Chronic Hypothyroidism Induces Abnormal Structure of High-Density Lipoproteins and Impaired Kinetics of Apolipoprotein A-I in the Rat

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Abnormal levels of plasma high-density lipoproteins (HDL) commonly reflect altered metabolism of the major HDL–apolipoprotein A-I (apo A-I). It is well known that thyroid hormones are involved in the regulation of lipoprotein metabolism, inducing significant changes in the concentration, size, and composition of plasma HDL. The purpose of this study was to evaluate the mechanisms responsible of the decreased HDL–apo A-I in chronic thyroidectomized rats (Htx) and to assess the role of HDL structure in apo A-I turnover. Htx rats were found to have a 3-fold increase in low-density lipoprotein-cholesterol (LDL-C), whereas HDL-C and apo A-I showed a 25.9% and 22.6% decrease compared to controls ($P < .05$), thus suggesting a defect in HDL metabolism. Turnover studies of apo A-I incorporated into normal HDL, using exogenous ^{125}I-radiolabeling, confirmed an altered fractional catabolic rate (FCR) in Htx rats (0.097 ± 0.009 d^{-1} *v* 0.154 ± 0.026 d^{-1} for Htx and control rats, respectively, $P < .005$). Apo A-I production rates calculated with autologous HDL data showed that apo A-I synthesis was decreased to a higher extent than the already reduced apo A-I catabolism, thus explaining the low apo A-I plasma levels in Htx rats. Composition analysis of HDL-Htx revealed increased phospholipid and apo E content, whereas apo A-IV was diminished. Such structural changes contribute to the reduced apo A-I catabolism as demonstrated with further kinetic turnover studies in normal rats treated with ^{125}I-radiolabeled apo A-I reincorporated into HDL isolated from plasma of Htx rats (FCR, 0.102 ± 0.017 *v* 0.154 ± 0.026 d^{-1}, for Htx and normal rats, respectively, $P < .005$). In summary, chronic hypothyroidism in rat a species that lacks cholesteryl ester transfer protein (CETP) activity is characterized by low HDL-C and apo A-I plasma levels as a result of a low apo A-I production rate that exceeds a decreased FCR. Both structural abnormalities of HDL and changes induced in the animal that affect HDL catabolism contribute to the low FCR of apo A-I in the hypothyroid state.

HIGH-DENSITY lipoprotein (HDL) and its major protein, apolipoprotein (apo) A-I play an important role in reverse cholesterol transport (RCT)[1] that explains their negative correlation with the incidence of atherosclerotic heart disease.[2,3] Considerable heterogeneity in both size and composition has been observed within HDL, some as the result of changes in lipid composition mediated by lipid-modifying enzymes and exchange proteins, others due to the variable composition of apolipoprotein distribution. The latter has a functional meaning; ie, small apo A-I– or apo E-containing HDL particles have been described as the initial cholesterol acceptors in the RCT to the liver.[4,5]

Low plasma levels of HDL may be the result of an abnormal biosynthesis of apo A-I.[6] In this context, it has been demonstrated that thyroid hormones upregulate apo A-I biosynthesis in vitro[7-10]; animals treated with L-triiodothyronine or thyromimetic compounds increase apo A-I expression.[11,12]. In contrast, thyroid hormone deficiency lowers intracellular apo A-I mRNA in rats,[13] and decreases HDL binding capacity in isolated rat liver cells, which may contribute to the reduced HDL clearance in hypothyroid animals.[14]

Despite of the role of thyroid hormones in the upregulation of the apo A-I gene, HDL-cholesterol (HDL-C) plasma levels are normal[15-17] or even increased,[18,19] in human hypothyroidism. In addition, thyroxine (T_4) therapy does not seem to increase serum HDL-C.[20] Low cholesteryl ester transfer protein (CETP) activities present in human hypothyroidism[21,22] provide an explanation to these paradoxical results. Low CETP activities induce cholesterol accumulation in HDL particles that results in high HDL-C plasma levels.[23,24] The latter probably counterbalance the decreased synthesis of apo A-I induced by hypothyroid status, as it has been demonstrated in vitro,[10,13] and consequently HDL-C plasma levels become normal or increased in hypothyroid patients.[15-19] This suggests that HDL metabolism is altered in hypothyroid patients, even if HDL-C plasma levels are normal.

HDL-C plasma levels are affected by a delayed HDL–apolipoprotein catabolism,[25] leading to an impaired RCT associated with an increased risk of coronary heart disease.[24] Lipoprotein structure is one factor affecting HDL–apolipoprotein catabolism.[26-29] Since structural changes in HDL subfractions have been described in hypothyroid status,[10,22] an altered HDL–apo A-I turnover could be expected in hypothyroid patients.

