

Rat plasma lipoproteins and apolipoproteins in experimental hypothyroidism

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Abstract Hyperlipidemia associated with hypothyroidism is well documented in man and several animal species. The effect of hypothyroidism on apolipoprotein metabolism in the absence of complicating factors such as high cholesterol or fat content in the diet is virtually unknown. Hypothyroidism was therefore induced in male Sprague-Dawley rats by radiothyroidectomy (RTx-treated) or treatment with propylthiouracil (PTU-treated). Both treatments resulted in an over 90% decrease in circulating thyroid hormone concentrations accompanied by a 50–100% increase in plasma cholesterol and a 20–40% reduction in plasma triglyceride concentrations. Plasma apo E and apo B concentrations increased by 100% in the PTU-treated group and 40–50% in the RTx-group. Apo A-I increased 10 and 30% in the RTx- and PTU-treated rats, respectively, while the concentration of apo A-IV was not altered. A large increase in the low-density lipoprotein (LDL) and high-density lipoprotein (HDL) protein was observed and accompanied by a marked reduction of very low density lipoprotein (VLDL) in the hypothyroid rats. The electrophoretic pattern of plasma lipoproteins in the hypothyroid rats was changed by the appearance of a slow pre- β band shown to be β -VLDL. A redistribution of apo B occurred within the lipoprotein fractions. Apo B content in the VLDL fraction decreased and a large increase was noted in LDL. The major portion of the apo E and apo A-I increment was recovered in the HDL and to a lesser degree in LDL. An accumulation of apo E-rich larger HDL particles, resembling HDL_c in apolipoprotein composition and distinct from the apo A-I-containing species, was observed by column chromatography. ■ The results presented are consistent with the hypothesis that hypothyroidism in the rat may induce an accelerated production of VLDL catabolic remnants, including LDL, but at the same time reduce the rate of removal of these lipoproteins from the circulation. — **Dory, L., and P. S. Roheim.** Rat plasma lipoproteins and apolipoproteins in experimental hypothyroidism. *J. Lipid Res.* 1981. **22:** 287–296.

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Hypothyroidism is a well-established cause of secondary hyperlipoproteinemia (1). The clinical features of hyperlipoproteinemia associated with hypothyroidism in humans have been described (2–6) and include increased plasma cholesterol levels accompanied by normal, moderately elevated, or very

high concentrations of triglyceride. These changes are reflected by significant elevations in plasma LDL, sometimes accompanied by raised plasma VLDL. Increased plasma LDL and occasionally VLDL concentrations observed in hypothyroidism have been chiefly attributed to decreased rate of degradation due to a) a defect in LDL removal (7) and b) decreased levels of PHLA (8). Hypertriglyceridemia associated with hypothyroidism may be caused by impaired removal of both endogenous (VLDL) (9) or exogenous (chylomicron) (9, 10) triglyceride, while triglyceride production appears to be normal (9). Perfused livers obtained from rats treated with propylthiouracil (PTU), an antithyroid agent, however, exhibit an increased rate of triglyceride secretion when compared to livers from euthyroid rats (11). Such apparently contradictory observations suggest that the action of thyroid hormones on lipid metabolism is complex and may, in some aspects, differ from species to species.

In the rat, thyroid hormones stimulate hepatic cholesterol biosynthesis, decrease hepatic triglyceride secretion, increase bile formation, increase excretion of cholesterol, and decrease intestinal cholesterol absorption (11, 12). The effects of hypothyroidism could be expected to act in the opposite direction, resulting in a net increase in plasma cholesterol concentration.

At the present time little is known about the effects of thyroid hormones on apolipoprotein metabolism. Hypothyroidism was shown to result in a decrease in LDL degradation, an observation reversed by administration of T₄ (7). Increased concentrations of apo B in IDL and LDL (6) and apo E in VLDL (13) of

Abbreviations: LDL, low density lipoproteins; VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; HDL, high density lipoproteins; PHLA, post-heparin lipolytic activity; PTU, propylthiouracil; T₄, thyroxine; T₃, 3,5,3'-triiodothyronine; RTx, radiothyroidectomized; EIA, electroimmunoassay; AU, arbitrary units; TMU, tetramethylurea; SDS-PAGE, sodium dodecyl-sulfate polyacrylamide gel electrophoresis; EDTA, disodium ethylene-diamine tetraacetate.

hypothyroid patients suggest that thyroid hormones may play an important role in regulating plasma apolipoprotein concentrations.

A number of studies have recently appeared dealing with changes in lipoprotein/apolipoprotein metabolism in cholesterol-fed animals in which hypothyroidism was also induced (14–17). Differentiation between the effects of cholesterol feeding and those induced by hypothyroidism, if possible, would contribute significantly to our understanding of lipoprotein metabolism. These considerations have led us to investigate and describe the changes in the lipid, lipoprotein, and apolipoprotein concentration and distribution in the plasma of hypothyroid rats. The observations reported below describe the strong influence of the thyroid status of the animal on plasma apolipoprotein concentration and distribution and on lipoprotein patterns. The presented data will provide the basis for future studies dealing with the mechanisms through which thyroid status influences lipoprotein metabolism.

