduced further at 44 days of treatment. Phospholipids are increased at 24 days, which is consistent with the predominance of smaller lipoprotein particles (LDL and HDL) in the serum. Surprisingly, serum cholesteryl es-

TABLE 1. Serum lipid levels in euthyroid and hypothyroid rats

Lipid	Euthyroid	Hypothyroid	
		24 Days	44 Days
		mg/dl	
Triglyceride	28.8 ± 4.1	8.3 ± 0.8[a]	2.4 ± 1.1[a]
Phospholipid	51.0 ± 1.9	77.9 ± 3.8[a]	57.7 ± 5.3
Cholesteryl ester	137.8 ± 4.3	130.5 ± 5.4	94.0 ± 9.1[a]
Cholesterol (free)	13.0 ± 0.8	28.2 ± 1.2[a]	23.5 ± 1.4[a]
Total cholesterol	93.8 ± 2.7	105.1 ± 4.2	78.8 ± 6.3[a]
Number of determinations	24	37	11

[a] Indicates a significant difference from the euthyroid fasted value at $P < 0.05$ or better.

Each value represents the mean ± SEM for the indicated number of determinations. The number of days indicates the time for which each group of rats received 0.1% PTU in their drinking water.

TABLE 2. Serum apoprotein levels of euthyroid and hypothyroid rats

Apoprotein	Euthyroid	Hypothyroid	
		24 Days	44 Days
		mg/dl	
ApoB	35.5 ± 1.4 (37)	64.2 ± 1.9 (55)[a]	48.6 ± 2.7 (11)[a]
ApoE	24.5 ± 0.9 (37)	28.3 ± 0.9 (45)[a]	31.1 ± 1.6 (11)[a]
ApoA-I	90.9 ± 1.8 (37)	89.4 ± 3.1 (38)	105.2 ± 6.1 (11)[a]

[a] Indicates a significant difference from the euthyroid value at $P < 0.05$.

Each value represents the mean ± SEM of the number of determinations in parentheses. The number of days indicates the time for which each group of rats received 0.1% PTU in their drinking water.

ters did not increase significantly and were lower than the euthyroid values after 44 days. Unesterified cholesterol, in contrast, showed a pronounced increase; however this was insufficient to produce an overall increase in total serum cholesterol previously reported for Sprague-Dawley rats (18). Apolipoproteins B and E are significantly increased in 24-day hypothyroid rats (**Table 2**). ApoB was somewhat lower at 44 days, which probably reflects the further reduction in serum VLDL noted at that time. ApoA-I did not significantly increase until 44 days. Sprague-Dawley rats, in contrast, showed a 30% increase in serum apoA-I after only 24 days of PTU treatment (18).