Because little is known about the apo A-I kinetic behavior in the hypothyroid status, the aim of this study was to evaluate the mechanisms responsible of the decreased HDL–apo A-I in chronic hypothyroidism, and to assess the role of HDL structure in apo A-I turnover. To avoid the secondary effects of low CETP activities on HDL metabolism on hypothyroid status, we selected the rat for our studies because this species does not express CETP,[30] and thus the direct effects of hypothyroidism on lipid profile and HDL metabolism could be determined. Our results demonstrated that chronic hypothyroidism is associated with an impaired HDL metabolism expressed by low HDL-C

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and apo A-I plasma levels, and that the modifications in the chemical composition of HDL induced by chronic hypothyroidism are associated to apo A-I catabolic rates.

MATERIALS AND METHODS

Induction of Hypothyroidism

Male Wistar rats weighing 250 to 300 g underwent surgical thyroidectomy with parathyroid reimplant, as previously described.[31] Briefly, under ether anesthesia, the trachea was exposed, and the parathyroid glands were visualized, dissected from the thyroid gland, and reimplanted into the surrounding neck muscles. The thyroid gland was then carefully dissected, to avoid injury to the laryngeal nerves, and completely excised. The effectiveness of this procedure was assessed by evaluation of serum calcium, phosphate, and T_4 concentrations in 10 sham rats (Ctrl) and 10 thyroidectomized (Htx) rats using standard techniques (calcium, 10.1± 0.7 Ctrl *v* 9.7 ± 0.7 mg/dL Htx, P = not significant [NS]; phosphate, 6.9 ±0.4 Ctrl *v* 6.5 ± 1.1 mg/dL Htx, P = NS; T_4, 5.1 ± 0.3 Ctrl *v* 1.3 ± 0.2 μg/mL Htx, $P < .05$; Diagnostic Products Corporation (Los Angeles, CA) radioimmunoassay [RIA]). The animals were used for further studies performed 16 weeks after thyroidectomy.

Blood Samples

Unless otherwise indicated, arterial blood from Htx and Ctrl male Wistar rats was obtained after a 12-hour fast by abdominal aortic puncture under pentobarbital anesthesia (30 mg/kg) and collected into EDTA tubes (1 mg/mL). Plasma was immediately separated by centrifugation at 4°C and conserved with 0.5 mmol/L phenylmethylsulfonyl fluoride (PMSF), 2 mmol/L benzamidine, 100 UI aprotinin, and 0.01% NaN_3. Lipoprotein fractionation was started within 2 hours of isolation.

Analytical Methods

Plasma cholesterol, triglycerides, phospholipids, and free cholesterol were determined by commercially available procedures (Menarini, Firenze, Italy; Boehringer-Mannheim, Mannheim, Germany). Lipoproteins cholesterol and triglycerides were assessed by sequential ultracentrifugation. Rat apo A-I and apo A-II were quantified by laser immunonephelometry using monoclonal antibodies. Total lipoprotein protein was estimated by a modification of the Lowry procedure.[32] Cholesteryl esters were calculated as the difference between total and free cholesterol multiplied by a factor of 1.68.[33]

Isolation and Analysis of Rat HDL

HDL were separated by ultracentrifugation in a Beckman Optima TLX table centrifuge at 110,000 rpm in 3.2-mL polycarbonate tubes. Total apo B-containing lipoproteins (density < 1.063 g/dL) were obtained after 2.16 hours, whereas total HDL (1.063 < density < 1.21 g/mL) took 2.5 hours. Under these conditions, 80% to 85% of total plasma apo A-I was recovered in the HDL fraction without apo B contamination. Unless otherwise indicated, HDL were dialyzed against 5 mmol/L ammonium bicarbonate buffer, pH 7.5.

HDL composition analysis was performed using albumin-free isolated lipoproteins in 0.09 mol/L Tris, 0.08 mol/L boric acid, 3 mmol/L EDTA buffer, pH 8.4. The homogeneity and hydrodynamic diameter of HDL was estimated by nondenaturing 5% to 30% gradient polyacrylamide gel electrophoresis using reference globular proteins (thyroglobulin, 17 nm; ferritin, 12.2 nm; catalase 10.4 nm; lactate dehydrogenase, 8.2 nm; and albumin, 7.1 nm; high molecular weight calibration kit, Pharmacia, Uppsala, Sweden).[34] Cholesterol and triglycerides in isolated HDL particles were determined as described in the analytical methods section.