MATERIALS AND METHODS

Animals

Male, Sprague-Dawley rats, weighing 200 ± 8 g were divided into a control and two experimental groups. The thyroid function of one of the experimental groups was ablated by continuous administration of 0.1% (w/v) PTU dissolved in the drinking water. To test for possible additional effects of PTU that may influence lipoprotein metabolism via a non-thyroid route, we felt that the use of another method of thyroid ablation should be employed and the effects compared. A second experimental group was therefore radiothyroidectomized by an intraperitoneal injection of 1 mCi of ^{131}I (New England Nuclear, Boston, MA) per rat, followed by the same treatment 1 week later. A second dose of ^{131}I was found necessary, as preliminary experiments showed that some rats recovered their thyroid function if only one injection was used. All three groups were maintained on normal Purina rat chow. Twenty-one to 25 days after treatment initiation, after an overnight fast, rats in all three groups were sacrificed in the morning, between 10–12 AM, and exsanguinated from the abdominal aorta. Blood was collected into syringes containing a solution of EDTA and sodium azide, pH 7.4, to obtain a final concentration of 0.01% EDTA and 0.01% sodium azide. Plasma was separated by centrifugation and stored at 4°C for the subsequent assays. The hypothyroid status of the experimental animals was confirmed by radioimmunoassay of T_3 and T_4 (18).

Anti- T_4 and anti- T_3 immunoglobulins were purchased from Calbiochem-Behring, Dallas, TX, and ^{125}I -labeled T_4 and T_3 (high specific activity) from New England Nuclear, Boston, MA.

Apolipoprotein quantitation

Plasma apolipoproteins were quantitated by electroimmunoassay (EIA) (19), as modified and previously described (20), except that both the samples and standards contained 1% NP-40 (v/v), and the agarose plates 0.05% of NP-40, a nonionic detergent (21). NP-40-treated samples were compared to similarly treated pooled standard reference rat plasma. Arbitrary units (AU) thus represent the percent concentration of the given apolipoprotein in the sample, when compared to the reference plasma. Antibodies to the individual apolipoproteins (B, E, A-I, and A-IV) were prepared as previously described (20).

The apolipoprotein content of the lipoprotein fractions was determined by measuring their concentration in the various density infranatants left after a single ultracentrifugation (20). Thus for example, the VLDL apolipoprotein content was determined by subtracting the content found in the $d > 1.006$ g/ml fraction from that found in the total plasma. Nonspecific losses of apolipoproteins in the infranatant fractions were quantitated and corrected for by the determination of albumin concentrations (also by EIA), which should remain constant. Such losses did not exceed 5% of the total.

Plasma apolipoprotein concentrations were also determined by scanning stained SDS-polyacrylamide gels of the $d < 1.21$ g/ml fraction of plasma and measuring areas under the peaks. The individual apolipoprotein bands were identified by comparison of their mobilities to those of purified, isolated apolipoproteins. In order to validate this method, a series of SDS-polyacrylamide gels with known and increasing apolipoprotein concentrations were run. The amount of the dye bound to each of the apolipoproteins was found to be directly proportional to the amount of apolipoprotein present in the concentration range used in these experiments. Total apo B content of the $d < 1.21$ g/ml fraction of the plasma was also determined by the TMU-precipitation technique (22).

Lipoprotein separation

A qualitative assessment of the changes in the lipoprotein pattern of the experimental animals was carried out by agarose gel electrophoresis (23) of plasma obtained from each animal. Ultracentrifugal separation of plasma lipoproteins was carried out by the method of Havel, Eder, and Bragdon (24) and the

TABLE 1. Plasma concentrations of T₃, T₄, cholesterol, and triglyceride in control, RTx-, and PTU-treated rats

Group	n	T ₄	T ₃	Cholesterol	Triglyceride
		μg/dl	ng/dl	mg/dl	
Control	10	3.9 (0.3)	43 (3)	47 (2)	49 (3)
RTx-treated	10	0.1 (0.5) ^a	3 (0.3) ^a	74 (2) ^a	38 (6) ^a
PTU-treated	10	<0.1 ^b	<1 ^b	95 (4) ^a	29 (3) ^a

^a Significantly different from the corresponding control values at least at $P < 0.05$.

^b The lowest tested values in each assay that resulted in readings (cpm) that were significantly different from the blank (0) tubes.

Numbers in parentheses represent \pm SEM.

isolated fractions were removed using a tube slicer. Total plasma lipoprotein fractions were prepared by adjusting the plasma density to 1.24 g/ml by addition of solid KBr and layering a KBr solution of $d = 1.21$ g/ml on top. Approximately 6 ml of the salt solution was layered on top of 5 ml of sample to keep albumin contamination minimal. To minimize losses of apolipoproteins due to ultracentrifugation, this fraction was not washed. SDS-polyacrylamide gel electrophoresis revealed only small amounts of albumin. These lipoprotein isolations were carried out in an SW-41 rotor for 48 hr at 100,000 g in an L5-50 Beckman ultracentrifuge.

To minimize changes in the apolipoprotein distribution due to excessive ultracentrifugation, the appropriate infranatant ($d >$) fractions were isolated for the determination of the apolipoprotein distribution. Thus 1 ml of plasma, adjusted to the desired density and layered with 0.8–1.0 ml of the appropriate salt solution, was centrifuged at 100,000 g at 5°C for 20–24 hr for the isolation of $d > 1.006$, $d > 1.019$, and $d > 1.063$ g/ml lipoprotein infranatant fractions and for 48 hr for the $d > 1.21$ g/ml fraction. A 40.3 Beckman fixed-angle rotor with adapters was used for these isolations.