Hepatic nascent lipoproteins

Table 3 shows the yield of hepatic secretory vesicles and Golgi cisternae membrane protein from normal and hypothyroid rat livers using the standard discontinuous sucrose gradient fractionation methodology previously reported (9). No significant difference could be detected in the yields of these membrane fractions between euthyroid and hypothyroid animals. These data are particularly significant upon inspection of the results in **Table 4,** which show the recovery of VLDL lipid, apoB, and apoE from the lysed secretory vesicles and Golgi cisternae of euthyroid and hypothyroid rat livers. Euthyroid hepatic secretory vesicles contain almost exactly twice the quantity of lipid and each apoprotein than that within the Golgi cisternae. This ratio is preserved upon induction of hypothyroidism, the Golgi cisternae containing half the lipid and apoprotein residing within the secretory vesicle fraction. However, in the fasted hypothyroid state, the quantity of lipids and apoproteins in both hepatic fractions is reduced to approximately one-third of that associated with the same fractions from euthyroid fasted rat livers. Analysis of the VLDL lipids recovered from the livers of hypothyroid rats fed ad libitum showed that the secretory vesicles and Golgi cisternae from these animals contained approximately twice the quantity of VLDL lipids associated with the fasted hypothyroid state. However, the values of 15.7 ± 3.1 μg/g liver (n = 2) for the secretory vesicle VLDL lipids and 8.7 ± 1.8 μg/g liver (n = 2) for the Golgi cisternae VLDL lipids were still significantly lower than the values in Table 4 for the normal fasted hepatic fractions. As previously reported (9), hepatic fractions from fed euthyroid rats contain 25.2 ± 3.6 μg/g liver secretory vesicle VLDL lipids and 12.2 ± 2.7 μg/g liver VLDL lipids in the Golgi cisternal fraction. VLDL apoB and apoE levels were proportionately increased in the hepatic fractions from fed hypothyroid rats but were still lower than those present in the fractions from fasted euthyroid animals. **Table 5** shows the percentage distribution of individual lipids between the VLDL and LDL lipoprotein density classes for the Golgi cisternae and secretory vesicles from 24-day hypothyroid rat livers. Insignificant quantities of lipids were present at densities greater than 1.063 g/ml and are not reported. Eighty-five percent of the total lipid mass within the secretory vesicles was present as VLDL, which also accounted for the major percentage of the individual lipid components. Within the Golgi cisternae 68% of the total lipid was present as VLDL, which also accounted for the majority of the triglycerides. The low quantities of cholesterol and cholesteryl esters associated with the Golgi cisternae lipoproteins were predominantly present in the LDL density range. The distributions of apolipoproteins B and E (**Table 6**) essentially parallel that observed for the total lipids.

TABLE 3. Yields of hepatic membrane fractions from euthyroid and hypothyroid rat livers

Animals	Secretory Vesicle Membrane Protein	Golgi Cisternae Membrane Protein
	mg/g liver	
Euthyroid rats	0.049 ± 0.003 (3)	0.255 ± 0.026 (3)
Hypothyroid rats (24 days)	0.048 ± 0.012 (7)	0.262 ± 0.027 (7)

Each value represents the mean ± SEM for the number of determinations in parentheses.

TABLE 4. The recovery of VLDL lipids and apolipoproteins from the hepatic fractions of euthyroid and hypothyroid rats

Fraction	Total Lipid	ApoB	ApoE
		VLDL μg/g liver	
Secretory vesicle			
Euthyroid	30.7 ± 3.7 (7)	1.90 ± 0.14 (15)	0.25 ± 0.04 (8)
Hypothyroid	9.8 ± 1.5 (6)[a]	0.74 ± 0.11 (7)[a]	0.07 ± 0.01 (5)[a]
Golgi cisternae			
Euthyroid	14.4 ± 0.8 (7)	1.05 ± 0.09 (15)	0.14 ± 0.02 (8)
Hypothyroid	4.8 ± 0.6 (6)[a]	0.34 ± 0.05 (7)[a]	0.04 ± 0.005 (5)[a]

[a] Indicates a statistically significant difference from the euthyroid values at $P < 0.001$.

Each value represents the mean ± SEM for the number of determinations in parentheses. All values are for fasted animals. Lipids and apolipoproteins were determined on the total mass of VLDL isolated from 65 g of liver.