Apolipoprotein composition of lyophilized HDL was analyzed by sodium dodecyl sulfate (SDS) 4% to 21% polyacrylamide gel electrophoresis (PAGE) according to the procedure used by Laemmli.[35] Similar amounts of total protein were loaded in the wells. Protein bands were visualized by staining in 0.045% Coomasie Blue R250. The relative amount of each apolipoprotein was estimated by quantifying Coomassie Blue R250 extracted from stained bands with pyridine as described previously.[29]

Rat apo A-I Purification and HDL Radiolabeling

Lyophilized HDL were delipidated with ethanol-ether 2:1 (vol:vol) and the HDL protein moiety was dissolved in 30 mmol/L Tris, 6 mol/L urea buffer, pH 8.0. Apo A-I was purified by anion-exchange chromatography using an UNO Q1 column (BioRad, Hercules, CA) coupled to a Bio-Rad Duo Flow chromatography system. Protein elution was accomplished by a linear gradient of the same buffer containing 100 mmol/L NaCl. Fractions of 250 μL were collected and those containing only apo A-I, assessed by SDS PAGE, were pooled, dialyzed against 5 mmol/L ammonium bicarbonate and lyophilized. Apo A-I concentration was estimated by spectrophotometry assuming a molar extinction coefficient of 1.13 mL · mg^{-1} · cm^{-1}. Purity was assessed to be at least 98% by scan Coomasie Blue–stained 4% to 21% PAGE.

Apo A-I radiolabeling was performed by the iodine monochloride method as modified by Bilheimer et al.[36] Briefly, 1 mg of rat apo A-I was mixed with 1 mCi of carrier-free $Na^{125}I$ and ICl to a final ICl:protein ratio of 2.5:1. Labeled apo A-I was separated from free iodine by passing the mixture over a 1.0 × 10 cm Sephadex G-25 column. Labeled ^{125}I-apo A-I was reincorporated into HDL obtained from normal, euthyroid rats (HDL-n) or from hypothyroid rats (HDL-Htx) as described elsewhere.[37] Briefly, ^{125}I-labeled apo A-I was combined with HDL (35 mg protein) and incubated for 1h at 37°C. The density of the mixture was adjusted to 1.25 with KBr and ultracentrifuged to separate the HDL from free apo A-I. By this method, about 0.35% of total HDL protein was ^{125}I-apo AI. Labeled HDL were dialyzed against sterile 0.15 mol/L NaCl solution. Virtually all of the counts in this fraction were precipitable with 10% trichloroacetic acid. The solution was sterile-filtrated using 0.22-μm filters (Millipore, Bedford, MA) and kept at 4°C until use.

Apo A-I Turnover Studies

Animals were maintained on a 12:12-hour light-dark cycle and fed Purina (St Louis, MO) rat chow throughout the study. All Ctrl animals were sham-operated. Four rat groups were included, 2 were Htx and 2 were Ctrl; 1 Htx and 1 Ctrl group received ^{125}I–apo A-I–labeled HDL-n, whereas the other 2 received ^{125}I–apo A-I–labeled HDL-Htx. Ten animals were lightly restrained with pentobarbital (15 mg/kg) and then 10 mg of HDL-n or HDL-Htx containing approximately 4×10^7 cpm of ^{125}I in a total volume of 0.3 mL was injected as a bolus into the penis vein. Blood samples of 200 μL were obtained from a tail vein 5 minutes after injection and then at 15 minutes, 30 minutes, and 1, 2, 5, 10, 15, 24, 32, 48, and 72 hours. Three 20-μL ^{125}I–apo A-I–labeled aliquots of serum were counted in a gamma counter for 1 minute. Measured gamma counts were corrected for natural radioactivity decay. ^{125}I decay curves were constructed by considering as 100%, the radioactivity in the 5-minute serum sample.

Percentages of initial radioactivity kinetics were fitted to biexponential functions using the computer program SAAM30.[38] The time length of the radiotracer turnover study allowed the use of this mathematical function in the kinetic analysis of the data. Residence times were obtained from the area under the curve and the fractional catabolic rate (FCR) is the reciprocal of the residence time. We assumed that animals were under steady-state as variances in the plasma levels of apo A-I are quite small in this type of study.[37] On the basis of this assumption, FCR equals the fractional synthetic rate (FSR). Next, apo A-I production rate (PR) by 100 g of weight was determined using the formula, PR =

(FSR × plasma apo A-I concentration × total plasma volume) × 100/(body weight). Plasma volume was assumed to be 0.04 mL/g body weight for both groups since hematocrit and total blood volume expressed as percentage of body weight in Htx are similar to Ctrl rats, as previously reported.[39,40]

Statistical Analysis

Data are expressed as the mean ± SD. Comparisons of experimental versus control groups were made using Student's *t* test. Associations between different parameters were determined by Pearson correlation analysis. Differences were considered significant at $P < .05$.

