Thyroid Hormone Increases Plasma Cholesteryl Ester Transfer Protein Activity and Plasma High-Density Lipoprotein Removal Rate in Transgenic Mice

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Thyroid dysfunction produces multiple alterations in plasma lipoprotein levels, including high-density lipoprotein (HDL). Cholesteryl ester transfer protein (CETP) and hepatic lipase (HL) are important proteins that modulate the metabolism of HDL. Thus, the effect of thyroid hormone on the activities of CETP and of HL was investigated using hypothyroid and hyperthyroid CETP transgenic (Tg) and nontransgenic (nTg) mice. Hyperthyroid Tg mice plasma lipoprotein (LP) profile analysis showed a significant increase in the very–low-density lipoprotein (VLDL) fraction ($P < .001$) and decrease in the HDL fraction ($P < .005$), whereas in the hypothyroid Tg mice an increase in low-density lipoprotein (LDL) was observed ($P < .02$). CETP activity was measured as the transfer of ^{14}C-cholesteryl ester (CE) from labeled HDL to LDL by an isotopic assay indicative of mass. Hyperthyroid Tg mice had twice as much plasma CETP activity as compared with their controls, while in hypothyroid Tg mice plasma CETP activity did not change. The role of CETP in determining the changes in LP profile of hyperthyroid animals was confirmed by showing that nTg wild-type hyperthyroid and euthyroid mice exhibited the same percent cholesterol distribution in LP. Postheparin HL activity measured in hyperthyroid Tg mice was significantly reduced ($P < .05$). ^{3}H-cholesteryl oleoyl ether (^{3}H-Cet)-HDL plasma fractional removal rate (FRR) was approximately 2-fold faster in the hyperthyroid Tg mice than in controls, but was not modified in hypothyroid animals. Tissue uptake of ^{3}H-Cet was examined in 10 tissue samples: levels were significantly increased in skeletal muscle and decreased in small intestine in hyperthyroid Tg mice, and decreased in the small intestine of hypothyroid Tg mice. In conclusion, the excess of thyroid hormone accelerates HDL metabolism in CETP transgenic mice mainly due to an increase in plasma CETP activity and independently from the HL activity. Hypothyroid status did not change CETP activity and HDL metabolism.

THYROID DYSFUNCTION is known to produce many alterations in lipoprotein metabolism. Hypothyroidism is generally associated with hypercholesterolemia mainly due to increased concentration of low-density lipoprotein (LDL).[1,2] This may contribute to premature development of atherosclerosis in untreated hypothyroid patients.[3,4] On the other hand, hyperthyroid patients tend to have low levels of very–low-density lipoprotein (VLDL)-, LDL-, and high-density lipoprotein (HDL)-cholesterol.[5-7] The changes in LDL levels have been explained by thyroid hormone modulation of LDL receptor activity.[8-10] However, the effects of thyroid hormones on HDL metabolism are less well understood. HDL-cholesterol has been reported to be either increased,[6,11,12] normal,[2,13,14] or even decreased[15] in hypothyroidism. Studies in hyperthyroid subjects showed that the plasma HDL-cholesterol concentration is reduced[5,6] or normal.[16] In contrast, experimental hyperthyroid rats exhibit hyperalphalipoproteinemia.[17-19]

Thyroid hormones may regulate HDL by altering the cholesteryl ester transfer protein (CETP) and/or the hepatic lipase (HL) activities. These proteins modify HDL composition and correlate inversely with the plasma HDL-cholesterol level. CETP facilitates the transfer and exchange of cholesteryl ester (CE), triacylglycerol, and phospholipids between lipoproteins, which results in decreased HDL-cholesterol levels, CE content, and size.[20] CETP action on lipoproteins may have an impact on the development of atherosclerosis as illustrated by its excess and deficiency states. Overexpression of simian CETP in transgenic (Tg) mice caused severe diet-induced atherosclerosis.[21] On the other hand, recent epidemiologic evidence has associated human genetic CETP deficiency with a higher incidence of coronary artery disease despite increased HDL-cholesterol levels.[22] Recent studies suggest that CETP activity is increased in hyperthyroid[23] and decreased in hypothyroid subjects,[23,24] while its concentration is unchanged in hypothyroidism.[25]