Lipid composition of nascent and serum lipoproteins

The lipid compositional analyses of the nascent hepatic and serum VLDL and LDL are shown in **Table 7** for the 24-day hypothyroid rats. The nascent hepatic lipoproteins were very triglyceride-rich, even when compared to those of the euthyroid rat (9). The nascent LDL, in particular, contained a significant percentage of this lipid and was relatively poor in cholesterol and cholesteryl esters. Analysis of the serum VLDL showed that it contained predominantly triglycerides but was somewhat enriched in total cholesterol when compared to the nascent hepatic VLDL and normal serum VLDL. The serum LDL fraction was, as expected, very rich in cholesteryl esters and free cholesterol and was relatively depleted in triglycerides. The lipid composition of the serum LDL of hypothyroid rats is thus very different from that of the nascent hepatic LDL fraction, unlike the situation in hypercholesterolemic animals where the nascent and serum LDL lipid compositions are very similar with the predominant lipid being cholesteryl ester (9). Prolongation of the PTU treatment for 44 days had no significant effect upon the lipid composition or distribution of the nascent hepatic lipoproteins when compared to the 24-day data. Similarly the lipid compositions of the serum VLDL and LDL were not significantly different from the 24-day analysis although the absolute mass of VLDL had decreased further at 44 days (data not shown). **Table 8** gives the free cholesterol to phospholipid molar ratios and the ratios of surface to core lipids for nascent and serum VLDL and the serum LDL fraction. Euthyroid serum VLDL has a cholesterol/phospholipid (C/PL) ratio of 0.6, which is similar to the value of 0.5 ± 0.02 recently reported by Schroeder et al. (31) for perfusate VLDL from normal rat livers. Serum VLDL from 24-day hypothyroid animals, in contrast, has a significantly higher C/PL ratio and a higher ratio of surface to core lipids. The high surface to core lipid ratio for the hypothyroid serum VLDL is reflected in its relatively small calculated particle diameter of 349 ± 12 Å (n = 19). The secretory vesicle VLDL from the hypothyroid animals, in contrast, has an observed particle diameter of 523 ± 7 Å which is significantly larger than the values of 408 ± 11

TABLE 5. Distribution of individual lipid classes between the VLDL and LDL from hepatic secretory vesicles and Golgi cisternae of hypothyroid rats

	Golgi Cisternae		Secretory Vesicles	
Lipid	VLDL	LDL	VLDL	LDL
		% ± SEM		
Triglyceride	90.3 ± 3.3	9.6 ± 3.3	91.6 ± 2.9	8.4 ± 2.9
Phospholipid	52.2 ± 9.1	47.8 ± 9.1	78.1 ± 3.2	21.8 ± 3.2
Cholesteryl ester	39.7 ± 4.7	60.3 ± 4.7	63.4 ± 4.5	36.6 ± 4.5
Cholesterol (free)	35.9 ± 6.9	64.1 ± 6.9	56.9 ± 4.1	43.1 ± 4.1
Total lipid	67.8 ± 5.7	32.2 ± 5.7	84.8 ± 2.7	15.2 ± 2.7
Total lipid μg/ 65 g liver	456.7 ± 39.9		743 ± 105.9	

Each value represents the mean % ± SEM for six experiments. Insignificant quantities of lipid were present at densities > 1.063 g/ml. Animals were maintained on 0.1% PTU in their drinking water for 24 days.

TABLE 6. Distribution of apoproteins B and E between the VLDL and LDL from hepatic fractions of hypothyroid rats

Lipoprotein Fraction	Golgi Cisternae		Secretory Vesicles	
	ApoB	ApoE	ApoB	ApoE
VLDL	43.7 ± 4.8	66.1 ± 7.3	70.3 ± 3.2	78.6 ± 4.2
LDL	56.2 ± 4.7	33.8 ± 7.3	29.7 ± 3.2	21.3 ± 4.2
Total apoprotein μg/65 g liver	51.5 ± 5.1	3.3 ± 0.3	69.3 ± 10.4	4.5 ± 1.3
N	7	4	7	3

Each value for VLDL and LDL represents the mean % ± SEM for the indicated number of experiments. Both apoB and apoE were detected in the d > 1.063 g/ml fraction, however, the quantities were too low for accurate quantitation by available methods.

Å and 413 ± 20 Å previously observed for secretory vesicle VLDL from euthyroid fed and hypothyroid hypercholesterolemic rats, respectively (9). The serum LDL fraction from hypothyroid animals has very high C/PL and surface to core lipid ratios with a calculated particle diameter of 250 ± 5 Å (n = 19).