RESULTS

Effect of Chronic Hypothyroidism on Plasma Lipid and Lipoprotein Parameters

Weight, lipids, and apolipoproteins plasma levels in hypothyroid and control rat groups are reported in Table 1. The hypothyroid group had several lipid and lipoprotein alterations compared to the control group; total plasma triglycerides were slightly but significantly reduced, mainly due to a reduction of about 37% in very-low-density lipoprotein (VLDL)-triglycerides (23.4 ± 7.6 *v* 37.2 ± 11.3 mg/dL for Htx and control groups, respectively, $P < .05$). No significant alterations were observed in triglycerides of other lipoprotein fractions (results not shown). In contrast with LDL-C, which was 3 times higher in the Htx group, HDL-C levels were about 25% lower than those of control group ($P < .05$) Redistribution of cholesterol between lipoproteins induced by the hypothyroid status resulted in a total plasma cholesterol value that tended to be increased, but without reaching statistical significance. Apo A-I plasma levels in Htx decreased to a similar extent as HDL-C (~22% lower than controls). Although Htx rats were smaller compared to controls at 16 weeks of follow-up, there was no correlation between weight and LDL-C ($r = -0.721$, P = NS for Htx; $r = 0.421$, P = NS for Ctrl). Similarly, weight did not correlate with any HDL parameter, ie,. HDL-C, apo A-I, and apo A-II.

HDL Structure Analysis

To investigate the possible HDL structure changes associated to hypothyroidism, we first estimated the hydrodynamic HDL diameter by electrophoresis in native polyacrylamide gradient. Densitometric profiles of the Coomassie Blue–stained gels are presented in Fig 1. The profile obtained from control animals (Fig 1A) indicates a distribution of HDL particles with a mean Strokes diameter size of 11.0 ± 0.1 nm (Table 2). The profile obtained from hypothyroid animals (Fig 1B) indicates a shift to larger sizes, with a mean Strokes diameter of 11.4 ± 0.1 nm ($P < .05$). The rise of HDL-Htx diameter was associated with a slight but significant phospholipid enrichment (Table 2).

Further characterization of hypothyroid rat HDL involved the apolipoprotein composition. HDL–apolipoproteins were separated by 4% to 21% SDS-PAGE and stained with Coomassie Blue. Apolipoprotein proportion was assessed by spectrometry of pyridine-eluted colorant for each protein band. The results obtained with HDL-n and HDL-Htx are illustrated in Fig 2. There was a decrease, although not significant, in apo A-I content in HDL-Htx. The total amount of apo E in HDL-Htx was, on average, 89% higher than in control HDL as opposed