HL hydrolyses the triacylglycerol in chylomicron remnants, intermediate-density lipoprotein (IDL), and HDL, as well as the phospholipids in HDL.[26] In addition, HL has been implicated in the interaction of lipoproteins with cell receptors and/or proteoglycans, as well as in modulating aortic lesion development in different animal models.[27] Like CETP, HL activity has been reported to be increased in hyperthyroid[28] and decreased in hypothyroid subjects.[1,2,28,29]

In the present study, we investigated the effects of the excess and deficiency of the thyroid hormones in modulating plasma CETP activity and how the latter modifies the metabolism of HDL. Tg mice expressing human CETP were used since wild-type mice do not express endogenous CETP. The CETP transgene introduced into the genome of these mice encompasses the native promoter and regulatory sequences that flank the CETP gene upstream and downstream. These mice exhibit physiologic levels of human CETP in plasma, as well as an authentic pattern of CETP tissue expression.[30] This animal model has been very useful to study the regulation of the CETP gene expression.[30-33] The present study shows that the excess of thyroid hormones stimulated plasma CETP activity, increased the intravascular removal rate of HDL-CE, and decreased the plasma level of HDL-cholesterol. These effects

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Submitted February 3, 2000; accepted November 16, 2000.

Supported by Fundação de Amparo à Pesquisa do Estado de S. Paulo (FAPESP) 96/10373-1, FINEP/PRONEX 134/97, and CNPq 520275/97-7.

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0026-0495/01/5005-0002$35.00/0

doi:10.1053/meta.2001.22514

were independent from the HL activity because the latter was reduced in hyperthyroid CETP Tg mice. Contrary to hyperthyroidism, the hypothyroid status did not modify the CETP activity and the metabolism of HDL. These results show that modifications of the metabolism of HDL by excess thyroid hormone is largely dependent on the activity of CETP.

MATERIALS AND METHODS

Animals

Experiments on animals were approved by the university's ethics committee and conformed with *the Guidelines on the Handling and Training of Laboratory Animals* published by the Universities Federation for Animal Welfare, 1992, UK.

The animals used in this study have been described elsewhere[30] and derived from the colony of Dr A.R. Tall's laboratory (Columbia University, New York, NY). Animals were maintained in a temperature-controlled room with alternating 12-hour periods of light and dark and had free access to food (rodent chow diet; Nuvital CR1, Paraná, Brazil) and water. Male and female heterozygous mice expressing human CETP (line 5203), from 8 to 11 months of age, were divided in 4 groups. Hyperthyroid animals were treated with daily intraperitoneal injections of triiodothyronine (T_3; Sigma, St Louis, MO), 250 μg/kg body weight during 7 consecutive days.[34] The same volume of saline was injected into control euthyroid mice. Hypothyroid CETP Tg mice were treated with 0.10% propylthiouracil (PTU; Sigma) in their drinking water over 3 weeks.[35,36] Their controls drank plain water. Control wild-type mice (C57Bl6 background) purchased from the State University of Campinas Animal Center (CEMIB/UNICAMP, São Paulo, Brazil) were also treated with T_3 or saline as described above.

Biochemical Analysis

Mouse plasma was isolated from blood collected from the retro-orbital plexus with heparinized hematocrit tubes. Total cholesterol, triacylglycerol, and nonesterified fatty acid levels were determined using enzymatic colorimetric assays (Wako Chemical, Neuss, Germany). Urea, creatinine, alanine aminotransferase, aspartate aminotransferase, gamma glutamyltransferase, and alkaline phosphatase were measured in a Mega automatic analyzer using Merck Diagnostic (Darmstadt, Germany) reagents.