Apoprotein analysis of nascent and serum lipoproteins

The ratios of immunoassayable apoB to apoE are reported in **Table 9** for the serum and nascent lipoproteins from normal and hypothyroid rats. No significant difference could be observed between the ratios for euthyroid and hypothyroid VLDL fractions. However, it is worthy of note that the secretory vesicle VLDL ratios for fasted euthyroid and hypothyroid animals are very much higher than the ratios of 5.8 ± 0.4 previously reported (9) for secretory vesicle VLDL from fed euthyroid rats. The ratios for the hepatic LDL fractions from hypothyroid animals are very high and contrast sharply with the ratios of 4.1 ± 0.5 and 5.4 ± 0.5 previously observed for the cholesteryl ester-rich nascent LDL from the hepatic secretory vesicles and Golgi cisternae, respectively, of fed hypercholesterolemic animals. Analysis of the apoB components present within the serum VLDL, IDL, and LDL fractions from hypothyroid and hypothyroid, cholesterol-fed rats was performed in an attempt to elucidate the origins of these particles. The results (**Fig. 2**) show that all fractions from the hypothyroid, cholesterol-fed animals contain the hepatic $apoB_{100}$ as the major component with $apoB_{48}$ as a relatively minor constituent. The LDL from hypothyroid rats, in contrast, contains only the hepatic $apoB_{100}$ component.

Agarose gel electrophoretic analysis of serum and nascent lipoproteins

Figs. 3A and **3B** show agarose gel electropherograms of the isolated VLDL and LDL fractions from the serum, hepatic Golgi cisternae, and secretory vesicles together with the autologous serum from euthyroid and 24-day hypothyroid rats. All hepatic nascent lipoprotein fractions exhibited pre-β or a slightly retarded pre-β migration. Lipoproteins with a β-migration could not be demonstrated within either the hepatic secretory vesicles or Golgi cisternae of hypothyroid rat livers although insufficient quantities of secretory vesicle LDL could be obtained for analysis by this technique. This observation is to be contrasted with the report of Swift et al. (32) who clearly demonstrated that the cholesteryl

TABLE 7. Lipid composition of serum and nascent lipoproteins from the hepatic Golgi cisternae and secretory vesicles of hypothyroid rats

Lipid	% Composition					
	Golgi Cisternae		Secretory Vesicles		Serum	
	VLDL	LDL	VLDL	LDL	VLDL	LDL
Triglyceride	67.5 ± 2.3	14.2 ± 2.4	71.7 ± 1.3	34.4 ± 6.3	55.7 ± 1.7	13.8 ± 0.9
Phospholipid	20.7 ± 2.3	42.6 ± 3.7	21.2 ± 0.7	35.7 ± 3.3	19.8 ± 0.7	22.3 ± 0.6
Cholesteryl ester	9.7 ± 0.6	33.9 ± 3.9	6.5 ± 0.8	24.1 ± 4.6	16.3 ± 1.2	51.3 ± 0.7
Cholesterol	2.1 ± 0.1	9.2 ± 0.9	1.2 ± 0.1	5.8 ± 0.9	8.1 ± 0.4	12.6 ± 0.1
Total lipid[a]	310.5 ± 42.7	146.0 ± 26.9	634.5 ± 100.8	108.8 ± 19.6	4.1 ± 0.6	41.3 ± 2.7
N	6		6		19	

[a] Values for total lipid are expressed in μg/65 g liver for the Golgi cisternae and secretory vesicles, and in mg/dl for the serum lipoproteins. Each value represents the mean ± SEM for the indicated number of determinations. Hypothyroid rats were maintained on PTU for 24 days.

TABLE 8. Molar ratio of lipids in nascent and serum lipoproteins

Lipoproteins	C/PL	(C + PL)/(CE + TG)
Serum VLDL		
Euthyroid	0.60 ± 0.10 (7)	0.32 ± 0.03 (7)
Hypo-T[a]	0.88 ± 0.05 (19)[b]	0.52 ± 0.02 (19)[b]
Secretory vesicle VLDL		
Euthyroid	0.38 ± 0.05 (7)	0.32 ± 0.01 (7)
Hypo-T	0.12 ± 0.01 (6)[b]	0.32 ± 0.01 (6)
Golgi cisternae VLDL		
Euthyroid	0.24 ± 0.02 (7)	0.31 ± 0.02 (7)
Hypo-T	0.23 ± 0.04 (6)	0.34 ± 0.04 (6)
Serum LDL		
Hypo-T	1.20 ± 0.03 (19)	0.63 ± 0.01 (19)