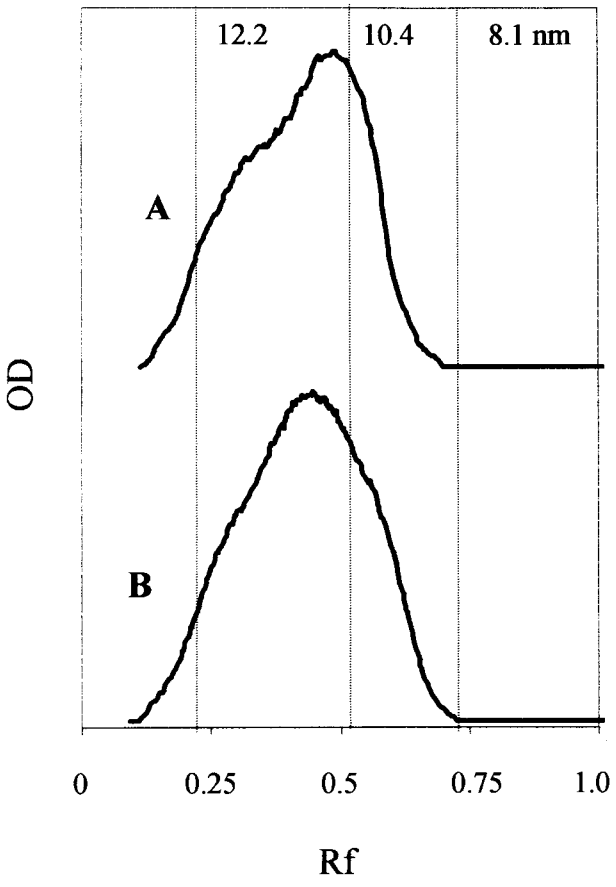

Fig 1. Gradient gel electrophoresis analysis of HDL isolated by sequential ultracentrifugation. Total HDL ($1.063 < \rho < 1.12$) were isolated and subjected to electrophoresis in polyacrylamide gradient (5% to 30%) gels for 24 hours at 120 V. The gels were then stained with Coomasie Blue and scanned using a Bio-Rad densitometer. The vertical axis represents arbitrary units of absorbance at 600 nm. Rf values shown are the distances traveled in the gel relative to lactate dehydrogenase and human albumin. The positions of standard proteins of known Strokes diameter are indicated. The profiles presented are representative results from (A) normal, and (B) Htx groups.

Table 1. Weight, Lipids, and Apolipoprotein Plasma Levels in Hypothyroid and Control Rat Groups

	Group	
	Hypothyroid (n = 11)	Control (n = 12)
Weight (g)	421 ± 28.6*	508.5 ± 49.9
Plasma triglycerides (mg/dL)	43.8 ± 8.2*	53.9 ± 12.6
Plasma cholesterol (mg/dL)	53.5 ± 14.3	47.9 ± 13.2
LDL-C (mg/dL)	24.8 ± 8.9*	8.3 ± 3.7
HDL-C (mg/dL)	22.9 ± 6.6*	30.9 ± 9.4
Apo A-I (mg/dL)	27.1 ± 2.6*	35.0 ± 6.4
Apo A-II (mg/dL)	18.3 ± 3.9	23.0 ± 11.0

NOTE. Values are mean ± SD.

*Significantly different from control ($P < .05$).

Table 2. Strokes Diameter and Mass Composition of Rat HDL

	HDL-n	HDL-Htx
Strokes diameter (nm)	11.0 ± 0.1	11.4 ± 0.1*
Free cholesterol (%)	4.3 ± 1.7	3.8 ± 0.6
Cholesterol esters (%)	33.8 ± 7.8	34.3 ± 4.0
Triglycerides (%)	2.4 ± 1.2	1.5 ± 0.7
Phospholipids (%)	22.1 ± 1.7	26.1 ± 1.9*
Proteins (%)	37.3 ± 3.4	34.3 ± 4.6

NOTE. HDL were isolated by sequential ultracentrifugation from control (HDL-n, n = 4) and chronic hypothyroid rats (HDL-Htx, n = 4), and analyzed separately. Composition is expressed as the percentage of the total dry mass. Results are expressed as mean ± SD.

*Significantly different from control ($P < .05$).

to a significant reduction (40%) in the amount of apo A-IV in HDL from hypothyroid rats.

Apo A-I Turnover Studies

Our first series of experiments was designed to determine the effect of chronic hypothyroidism on the in vivo metabolism of normal ^{125}I–apo A-I incorporated into HDL-n. Radioactivity decay curves of the mean ± SD of each time point for both groups are shown in Fig 3A. In Htx rats (n = 8), the apo A-I radioactivity decay curve had a shallower slope and a higher final radioactivity than did the mean of 8 control rats, indicating a delayed apo A-I catabolism. The calculated FCR of labeled

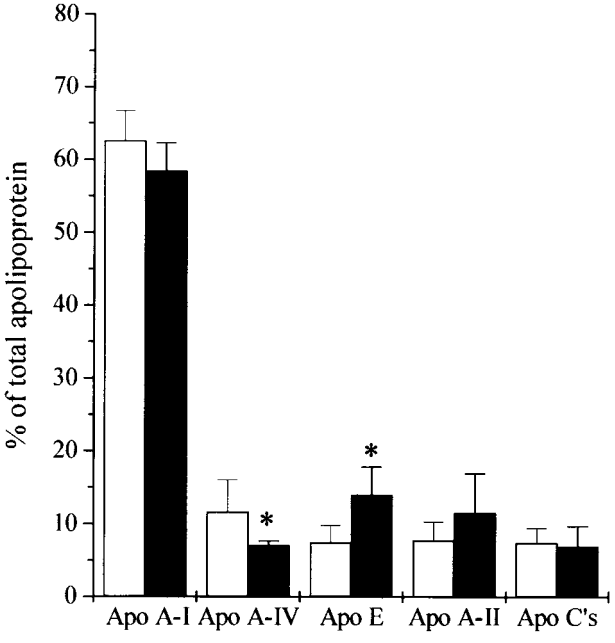

Fig 2. Percentage of apolipoprotein composition of plasma HDL-n (□) and HDL-Htx (■) isolated by sequential ultracentrifugation. HDL apolipoproteins were separated by polyacrylamide gradient gel electrophoresis in presence of sodium dodecyl sulfate. Proteins were stained with Comassie Blue and the fixed colorant was eluted in pyridine. The absorbance of the pyridine-eluted colorant at 605 nm was measured on every protein band. Protein percentage was calculated on the basis of total protein absorbance. Bars are the percentage means ± SD. *$P < .05$.