Lipoproteins of the $d < 1.21$ g/ml fractions were further subfractionated by column chromatography on a 0.90×90 cm column of 6% agarose (Bio-Gel A-5m, 200–400 mesh, Bio-Rad Laboratories, Richmond, CA), using 0.15M NaCl, 0.01% EDTA, 0.01% Na azide, pH 7.4, as equilibrating and running buffer. The column was operated at 23°C and calibrated with blue dextran and ultracentrifugally isolated lipoproteins. The distribution of the various apolipoproteins within the various column fractions was determined by EIA of each fraction.

Analytical methods

Protein concentrations were determined by the method of Lowry et al. (25) using bovine serum albumin as a standard. Cholesterol and triglyceride

concentrations were determined enzymatically, based on the method of Allain et al. (26) and Bucolo and David (27), respectively, with reagents purchased from Calbiochem-Behring, Dallas, TX. SDS-PAGE was carried out according to Shapiro, Viñuela, and Maizel (28). The gels were stained with 0.1% Coomassie Blue G-250 (in 50% methanol and 10% acetic acid) for 2 hr at 60°C, destained in 10% acetic acid at room temperature overnight, and scanned in an E-C Apparatus Corp. densitometer. The Student's t -test was used to establish significant differences between experimental and control values.

RESULTS

Plasma thyroid hormones and lipid concentrations

As Table 1 indicates, no detectable thyroid hormones were found in the circulation of the PTU-treated animals and only traces of these hormones were detected in the animals from the RTx group. Animals from both experimental groups became hypophagic within a few days after treatment initiation, but continued to gain weight at a reduced rate. The initial weight of the animals in all three groups was 202 ± 8 (\pm SEM) g; at the end of the experimental period the control rats weighed 343 ± 8 g while the RTx- and PTU-treated rats weighed 301 ± 5 g and 261 ± 3 g, respectively. As shown in Table 1, development of hypothyroidism was accompanied by a significant rise in plasma cholesterol concentrations, from 47 mg/dl for control rats to 74 mg/dl and 94 mg/dl in RTx- and PTU-treated rats, respectively. A 20–40% decrease in plasma triglyceride concentrations was also observed in both experimental groups.

Plasma apolipoprotein concentrations

Both hypothyroid groups of rats had increased plasma concentrations of all apolipoproteins except apo A-IV (Table 2). Apo E and apo B increased by 60% and 30%, respectively, in the RTx-treated

TABLE 2. Total plasma apolipoprotein concentrations in control, RTx-, and PTU-treated rats

Apolipoprotein	n	Control	RTx-treated	PTU-treated
(AU)				
Apo B ^a	10	91 (4)	121 (6) ^c	171 (9) ^c
Apo E ^a	10	60 (3)	96 (5) ^c	113 (5) ^c
Apo A-I ^a	10	53 (3)	58 (3) ^c	69 (6) ^c
Apo A-IV ^a	10	20 (1)	21 (1)	19 (1)
mg/dl				
Apo B ^b	3	11 (2)	19 (2) ^c	22 (2) ^c

^a As determined by electroimmunoassay (EIA). Plasma from individual animals was used for the determination of the apolipoprotein concentrations.

^b TMU-Precipitable protein. Apo B concentrations were also determined as TMU-precipitable protein in the $d < 1.21$ g/ml fraction, obtained from plasma pooled from three to four animals. Duplicate determinations were carried out on three pools from each group of animals.

^c Significantly different at least at $P < 0.05$ from corresponding control values.

Numbers in parentheses represent \pm SEM.

group, while the plasma concentrations of both apolipoproteins nearly doubled in the PTU-treated group. The percent increase in plasma apo A-I concentrations was 10%–30% in both hypothyroid groups.

Tetramethylurea-precipitable protein (apo B) concentration in the $d < 1.21$ g/ml fraction of plasma (shown in Table 2) also increased in the hypothyroid animals, confirming the changes in apo B concentration determined by EIA. The plasma apolipoprotein concentrations were also estimated by scanning the SDS polyacrylamide gels of the $d < 1.21$ g/ml fractions, as shown in Fig. 1A–C. Since the

proportion of the TMU-soluble proteins and the total protein content applied to each gel was known, the areas of the peaks on the scans were used to calculate the amounts of the major TMU-soluble plasma apolipoproteins (Table 3). These observations were in general agreement with the data obtained by EIA, confirming increased plasma concentrations of apo E and apo A-I in the hypothyroid animals.

Plasma lipoproteins

Agarose electrophoresis, gel filtration, and ultracentrifugation were used simultaneously in order to study the changes in lipoprotein distribution based on their a) electrophoretic mobility, b) hydrated density, and c) size. Agarose electrophoresis of the whole plasma of individual animals revealed significant changes in the lipoprotein patterns of the hypothyroid rats. An agarose electrophoresis pattern of plasma obtained from a control and hypothyroid rat is shown in Fig. 2A. The α -migrating HDL and the pre- β migrating VLDL are the predominant lipoproteins of the control animals. The plasma lipoprotein pattern of the hypothyroid animals on the other hand is characterized by a heavy, β -migrating LDL band, accompanied by a disappearance of the pre- β VLDL and the appearance of a new lipoprotein band with a retarded pre- β mobility. A comparison of the electrophoretic mobilities of ultracentrifugally isolated lipoproteins (Fig. 2B) reveals that this new lipoprotein band is due to the appearance of β -VLDL.