[a] Hypo-T indicates animals rendered hypothyroid for 24 days.
[b] Indicates a significant difference from the normal value at $P < 0.005$.
Each value represents the mean molar ratio of the indicated lipids ± SEM for the number of determinations in parentheses. C, cholesterol; PL, phospholipids; CE, cholesteryl esters; TG, triglycerides.

ester-rich LDL from the hypercholesterolemic rat hepatic Golgi apparatus has a β-migration upon agarose gel electrophoresis and our own prior observations of the secretion of β-migrating lipoproteins by perfused livers of hypothyroid cholesterol-fed rats (10).

DISCUSSION

Experimental hypothyroidism induced by PTU treatment in male Long-Evans rats is characterized by the accumulation of a β-migrating plasma LDL, decreased VLDL and plasma triglycerides with normal levels of cholesteryl ester, elevated unesterified cholesterol, and phospholipids. These changes are reflected in the elevation of serum apoB and apoE levels and a small increment in apoA-I. Similar effects have been noted in Sprague-Dawley rats (18) although strain differences are apparent in both the euthyroid serum lipid and apoprotein levels and the degree to which each responds to hypothyroidism. A rather surprising result was the lack of significant elevation of serum cholesteryl esters upon induction of hypothyroidism. Serum unesterified cholesterol, in contrast, was markedly elevated, the serum LDL had a very high cholesterol/phospholipid ratio (Table 9), and the serum levels of cholesteryl arachidonate did not increase upon fasting as they do in euthyroid controls (results not shown). As serum cholesteryl arachidonate is derived primarily from the transesterification of arachidonic acid from 2-arachidonyl phospholipids by the action of LCAT (33), these observations are consistent with a functional LCAT deficiency in hypothyroidism possibly associated with a decreased hepatic secretion of the enzyme.

TABLE 9. Ratio of immunoassayable apoB to apoE in nascent and serum lipoproteins from normal and hypothyroid rats

Fraction	Normal	Hypothyroid
Golgi cisternae		
VLDL	9.4 ± 0.8 (8)	10.6 ± 1.2 (5)
LDL	ND	29.8 ± 3.7 (4)
Secretory vesicles		
VLDL	10.3 ± 2.3 (8)	11.9 ± 0.7 (4)
LDL	ND	39.2 ± 2.9 (3)
Serum		
VLDL	3.6 ± 0.2 (11)	3.9 ± 0.4 (15)
LDL	ND	32.7 ± 3.4 (15)

Each value represents the mean ± SEM for the number of determinations in parentheses.

The primary focus of this report was to evaluate the hepatic contribution to the plasma lipoproteins in experimental hypothyroidism with particular reference to the origin(s) of the plasma LDL. The data obtained clearly establish that the hepatic secretory vesicles and Golgi cisternae from hypothyroid rats contain triglyceride-rich (Table 7) apoE-deficient (Table 8) lipoproteins that were isolated predominantly as VLDL (Table 5). Despite the fact that these lipoproteins were present in much lower quantities than in euthyroid hepatic fractions (Table 4), the distributions of total lipids and apoproteins B and E between the VLDL and LDL density ranges closely approximated that observed in he-