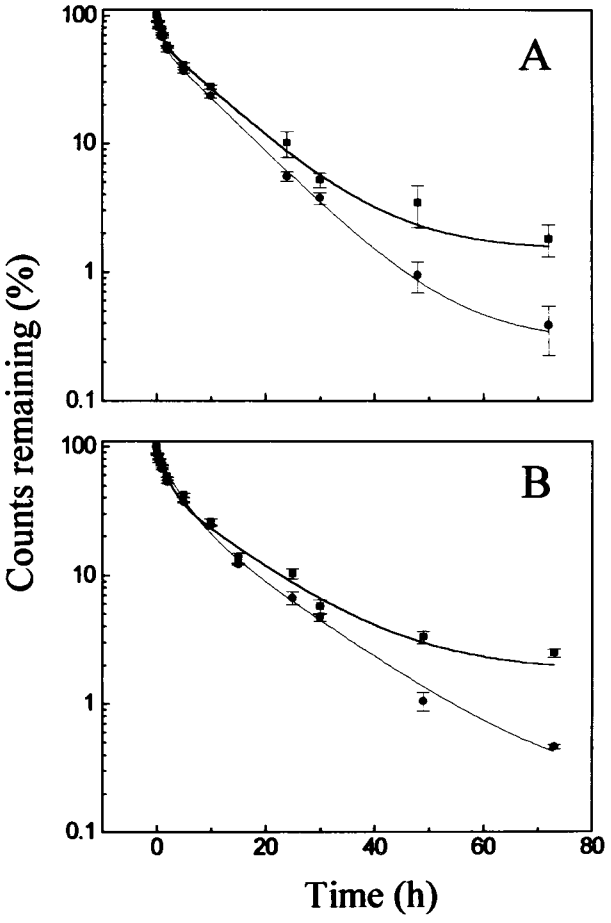

Fig 3. Plasma radioactivity decay curves of ^{125}I–apo A-I in (A) HDL-n and (B) HDL-Htx. Data are given as mean ± SD for normal (●) and Htx rats (■).

apo A-I incorporated into HDL-n was markedly lower for the Htx group (37%) than for the control group (Table 3).

Since it has been reported that hypothyroidism induces structural changes in HDL, we decided to further investigate whether these changes influence the catabolism of apo A-I as well. Therefore, our second series of turnover studies was performed with labeled apo A-I incorporated into HDL from

Table 3. Kinetic Parameters of ^{125}I–apo A-I Incorporated Into HDL-Htx or HDL-n

	^{125}I–apo A-I		
	FCR (d^{-1})		PR (mg [100 g]$^{-1}$ · d^{-1})
Recipient	HDL-n	HDL-Htx	
Hypothyroid (n = 8)	0.097 ± 0.009*	0.082 ± 0.006	0.106 ± 0.015
Control (n = 8)	0.154 ± 0.026†	0.102 ± 0.017	0.253 ± 0.047
	$P < .005$	$P < .05$	$P < .005$

NOTE. FCRs were calculated from radioactivity decay curves as described in the Methods. Production rates from data obtained with autologous HDL. Values are given as the best estimate ± SD.

*$P < .005$ for FCR between HDL-Htx and HDL-n in Htx rats.

†$P < .005$ for FCR between HDL-Htx and HDL-n in control rats.

hypothyroid rats. Our results show that during the 72 hours of the study, radioactivity decay of ^{125}I-labeled apo A-I in HDL-Htx was lower in hypothyroid rats than in control rats (Fig 3B). Thus, the calculated apo A-I FCR for the hypothyroid group was about one fifth lower than that of the control group (Table 3). Table 3 shows that the apo A-I FCRs were always, and independently from the recipient, lower when HDL-Htx were used compared to the values obtained with HDL-n. The most striking difference in apo A-I FCR (1.9-fold decrease) was observed when homologous HDL were used, ie, HDL-Htx in hypothyroid rats and HDL-n in the control group. Since apo A-I FCR was dependent of the source of HDL, and PR was calculated using the values of apo A-I FCR obtained with homologous HDL, we determined that the apo A-I PR of hypothyroid rats was 2.4-fold lower than that of the control group (Table 3).