Fast Protein Liquid Chromatography

Pooled plasma (250 μL) from treated and control mice was fractionated on a Superose 6 column (Pharmacia Biotech, Uppsala, Sweden) using tris-buffered saline, pH 7.2, as previously described.[37] Cholesterol was determined enzymatically in a Cobas automatic analyzer (Hoffman-La Roche, Basileia, Switzerland) using Boehringer Mannheim reagents (Mannheim, Germany).

HDL Labeling

The plasma fraction of density (d) higher than 1.125 g/mL was isolated from a pool of normolipidemic human blood donors by ultracentrifugation.[14] C-cholesteryl oleate (50 μCi) or ^{3}H-cholesteryl oleoyl ether (^{3}H-Cet; Amersham Life Science, Buckinghamshire, England) dissolved in ethanol (100 μL) was added dropwise to the d $>$ 1.125 plasma fraction with gentle mixing and incubated at 37°C for 20 hours in a tube rotator. After adjustment to d = 1.21 g/mL, the labeled HDL_3 was isolated by spinning for 40 hours at 40,000 rpm at 4°C on a 50-Ti rotor in a L8 Beckman ultracentrifuge (Beckman Instruments, Palo Alto, CA). Labeled HDL_3 was purified by another 40-hour ultracentrifugation run. ^{3}H- or ^{14}C-HDL fractions were dialyzed against saline-EDTA, sterilized in a 0.22-μm Millipore (Bedford, MA) filter, sprayed with nitrogen, and stored at 4°C.

HDL Kinetics and Tissue Uptake Studies

Mice were injected intraperitoneally with 10^6 dpm of HDL labeled with ^{3}H-Cet. Blood (50 μL) was taken from the tail at 0.5, 1, 2, 3, 4, 6, 8, and 24 hours for determination of radioactivity in plasma. Animals had free access to food and water during the experiment. Twenty four hours after ^{3}H-HDL injection, mice were anesthetized with a mixture of 16 mg/kg of Rompun (Bayer, São Paulo, Brazil) and 50 mg/kg of Vetanarcol (Konig, Avellaneda, Argentina) intraperitoneally, blood was drawn from the retro-orbital plexus, organs were perfused with 10 mL of saline solution through the right ventricle and section of the distal abdominal aorta, and 10 tissues were excised: liver, small intestine, spleen, lungs, heart, kidneys, adrenals, testis or ovaries, adipose tissue, and leg skeletal muscle. The fractional removal rates (FRRs) were calculated as the slope of the disappearance curves of ^{3}H-Cet in whole plasma from peak radioactivity (1 or 2 hours) until 8 hours after ^{3}H-HDL injection using nonlinear least-squares curve fitting. Total tissue lipid was extracted[38] and radioactivity was measured by liquid scintillation in a LS6000 Beckman Beta Counter.

CETP Activity Assay

One hundred micrograms of a mixture of human VLDL and LDL protein were incubated with 10,000 dpm of human HDL_3 labeled with [^{14}C]-CE and 5 μL of diluted CETP Tg mice plasma as the source of CETP in a final volume of 100 μL. Blanks were prepared with tris/saline/EDTA buffer (10 mmol/L, 140 mmol/L, 1 mmol/L), pH 7.4, and negative controls with nontransgenic (nTg) mice plasma. The incubations were carried out at 40°C for 1 hour. After these periods, the apolipoprotein B–containing lipoproteins were precipitated with a 1.6% dextran sulfate/1 mol/L $MgCl_2$ solution (1:1) and the radioactivity measured in the supernatant with scintillation solution Ultima Gold (Eastman Kodak, Rochester, NY) in a LS6000 Beckman Beta Counter. The percent CE transferred from [^{14}C]-CE-HDL to VLDL+LDL was calculated as the subtraction between dpm remaining in the blank tube and dpm remaining in the sample supernatant divided by dpm remaining in the blank tube and multiplied by 100.