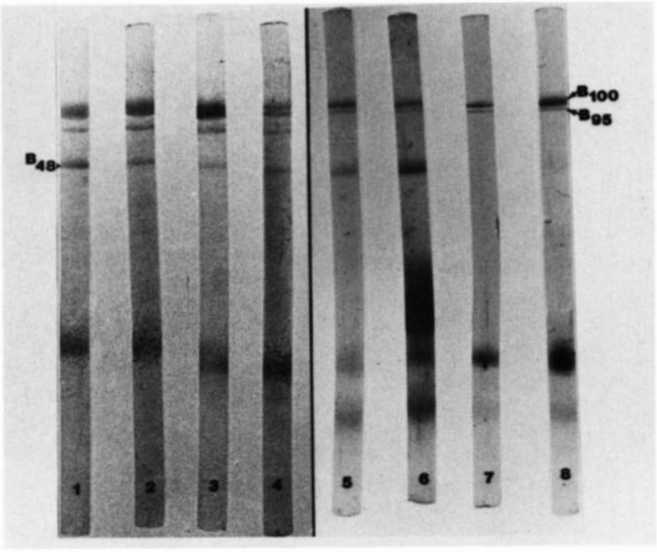

Fig. 2. Sodium dodecylsulfate-3% polyacrylamide gel electrophoretic analysis of the apoB components of lipoproteins from hypothyroid, hypercholesterolemic, and hypothyroid rat sera. ApoVLDL (lane 1), IDL (lanes 2 and 3), and LDL (lane 4) from hypothyroid, hypercholesterolemic rats. Lane 5, euthyroid rat serum apoVLDL. ApoVLDL (lane 6) and apoLDL (lanes 7 and 8) from 24-day hypothyroid rat serum. Note the lack of the lower molecular weight $apoB_{48}$ in the LDL from the rats maintained on PTU alone.