The plasma concentrations of ultracentrifugally isolated lipoprotein-proteins are shown in Table 4. A notable decrease in the VLDL concentration in

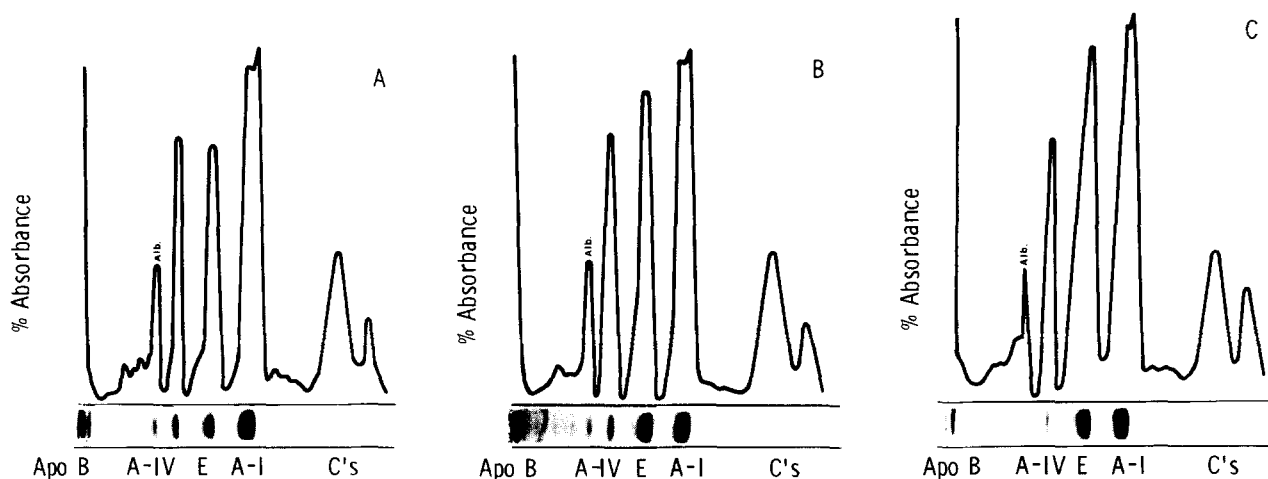


Fig. 1. Scanning of the SDS-polyacrylamide gels of the separated $d < 1.21$ g/ml apolipoproteins of (A) control, (B) RTx- and (C) PTU-treated rats. 50 μ g of total protein was applied to each gel and the amount of TMU-soluble apolipoproteins was determined for each sample beforehand. The Coomassie Blue-stained gels were scanned at 660 nm and the areas under the peak were used to calculate the apolipoprotein concentrations presented in Table 3.

TABLE 3. Plasma apolipoprotein concentrations in the $d < 1.21$ g/ml fraction of control, RTx-, and PTU-treated rats

Apolipoprotein	n	Control	RTx-treated	PTU-treated
			<i>mg/dl</i>	
Apo E	3	11 (1)	20 (2) ^a	23 (2) ^a
Apo A-I	3	25 (1)	26 (2)	35 (1) ^a
Apo A-IV	3	6 (0.3)	5 (0.6)	8 (1)

^a Significantly different from corresponding control values at least at $P < 0.05$. The apolipoprotein concentrations in the $d < 1.21$ g/ml fractions were determined by densitometric scanning of SDS gels (see Fig. 2 A-C). The concentrations were calculated on the basis of total TMU-soluble protein applied to the gels. Each group of animals was divided into three pools of three to four animals each. Two gels of the material from each pool were scanned to obtain the relative proportion of the various TMU-soluble apolipoproteins applied. It is possible that this method of measurement of plasma apolipoprotein concentrations underestimates their true concentrations due to some losses of the apolipoprotein during ultracentrifugation (36, 37).

Numbers in parentheses represent \pm SEM.

both hypothyroid groups of rats was accompanied by a two to four-fold increase in LDL concentrations. The plasma HDL levels in both hypothyroid groups were elevated by 25%. Distribution of the cholesterol within the various lipoprotein classes is shown in Table 5. The greatest increase in cholesterol concentrations was found in LDL and HDL, but VLDL and IDL also contained higher concentrations.

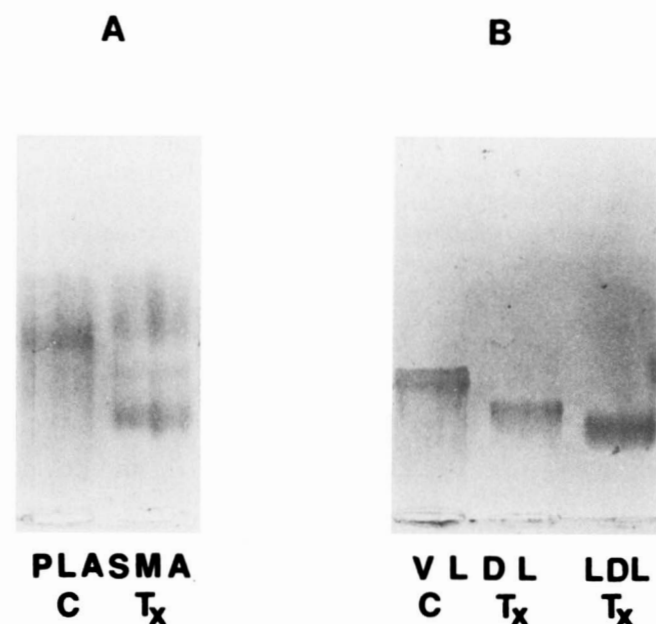


Fig. 2. A, Agarose electrophoresis of 9 μ l of plasma obtained from a control (C) and hypothyroid (Tx) rat. B, Agarose electrophoresis of ultracentrifugally isolated VLDL from a control (C) and hypothyroid (Tx) rat and LDL (d 1.019–1.063 g/ml) from a hypothyroid rat. Control LDL is now shown but comigrates with hypothyroid LDL.