HL Activity

HL was determined according to Ehnholm and Kuusi.[39] Briefly, plasma samples from mice fasted overnight, collected pre (basal) and 10 minutes after heparin intraperitoneal injection (100 U/kg body weight), were incubated with a ^{3}H-triolein/arabic gum substrate ([9,10 ^{3}H (N)]-triolein; New England Nuclear, Boston, MA), 2 mol/L NaCl, pH 8.5, 37°C, during 1 hour. The hydrolyzed labeled free fatty acids were extracted with methanol/chloroform/heptane (1.4:1.25:1), pH 10.5, dried under N_2, and their radioactivity determined with a liquid scintillation solution in a LS6000 Beckman Beta Counter.

RESULTS

CETP Tg mice were made hypothyroid by treatment with 0.10% PTU in their drinking water for 3 weeks, or hyperthyroid by daily intraperitoneal injections of T_3 ($\sim$7.5 μg) during 7 consecutive days. The efficacy of the treatments was confirmed by measuring plasma levels of thyroxine, which were suppressed by both T_3 and PTU treatments (Table 1). At the end of the PTU treatment, there was a 17% reduction of the body weight of the hypothyroid Tg mice ($P < .02$), which was the result of a decrease in the water and food intake ($P < .01$). Hyperthyroid T_3-treated Tg mice did not have their body weight modified, despite presenting a higher food and water intake during treatment ($P < .01$) (data not shown). Plasma levels of hepatic transaminases (aspartate and alanine) and urea did not change after both treatments (data not shown). The

Table 1. Nonfasting Plasma Concentration of Thyroxine, Alkaline Phosphatase, Cholesterol, Triacylglycerol, and CETP Activity in CETP Tg Mice Made Hyperthyroid (T_3) or Hypothyroid (PTU) and Their Respective Controls (saline and H_2O)

	Control (saline)	Hyperthyroid (T_3)	Control (H_2O)	Hypothyroid (PTU)
Thyroxine (μg/dL)	5.42 ± 0.8 (4)	0.79 ± 0.05 (7)†	7.9 ± 1.1 (4)	2.8 ± 0.4 (9)†
Alkaline phosphatase (U/L)	130 ± 21 (4)	380 ± 49 (4)*	185 ± 33 (7)	197 ± 18 (7)
Cholesterol (mg/dL)	95 ± 5 (4)	68 ± 3 (4)*	75 ± 7 (7)	65 ± 7 (7)
Triacylglycerol (mg/dL)	74 ± 9 (4)	236 ± 37 (4)*	147 ± 28 (7)	136 ± 25 (7)
CETP activity (% CE transfer)	25.4 ± 3.0 (7)	47.6 ± 2.1 (9)‡	30.6 ± 1.7 (9)	33.5 ± 3.8 (8)

NOTE. Data are the mean ± SE (n).

Comparison between treated and respective control groups by Mann-Whitney test: * $P < .03$, † $P < .006$, ‡ $P < .0005$.

plasma concentration of creatinine was significantly reduced ($P < .05$) in hyperthyroid Tg mice, suggesting an elevated renal clearance in these animals. A significant increase in serum alkaline phosphatase was observed in hyperthyroid Tg mice (Table 1), which is considered a clinical marker of T_3 action in liver and bone.[40] Total cholesterol and triacylglycerol concentrations in plasma were similar in PTU-treated and control Tg mice, while total cholesterol decreased and triacylglycerol and free fatty acids levels increased in T_3-treated Tg mice as compared with their controls (Tables 1 and 2).