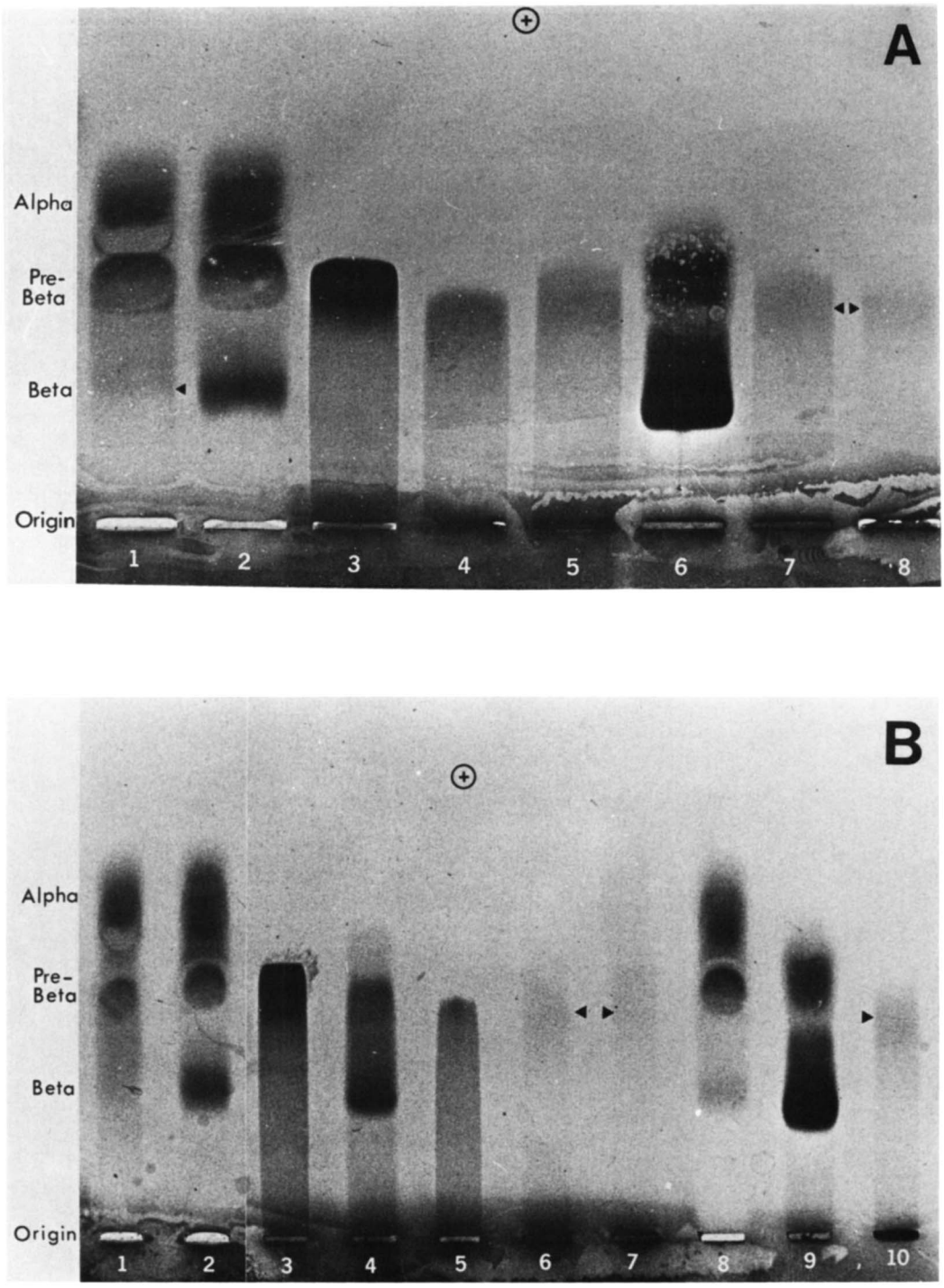

Fig. 3. Agarose gel electrophoresis of serum and hepatic nascent lipoproteins from fasted euthyroid and hypothyroid Long-Evans rats. **A and B:** lane 1, euthyroid rat serum; lane 2, serum from hypothyroid rats maintained on 0.1% PTU for 24 days; lane 3, serum VLDL isolated from euthyroid rats; lanes 6 and 9, d 1.006–1.063 g/ml fraction from 24-day hypothyroid rat serum. **A:** lane 4, hepatic secretory vesicle VLDL from euthyroid rats; lane 5, hepatic secretory vesicle VLDL from 24-day hypothyroid rats; lane 7, hepatic Golgi cisternae VLDL from euthyroid rats; lane 8, hepatic Golgi cisternae VLDL from 24-day hypothyroid rats. **B:** lane 4, d 1.006–1.063 g/ml fraction from euthyroid rat serum; lane 5, isolated serum VLDL from 24-day hypothyroid rats; lane 6, hepatic Golgi cisternae LDL from euthyroid animals; lane 7, hepatic Golgi cisternae LDL from hypothyroid rats; lane 8, d > 1.063 g/ml fraction from hypothyroid rat serum; lane 10, hepatic secretory vesicle LDL from euthyroid rats. Gels were stained as in Fig. 1.

patic fractions from euthyroid rats (9). In contrast with hypothyroid hypercholesterolemic rats (9), there was no observable accumulation of cholesteryl ester and apoE-rich lipoproteins within the LDL fraction of the hepatic Golgi cisternae or secretory vesicles of hypothyroid rats. Similarly, lipoproteins of β-migration were not observed, all nascent lipoproteins having pre-β or slightly retarded pre-β migration on agarose gel electrophoresis (Fig. 2). Euthyroid and hypothyroid hepatic secretory vesicles both contain 25–30% of the total lipid and apoprotein mass as particles that isolate in the LDL density range. These lipoproteins were richer in triglyceride and poorer in cholesteryl ester than their serum counterparts and did not exhibit characteristic β-migration on agarose gel electrophoresis. We therefore consider that these lipoproteins represent small, possibly immature, VLDL rather than a true species of LDL. As lipoproteins present within the hepatic secretory vesicles are known to be the immediate precursors of their serum counterparts (23), we conclude that the induction of hypothyroidism alone does not result in the hepatic biosynthesis and secretion of cholesteryl ester and apoE-rich lipoproteins, as is the case in cholesterol-fed hypothyroid animals (9). The quantities of VLDL lipid isolated from equal amounts of hepatic secretory vesicles and Golgi cisternae were consistently depressed in fractions from hypothyroid rat livers (Table 4). Ad libitum feeding increased the yield of VLDL from these fractions in euthyroid and hypothyroid animals; however the level of VLDL present in hepatic fractions from fed hypothyroid animals failed to achieve that within the hepatic fractions from fasted euthyroid rats. We interpret this observation as indicative of very significantly depressed hepatic VLDL secretion in hypothyroid rats in vivo.