TABLE 4. Plasma lipoprotein concentrations^a in control, RTx-, and PTU-treated rats

Lipoprotein	Density	Control	RTx-treated	PTU-treated
	<i>g/ml</i>		<i>mg/dl</i>	
VLDL	<1.006	4.1	2.4	0.4
IDL	1.006–1.030	4.6	3.5	3.4
LDL	1.030–1.063	4.2	9.2	16.2
HDL	1.063–1.210	39.0	50.0	50.0

^a Lipoproteins from each group of animals were isolated from pooled plasma of ten rats by sequential ultracentrifugation. Their protein content was determined by the method of Lowry et al. (25).

Fractionation of the $d < 1.21$ g/ml lipoproteins by column chromatography confirmed and extended the data obtained by ultracentrifugal or electrophoretic methods presented above. As Fig. 4A indicates, there was a marked decrease in the VLDL concentrations in the hypothyroid animals. During the separation of lipoproteins from control animals, LDL does not appear as a distinct peak, measurable by an increase in the absorbance at 280 nm, but the increase in LDL in the hypothyroid animals was sufficient to produce a distinct peak. The HDL fraction of the hypothyroid animals also increased, with an apparent shift to the larger particle size range. The greatest change occurred in the PTU-treated animals, but remained significant in the RTx-treated group as well.

Distribution of apolipoproteins in the lipoprotein fractions

The distribution of the apolipoproteins among the main lipoprotein fractions was determined as described in the Methods. In the control animals, about 50% of apoB was found in LDL and about 30 and 20% in VLDL and IDL, respectively (Fig. 3A). In the hypothyroid animals there was more than a 100% increase in the apoB concentration (see also Table 2), both in IDL and LDL, the greatest

TABLE 5. Distribution of cholesterol among the various plasma lipoprotein fractions of control, RTx-, and PTU-treated rats

Lipo-protein	Density	n	Control	RTx-treated	PTU-treated
	<i>g/ml</i>		<i>mg/dl</i>		
VLDL	1.006	3	1.2 (0.4)	4.2 (0.2)	3.6 (0.2)
IDL	1.006–1.030	3	2.3 (0.2)	6.8 (0.1)	8.8 (3.9)
LDL	1.030–1.063	3	14.0 (1.5)	20.0 (2.5)	31.5 (1.8)
HDL	1.063–1.210	3	22.7 (2.0)	36.8 (3.0)	40.8 (4.0)

Numbers in parentheses represent \pm SEM.

Plasma pooled from three to four animals was used for each determination. Duplicate measurements were carried out on three pools from each group of animals. Cholesterol determinations were performed on the infranatant fractions used for the determination of the apolipoprotein distribution (see Fig. 4). The procedure is described in the Methods section.