Plasma lipoprotein profiles were obtained by fast protein liquid chromatography (FPLC) and are shown in Fig 1 (absolute values) and Fig 2 (relative values). In PTU-treated Tg mice the only observed change was a higher LDL-cholesterol level compared with control mice ($P < .02$). This finding agrees with the most consistent lipoprotein alteration known to occur in hypothyroid subjects, which is attributed to a reduction in LDL receptor number. On the other hand, CETP Tg mice responded to T_3 treatment with significant increases in their VLDL-cholesterol ($P < .001$) and decreases in their HDL-cholesterol levels ($P < .005$) (Fig 2). Considering the total cholesterol concentration in the plasma pools injected into the gel filtration column, absolute values of cholesterol (mg/dL) in VLDL, LDL, and HDL are, respectively, 18.8 ± 0.66, 29.9 ± 0.66, and 45.3 ± 0.68, for control plasma and 24.4 ± 0.74, 29.3 ± 0.84, and 39.3 ± 1.5 for the T_3-treated plasma. Interestingly, FPLC profiles show that in both cases, hyperthyroidism and hypothyroidism, VLDL and LDL fractions shifted to the left in relation to the control lipoprotein fractions, suggesting that those particles have larger sizes than the controls. Hyperthyroid animals were hypertriglyceridemic, a fact that may explains their larger apolipoprotein B–containing lipoproteins. Others have shown that thyroid status can lead to lipoprotein heterogeneity, such as an increase of lipid (total fatty acids) content of the LDL particles in hypothyroidism.[41,42]

Two plasma proteins are potent modifiers of the HDL-cholesterol plasma levels, namely, CETP and HL. An isotopic assay in which CETP activity is independent from endogenous lipoproteins was used and therefore correlates with CETP mass.[43] As shown in Table 1, plasma CETP activity in hyperthyroid mice doubled the control plasma value. No differences were found in plasma CETP activity between hypothyroid and control Tg mice.

To verify the relative role of CETP on the plasma lipoprotein response to the hyperthyroidism, nTg wild-type mice were treated with T_3 and their lipoprotein profiles were compared with those of saline-treated nTg mice. There was a marked reduction (40%) in the total cholesterol concentration of T_3-treated (39 ± 3 mg/dL, $P < .0001$) as compared with saline-treated nTg mice (67 ± 5 mg/dL). However, as opposed to the CETP Tg response, the percent cholesterol distribution among the plasma lipoproteins was not statistically different between hyperthyroid and euthyroid nTg mice. The relative values of cholesterol (%) in VLDL, LDL, and HDL were, respectively, 3.1 ± 0.8, 12.6 ± 2.2, and 84.3 ± 1.9 for control plasmas, and 7.0 ± 1.6, 9.0 ± 2.2, and 84.0 ± 3.2 for T_3-treated nTg mice (n = 4).

To determine the activity of HL, two other groups of CETP Tg mice, hyperthyroid (T_3) and control (saline), were studied in a fasting basal state and 10 minutes after heparin intraperitoneal injection (100 U/kg body weight). Results are shown in Table 2. Total triacylglycerol and nonesterified fatty acid levels were significantly elevated in hyperthyroid Tg mice, probably reflecting lower lipoprotein lipase (LPL) and higher hormone-sensitive intracellular lipase activities, respectively. There were no statistical differences between basal and postheparin HL

Table 2. Fasting Plasma Levels of Nonesterified Fatty Acids, Triacylglycerol, and Hepatic Lipase Activity in the Basal State and After Intraperitoneal Injection of Heparin (100 U/kg body weight) in Hyperthyroid (T_3) and Control (saline) CETP Tg Mice

	Control (saline)		Hyperthyroid (T_3)	
	Basal	Postheparin	Basal	Postheparin
Nonesterified fatty acids (mmol/L)	1.33 ± 0.17 (8)	1.16 ± 0.18 (8)	2.0 ± 0.21 (7)[a,b]	1.7 ± 0.22 (7)[c]
Triacylglycerol (mg/dL)	72.0 ± 10.3 (7)	72.3 ± 16.1 (7)	157.2 ± 13.6 (6)[d,e]	113.6 ± 10.1 (6)[f,g,h]
Hepatic lipase (μmol/mL/h)	1.58 ± 0.18 (8)	1.71 ± 0.22 (8)	1.16 ± 0.11 (9)[i,j]	1.22 ± 0.09 (9)[k]

NOTE. Data are the mean ± SE (n).