The lipid and apoprotein compositions of the serum VLDL and LDL of hypothyroid rats are worthy of some comment. The serum VLDL, which is present at low levels, is less rich in triglycerides, contains proportionately more cholesteryl esters (Table 7), and has a higher C/PL and surface/core lipid ratio (Table 9) than normal serum VLDL. These data are consistent with partial lipolysis and indicate that the serum VLDL of hypothyroid rats is itself a partial remnant. This would also account for the retarded or slow pre-β migration of this lipoprotein upon agarose gel electrophoresis noted by ourselves (Fig. 3) and Dory and Roheim (18). The serum LDL from hypothyroid animals contained 51% of its lipid mass as cholesteryl ester; however the ratio of apoB to apoE for this fraction was remarkably high (Table 8), indicating a deficiency in apoE relative to apoB. The LDL that accumulates in the serum of cholesterol-fed hypothyroid rats, in contrast, contained 0.8% triglyceride, 69% cholesteryl ester, and had an apoB/apoE ratio of 8.0 (9). The LDL of hypothyroid serum thus more closely resembles the euthyroid rat serum LDL which contains 21% triglyceride, 47% cholesteryl ester and has an apoB/apoE ratio of 34 (9), and is known to be derived from hepatic VLDL by lipolysis (34). Furthermore, the LDL from hypothyroid rats contained exclusively $apoB_{100}$ (Fig. 2) which, unlike $apoB_{48}$, is only synthesized by the liver in the rat (35). Hypothyroidism would thus not appear to perturb the normal metabolism of VLDL in the rat (34) and result in the appearance of chylomicron remnants (containing $apoB_{48}$) in the serum LDL fraction. Rat liver, unlike human, synthesizes both $apoB_{100}$ and $apoB_{48}$ (35, 36). Thus the presence of $apoB_{48}$ in a serum lipoprotein fraction cannot necessarily be interpreted to reflect the presence of intestinally derived lipoproteins. The predominant B apolipoprotein of the IDL and LDL fractions from hypothyroid, cholesterol-fed rats is the high molecular weight $apoB_{100}$ (Fig. 2), which indicates a major hepatic contribution to these fractions. $ApoB_{48}$, however, is also present at significant levels which may infer the presence of chylomicron remnants or, alternatively, the hepatic $apoB_{48}$ component which, like the $apoB_{100}$-containing lipoproteins, accumulates in the plasma compartment. Credence is lent to the second possibility by the observations that chylomicron remnant removal does not appear to be depressed in hypothyroid cholesterol-fed rats (20). A similar mechanism is clearly not operative in the hypothyroid state where the LDL contains only $apoB_{100}$ and is clearly of hepatic origin. Chylomicron remnants in the rat are known to be cleared by the liver through the mediation of a specific apoE receptor (37) which, to date, has not been shown to be hormonally regulated (38). Rat plasma contains little LDL. VLDL catabolites appear to be cleared at the remnant stage prior to their conversion to LDL (39, 40). The lipoprotein receptor responsible for VLDL remnant clearance has not yet been characterized; however available evidence would suggest that this may occur via the apoB, E or LDL receptor. This receptor is known to be present in rat liver and other peripheral tissues (38) and to be down-regulated in human hypothyroidism (41). If VLDL remnants are indeed cleared by this mechanism, hypothyroidism would increase the plasma residence time of VLDL remnants and thereby promote their conversion to LDL. Due to the absence of any effect upon the apoE receptor, chylomicron remnant removal would not be influenced. This hypothesis is currently under active investigation in our laboratory.

These studies were supported by grant No. MA 5999 to PJD from the Medical Research Council of Canada. The authors

are indebted to Dr. W. C. Breckenridge for his assistance with the gas–liquid chromatographic total lipid analyses and many fruitful discussions.

Manuscript received 15 June 1982 and in revised form 20 January 1983.

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