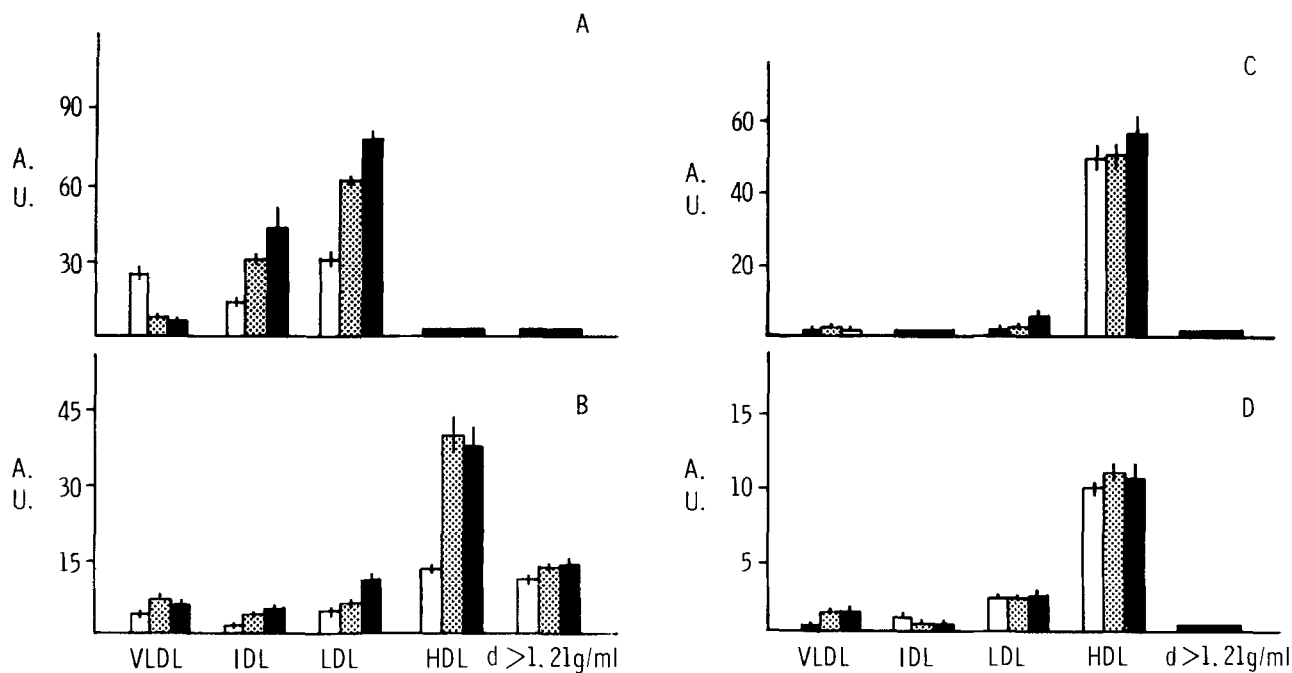


Fig. 3. Distribution of the apolipoproteins among plasma lipoproteins separated by ultracentrifugation: (A) apo B, (B) apo E, (C) apo A-I, and (D) apo A-IV, in control (□), RTx- (▨), and PTU- (■) treated rats. The apolipoprotein content of the various lipoproteins was determined by measuring their concentrations in the various density infranatants left after a single ultracentrifugal run. For example, VLDL apolipoprotein concentration was determined by subtracting the amount found in the $d > 1.006$ g/ml fraction from that found in the total plasma. The difference between the apolipoprotein content of the $d > 1.006$ g/ml and $d > 1.019$ g/ml is the apolipoprotein concentration of IDL. S.E.M.s are indicated.

increases occurring in the PTU-treated animals. Despite the increase in the total plasma concentrations of apoB, the concentration of this apolipoprotein markedly decreased in VLDL, resulting in a significant alteration in its plasma distribution. The concentration of apoE increased in all lipoprotein fractions (Fig. 3B), but the largest increase occurred in the HDL fraction, where most of apoE is also found in the control animals. While small changes in similar direction were observed for apo A-I (Fig. 3C), no changes in the distribution of apo A-IV took place (Fig. 3D).

As described in the Methods, EIA was also used to determine the apolipoprotein distribution within the lipoprotein fractions obtained by column chromatography. A typical distribution pattern for apo B is shown in Fig. 4B. A decrease of apo B in VLDL and a marked increase in LDL was consistently found. The elution profile of the apo B-containing particles obtained from the plasma of hypothyroid rats suggested a decrease in the average diameter of these particles. On the other hand, plasma apo E concentration in the hypothyroid animals significantly increased in the HDL and LDL region (Fig. 4C), and a shift in its distribution towards a larger particle size range was observed. The apo A-I elution profile of the hypothyroid animals, however, shown in

Fig. 4D, indicated an increase in the concentration of this apolipoprotein in the HDL region with no apparent change in the size of the particles associated with it. Although not shown, induction of hypothyroidism did not alter the elution profile of apo A-IV.

Since the distribution of apolipoproteins in the lipoprotein fractions was obtained by the use of EIA, an independent method was used to confirm the data. Qualitative changes in the apolipoprotein composition of the individual fractions of the column eluates were determined by SDS-PAGE (Fig. 5). On comparing the SDS-PAGE patterns of the lipoprotein fractions obtained from control and PTU-treated animals, the overall increase in apo E is obvious. In the control, apo A-I was the major apolipoprotein of each HDL column fraction, with little or no other apolipoprotein present in the smaller HDL. In contrast, in the PTU-treated animals, each of the HDL column fractions contained apo E as a major component. In the larger HDL region, in addition to some apo B, due to presence of LDL, apo E was the major apolipoprotein, with only trace amounts of apo A-I. The apolipoprotein composition of this lipoprotein species resembles HDL_c (15). While SDS-PAGE patterns of column fractions shown in Fig. 5 are only from control and PTU-