DISCUSSION

Hypercholesterolemia is a common feature in hypothyroidism since thyroid hormones upregulate LDL-receptor expression.[41-43] High LDL-cholesterol levels were the most striking lipoprotein abnormality in our hypothyroid animal model. However, total cholesterol plasma levels remained similar to that of the control group because of the concomitant decrease in the HDL-C fraction. Thyroidectomized rats indeed had significantly lower HDL-C and apo A-I plasma levels than controls, reinforcing the effect of hypothyroid status on HDL metabolism. Nevertheless, low HDL-C is not a common finding in human hypothyroidism. One important difference between HDL metabolism in the rat and in primates is the absence in the former of CETP.[30] As a result, the rat does not transfer the cholesterol ester–laden core of HDL to VLDL, thus providing a suitable model for the study of HDL metabolism without the influence of changes in the CETP activity; it is well known that this activity has an inverse correlation with HDL-C plasma levels,[44,45] and thus low CETP activities contribute to the increase in HDL-C reported in human hypothyroidism.[21,22] In the present study using chronic Htx rats we have demonstrated an altered metabolism of HDL that results in low HDL-C and apo A-I plasma levels. It is possible that this altered HDL metabolism might be present in human hypothyroidism, the difference being that there are low CETP activities[21,22] that may mask the expected low HDL plasma levels. This hypothesis is supported by the stimulating effect of thyroid hormones on CETP plasma activity that decreases HDL-C plasma levels, recently demonstrated in vivo using transgenic human CETP mice.[46]

Since it is well known that the lack of thyroid hormones limits growth,[47] and the rats were thyroidectomized when they reached half of their maximal weight, Htx animals grew less than the controls, as expected. However, it can be argued that Htx rats were smaller than controls because of less food intake and that this may be the origin of the observed HDL alterations in the former. Hypocaloric diets frequently result in an increase of HDL-C and a decrease of LDL-C.[48] We observed an opposite situation in Htx rats, ie, low HDL-C and elevated LDL-C; therefore, we can conclude that the origin of the lipid alterations was not a low food intake. Moreover, statistical analysis was not able to show any correlation between weight and HDL parameters. Another environmental parameter that may alter the lipid profile is exercise. Even if Htx rats were more lethargic than sham controls, the small area of the cage limited physical activity in the latter. In addition, mild exercise during a period comparable to that of our experiments is not enough to modify the lipid profile.[49] Thus, increased physical activity in sham rats compared to the Htx group as the origin of the lipid abnormalities can be ruled out.

Significant changes occurred in the size and composition of plasma HDL particles in hypothyroid rats. In accordance with previous reports,[10,50,51] we found larger HDL particles in the plasma of hypothyroid rats. Associated with this, the content of apo E was significantly increased as opposed to apo A-IV, which was decreased. HDL apo E enrichment has already been observed in Htx rats[8,10] and might be secondary to the lack of thyroid hormones, since an enhanced degradation of mature apo E mRNA has been demonstrated in thyroid hormone-treated HepG2 cells.[52] Our results showed an increase in apo A-IV content. In this regard, chronic administration of triiodothyronine increases the amount of apo A-IV mRNA in the cytoplasm of rat liver cells[53]; therefore, it is possible that the lack of thyroid hormones might result in diminished apo A-IV synthesis[10] and apo A-IV–poor HDL. Another difference between HDL from Htx rats and controls was the increased phospholipid content probably due to an impaired phospholipid transfer protein (PLTP) activity associated with low HDL-C[54,55]; however, when PLTP activity was measured using an external phospholipid donor, no difference was observed between groups (data not shown). Thus, the slight HDL phospholipid enrichment in Htx rats might be attributed to the changes in apolipoprotein composition (apo E and apo A-IV).

Turnover studies performed in Htx rats demonstrated a low catabolism of labeled apo A-I incorporated into HDL-n. Since low HDL-binding capacity has been demonstrated in isolated liver cells from Htx rats,[14] it is possible that chronic hypothyroidism might affect the catabolic sites of apo A-I, contributing to the diminished clearance of HDL.

A high catabolic rate, a low synthetic rate, or both may explain the low apo A-I plasma levels in Htx rats. Turnover studies using autologous HDL, ie, HDL-Htx in Htx rats and HDL-n in a control group, revealed a 1.9-fold decrease in the catabolism of apo A-I in Htx. This low catabolism is consistent with low apo A-I plasma levels only when protein synthesis diminishes to levels below the catabolic rate. Thus, low apo A-I plasma levels in Htx rats are based on an unbalance between synthesis and catabolism, ie, a decreased apo A-I production rate (2.4-fold decrease) that exceeds the low FCR (1.9-fold decrease). Our results are consistent with a low synthesis and a low quantity of intracellular apo A-I mRNA in hypothyroid rats,[13] and with a decrease in ^{14}C-leucine incorporation into apo A-I after 2 hours of labeled aminoacid injection in propylthiouracil (PTU) hypothyroidism-induced rats.[10] Furthermore, since thyroid hormones increase the stability of nuclear apo A-I mRNA precursors,[13] the lack of such hormones would result in a low apo A-I synthesis as observed in Htx rats.