Mann-Whitney test: [a]$P = .02$ *v* control basal; [b]$P = .02$ *v* control postheparin; [c]$P = .04$ *v* control postheparin; [d]$P = .02$ *v* control basal; [e]$P = .01$ *v* control postheparin; [f]$P = .02$ *v* control basal; [g]$P = .03$ *v* control postheparin; [h]$P = .04$ *v* T_3 basal; [i]$P = .04$ *v* control postheparin; [j]$P = .06$ *v* control basal; [k]$P = .03$ *v* control postheparin.

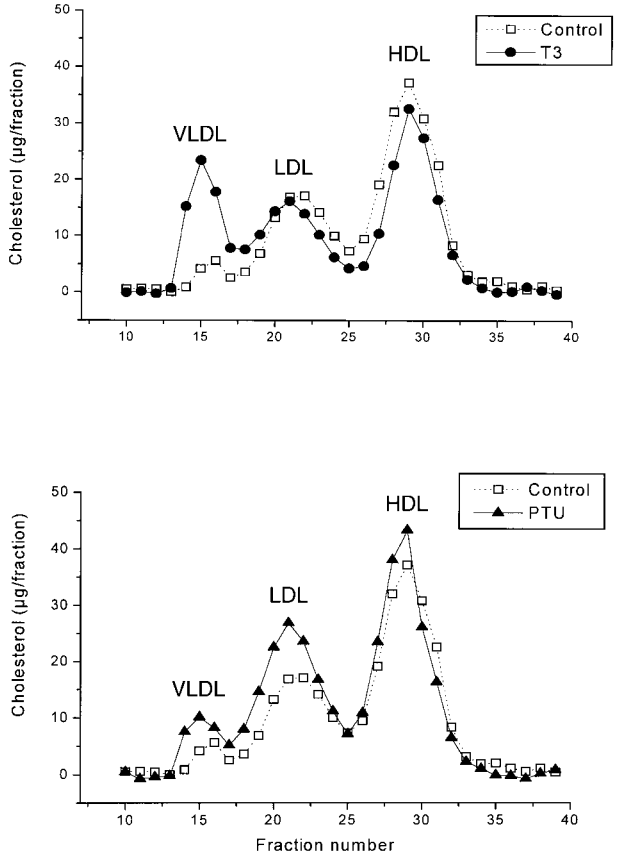

Fig 1. Plasma lipoprotein cholesterol profile of hypothyroid (PTU, n = 3), hyperthyroid (T_3, n = 3), and control (n = 6) CETP Tg mice. Fresh plasma samples were pooled, fractionated by FPLC on a Superose 6 column, and the cholesterol content of each fraction determined. The positions of VLDL, LDL, and HDL are indicated. Each point represents the mean of 3 pools of 5 animals for hypothyroid (PTU) and hyperthyroid (T_3) Tg mice groups and 6 pools of 3 to 5 animals for the control group.