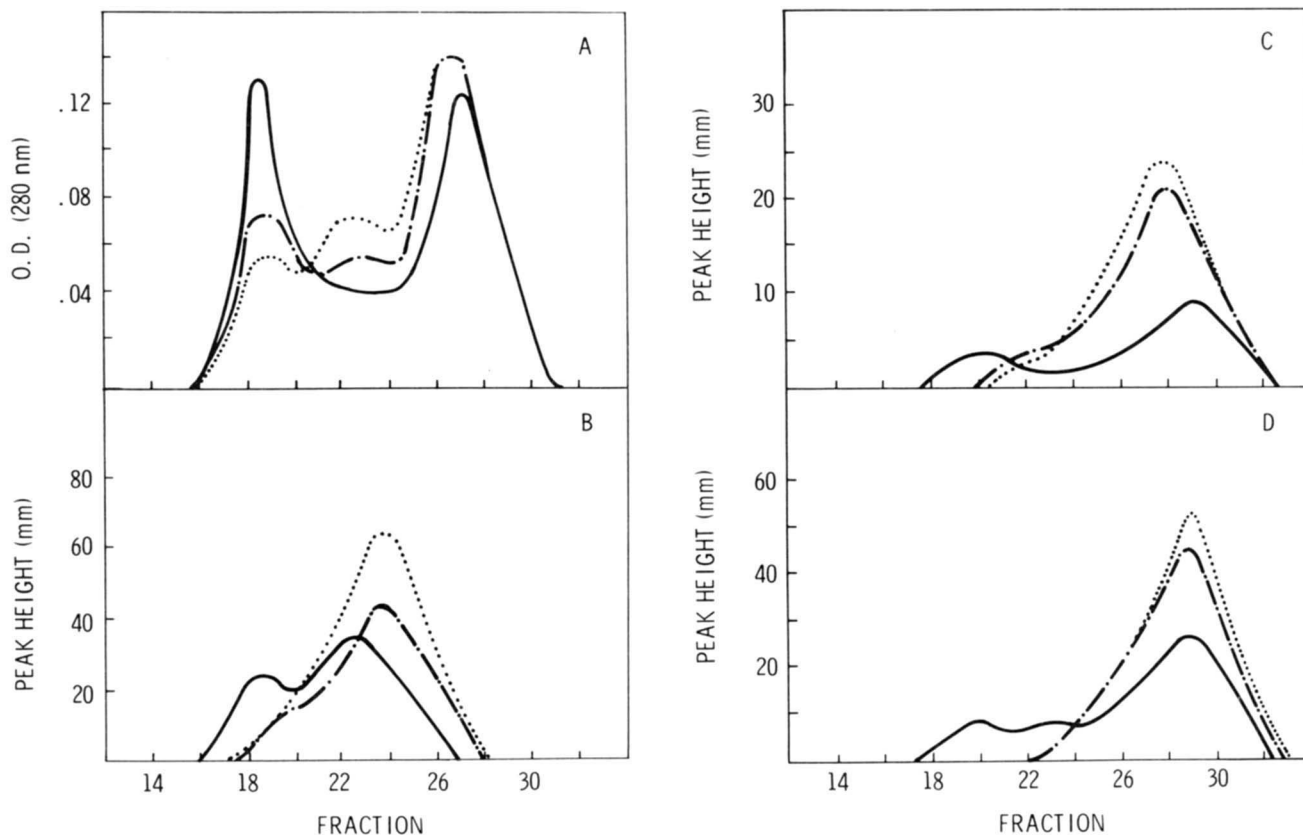


Fig. 4. Distribution of the apolipoproteins among the $d < 1.21$ g/ml lipoproteins separated by agarose gel chromatography, obtained from control (—), RTx(- - -) and PTU(· · · · ·) treated rats. (A) Total protein elution profile. The locations of the various lipoproteins were identified by chromatography of ultracentrifugally purified VLDL, LDL, and HDL. All lipoprotein fractions were obtained from 5 ml of plasma and were concentrated to 2 ml prior to chromatography. The elution profiles are thus directly comparable and reflect the relative differences in lipoprotein concentrations in the three groups of animals. The elution profiles of apo B (B), apo E (C), and apo A-I (D) are also shown and were obtained as described and shown in Fig. 1. Peak heights refer to the heights of the rockets obtained by EIA. Arbitrary units (AU) represent the percent concentration of a given apolipoprotein in the sample, when compared to a pooled reference plasma.

treated animals, such analyses were also done on samples obtained from the RTx-treated animals. The resulting patterns were very similar to those obtained from the PTU-treated animals, except that the apparent increases in apo E were not as great.

DISCUSSION

In order to investigate the influence of thyroid hormones on the lipoprotein pattern of the rat, our approach was to block the hormone-producing organ so that the effect of the absence of the hormones could be first established. It is possible that in the hypothyroid state the drastic reduction of circulating thyroid hormone concentrations may result in changes in the concentration of or tissue sensitivity to other hormones in plasma. Changes secondary to these effects cannot presently be ruled out and the reported observations are therefore considered to be

the overall result of the hypothyroid state. The data indicate that hypothyroidism induced by both radiothyroidectomy or PTU administration resulted in

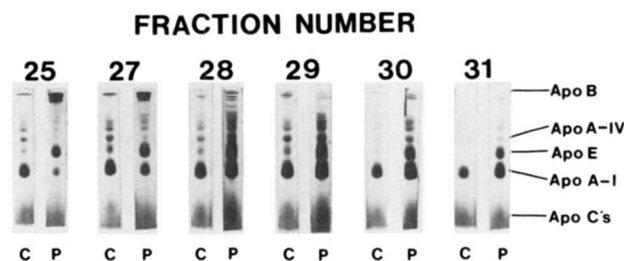


Fig. 5. Comparison of the apolipoprotein composition of the indicated column fractions for control (C) and PTU-treated (P) rats by SDS-PAGE. The lipoprotein fractions were obtained from 5 ml of plasma. Equal aliquots of each fraction tube were applied to the SDS polyacrylamide gels, even if in some cases this resulted in overloading. The apolipoprotein composition of and concentration in each fraction are therefore directly comparable and reflect the differences in total lipoprotein protein content.

qualitatively similar changes in the lipoprotein concentration and composition. The more pronounced effects of PTU administration on most of the parameters measured may be due to a greater depression of the circulating thyroid hormone levels in these animals (Table 1) due to the additional action of PTU in blocking peripheral T_4 conversion to T_3 .

To detect changes in the different physicochemical properties of lipoproteins induced by hypothyroidism, several methods of lipoprotein isolation were simultaneously used: agarose electrophoresis, agarose gel chromatography, and ultracentrifugation. It should be emphasized that these methods are complementary and comparable, but not equivalent. Thus, for instance, increased concentrations of HDL were demonstrated by all three methods, but analysis of data obtained by agarose gel chromatography indicated, in addition, an increase in the mean HDL diameter (Fig. 4A) and changes in the apolipoprotein composition of HDL particles of different sizes (Fig. 5). Whenever possible, independent analytical methods were also used when measuring plasma apolipoprotein concentrations. Quantitative changes in apolipoprotein concentrations determined by EIA were confirmed by scanning of the SDS polyacrylamide gels or, in the case of apo B, by the method of TMU-precipitation (22). In all cases good agreement has been found between the different methods of apolipoprotein estimation.