Kinetic studies performed in subjects without a history of coronary heart disease and very low levels of apo A-I[29,56,57] showed that low apo A-I plasma levels are the consequence of hypercatabolism and unaffected synthesis. These patients do

not develop atherosclerosis probably because their RCT is enhanced, ie, less HDL particles transport a normal amount of cholesterol to the liver because they do it much faster. In contrast, a decreased apo A-I catabolism could be associated with the development of coronary heart disease, as in CETP deficiency.[24] Low catabolism and normal synthesis of apo A-I in CETP-deficient patients result in high levels of HDL-C. Despite the high HDL-C levels, cholesterol accumulates in coronary arteries, which may be indicative of impaired RCT. Our study demonstrates that the lack of thyroid hormones has a primary effect on HDL metabolism, particularly decreased apo A-I synthesis and catabolism. Such a kinetic unbalance may result in an impaired RCT, enhancing the atherosclerotic effect of the concomitant high LDL-C in the hypothyroid status.

Since hypothyroidism induced changes in the HDL structure, we were concerned whether these structural variations influenced the kinetic behavior of apo A-I. We therefore performed a second series of kinetic studies reincorporating apo A-I into HDL isolated from Htx animals. In agreement with the first series of turnover studies where we used HDL-n, apo A-I FCR was decreased in Htx animals, thus reinforcing the possibility of altered catabolic sites for apo A-I. Further analysis comparing the apo A-I FCR values obtained with control rats demonstrated that apo A-I in HDL-Htx was catabolized at lower rates than in HDL-n, which strongly suggests that both HDL structure and composition influence apo A-I turnover. Similar results were obtained with the groups of Htx rats. Apo A-I in HDL-n was catabolized faster than in HDL-Htx, supporting the idea that changes in the structure of HDL induced by hypothyroid status contributes to the low apo A-I FCR. Changes in apolipoprotein size, composition, or both might account for the low turnover of HDL-Htx. Increased apo A-I FCR has been demonstrated in severe hypoalphalipoproteinemic patients possessing small HDL particles.[56,57] Moreover, large HDL detected in human CETP deficiency are associated with delayed catabolism of apo A-I and apo A-II.[25] As a consequence, one would expect low catabolism of the large HDL particles in Htx rats.

Large HDL particles are not always associated with low apo A-I catabolic rates; Lewis et al[58] demonstrated, in rats, an increased apo A-I FCR using large HDL particles rich in triglycerides. These results suggest that HDL apolipoprotein composition plays an important role on apo A-I catabolism; thus, structure may determine the efficiency of HDL in the transport of cholesterol to the liver. The main difference between Htx and HDL-n was the apolipoprotein distribution. Our results showed that HDL-Htx were enriched in apo E. Nevertheless, despite the high content of apo E, which enhances HDL clearance,[25,56,59] the low catabolic rates of apo A-I in HDL-Htx seem to be the consequence of both low content of apo A-IV, which has been associated with HDL hypercatabolism[60] and increased size of the particle. It has been suggested that the HDL alterations associated with thyroid dysfunction are in part the result of low CETP activities.[21,22] Our results using rats, a species lacking CETP activity, demonstrated that the changes in apolipoprotein composition and particle size contribute to the abnormalities in apo A-I turnover, which may impact RCT, thus enhancing the atherosclerotic process frequently associated with hypothyroidism.

In summary, this study demonstrates that chronic hypothyroidism in the rat, an animal that lacks CETP activity, is characterized by low HDL-C and apo A-I plasma levels as a consequence of the low apo A-I synthesis rate that exceeds the low FCR. Moreover, slight structural modifications of HDL and changes induced in the animals that affect HDL catabolism significantly contributed to the decrease in apo A-I catabolism in the hypothyroid state. These results raise the possibility that an impaired HDL metabolism may exist in human hypothyroidism, even in the absence of altered HDL or apo A-I plasma levels. Whether these changes affect cholesterol transport from peripheral cells to the liver and contribute to the atherosclerotic process frequently present in hypothyroid patients remains to be elucidated.

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