In the hypothyroid rats, plasma cholesterol concentrations increased while triglyceride concentrations decreased (Table 1). These observations are consistent with the findings of decreased plasma VLDL and increased LDL and HDL concentrations. While it is possible that the decreased plasma triglyceride levels found in the hypothyroid rats were partly due to their decreased food intake, it should be pointed out that at the time of killing (after an overnight fast), most of the circulating triglyceride was of endogenous origin, thus minimizing the effect of decreased food intake. The rates of triglyceride secretion may be altered, but the evidence for this is conflicting. Increased triglyceride secretion by perfused livers obtained from PTU-treated rats when compared to livers from pair-fed controls was reported by Keyes and Heimberg (11), while no change was observed by Kris-Etherton and Cooper (29). On the other hand, studies in our laboratory indicate that hypothyroidism induces an overall increase in the peripheral LPL activity of the rat¹, a phe-

¹ Dory, L., M-Y. Chuang, and P. S. Roheim. Unpublished observations.

nomenon possibly responsible for the decreased plasma triglyceride concentrations.


A major observation of significantly increased LDL levels is consistent with a reduction in the peripheral or hepatic removal of the products of VLDL catabolism by LDL, i.e., VLDL remnants and LDL. Data obtained with cultured human fibroblasts in the presence of T_3 demonstrated an increased rate of ¹²⁵I-labeled LDL uptake (30). This observation, and the observation of increased concentrations and decreased turnover of LDL in hypothyroid humans (6) and monkeys (2, 7) are consistent with the hypothesis that the increased levels of LDL are, at least partially, the result of decreased peripheral catabolism. Increased plasma LDL concentrations may also be due to a direct synthesis and secretion by the liver of the hypothyroid animal, and the present studies cannot exclude this possibility.

The appearance of β -VLDL in the plasma of hypothyroid rats is an important observation, especially in light of recent findings of Goldstein et al. (31) that β -VLDL is the only known naturally occurring lipoprotein species taken up by macrophages by a receptor-mediated process, leading to cholesteryl ester accumulation. We and others (32, 33) have previously shown that catabolism of VLDL results in a production of remnants of varying hydrated densities, relatively rich in cholesterol and apo E and depleted of apo C, having an altered electrophoretic mobility. The presence of β -VLDL, as well as increased apo E, cholesterol, and IDL concentrations, may therefore indicate a reduction in the rate of removal of VLDL remnants. Direct synthesis and secretion of β -VLDL by the livers of hypothyroid rats cannot be ruled out, however, and the origin of β -VLDL in these experiments needs further clarification.

The extended residency of the VLDL catabolic products in the circulation may, at least partially, account for the appearance of smaller size apo B-containing particles in the LDL range (Fig. 4B). Similarly, increased production of "surface" remnants via VLDL catabolism (34, 35) may contribute to the increased concentrations of the HDL observed in the hypothyroid rats.

Changes in plasma apolipoprotein concentration and distribution in hypothyroid rats are in agreement with the changes in the concentration and distribution of lipoproteins and generally support our interpretation of the data. With the exception of apo A-IV, the concentration of all other plasma apolipoproteins increased significantly. Significant changes in apolipoprotein distribution also occurred.

A decrease of apo B in the VLDL fraction, was accompanied by an increase in the IDL and LDL range (Figs. 3A and 4B), in agreement with our findings on whole lipoproteins. In addition, as previously discussed, analysis of data obtained by gel filtration suggested that most of the increase in apo B concentration was localized to the smaller-sized LDL (Fig. 4B). The greatest increase in apo E concentration was found in HDL of larger size, and to a lesser extent in LDL and IDL. A shift of the apo E-containing HDL particles and also the apo C-containing particles (not shown) towards a larger particle size, as indicated by gel filtration, is of interest, since such a shift did not occur in the distribution of the apo A-I despite a large increase in the concentration of this apolipoprotein (Fig. 4D). When the apolipoprotein composition of the individual lipoprotein fractions isolated by column chromatography was analyzed by SDS-PAGE, apo E was the major component of the larger "HDL" fraction. The apolipoprotein composition of this lipoprotein suggests the appearance of HDL_c, a lipoprotein previously found only in rats fed large amounts of fat and cholesterol, in addition to PTU (15). The cholesterol-rich HDL_c may therefore appear in the circulation, even in the absence of a large exogenous cholesterol load. Alternatively, apo E may accumulate in this region as a surface component product shown to arise from VLDL catabolism (38).

Our findings of increased plasma concentrations of apo E, IDL, and LDL apo B and the appearance of apo E-rich HDL and β -VLDL, are similar to those obtained in rats fed a high cholesterol and PTU-supplemented diet (15, 16). Such findings not only emphasize the need to differentiate the effects of hypothyroidism from those of cholesterol feeding, but also point to the potential importance of thyroid hormones in regulating lipoprotein and apolipoprotein metabolism. The data presented are consistent with the hypothesis that hypothyroidism in the rat causes a significant increase in the plasma concentrations of VLDL remnants, possibly through increased VLDL catabolism and/or a marked reduction in their clearance from the plasma. To test this hypothesis, studies of VLDL and LDL turnover will be necessary. 

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