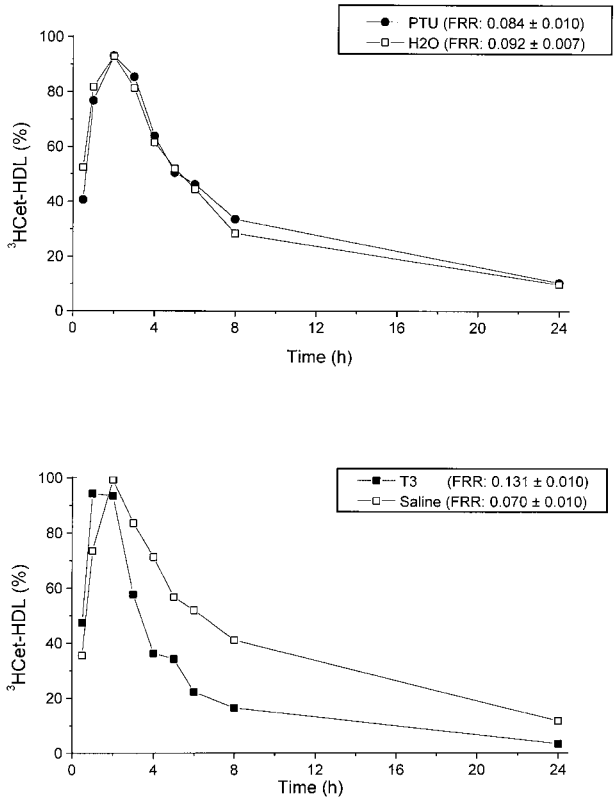

Fig 3. Kinetics of ^{3}H-cholesteryl ether (^{3}H-Cet)–labeled HDL injected intraperitoneally into hypothyroid (PTU) and hyperthyroid (T3) CETP Tg mice and their respective controls, H_2O and saline. The amounts of radioactivity in the whole plasma were determined at the indicated time after the injection. Each curve represents the average of normalized data (peak = 100%) from 6 animals per group. The mean FRR of each group is shown in the inserts and was calculated as the slopes of the disappearance curves from peak radioactivity until 8 hours after ^{3}H-Cet–HDL injection using nonlinear least-squares curve fitting. *P< .005 for FRR T_3-treated mice as compared with saline-treated animals by Mann-Whitney test.

activities within each group. Basal levels of HL activity tended to decrease (P = .06) and postheparin HL activity was significantly reduced (P < .05) in hyperthyroid as compared with control CETP Tg mice.

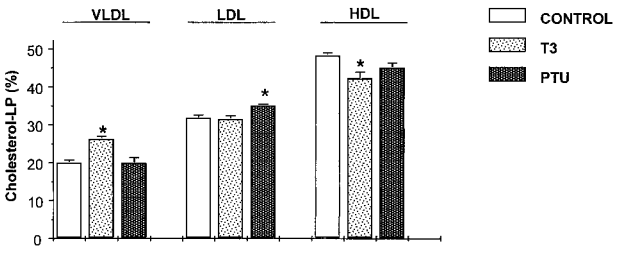

Fig 2. Percent distribution of cholesterol in plasma lipoprotein of hypothyroid (PTU, n = 3), hyperthyroid (T_3, n = 3), and control (n = 6) CETP Tg mice. Data were calculated as the area under the VLDL, LDL, and HDL peaks of the FPLC profile shown in Fig 1. *P < .001 for VLDL T_3, P < .02 for LDL PTU, and P < .004 for HDL T_3 compared with their respective control fractions, according to the Mann-Whitney test.

The plasma removal rate and tissue uptake of ^{3}H-Cet–labeled HDL were determined in hypothyroid and hyperthyroid CETP Tg mice and their respective controls. Intravascular ^{3}H-Cet-HDL metabolism was approximately 2-fold faster in hyperthyroid than in control Tg mice, as shown by their FRR, presented in Fig 3. No change was observed in ^{3}H-Cet–HDL plasma removal rates in hypothyroid Tg mice (Fig 3). ^{3}H-Cet tissue uptake was evaluated 24 hours after ^{3}H-Cet–HDL injection into the 4 groups of animals. These results are presented in Fig 4 (hyperthyroid *v* control) and Fig 5 (hypothyroid *v* control). Considering the whole organs, the main tissues that took up ^{3}H-Cet in the 4 groups of animals were liver, adipose tissue, and skeletal muscle. In hyperthyroid Tg mice, among the 10 tissues studied, there was a higher ^{3}H-Cet uptake by skeletal muscle and a lower uptake by the small intestine (Fig 4). In hypothyroid Tg mice there was a significant decrease in small intestinal ^{3}H-Cet uptake (Fig 5).

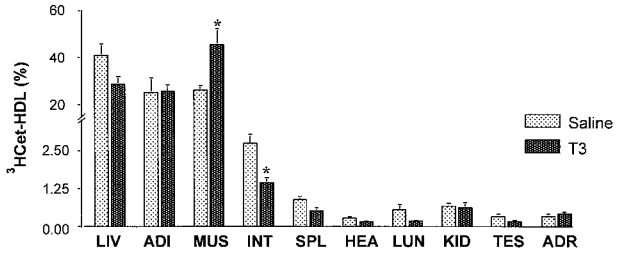

Fig 4. Percent distribution of ^{3}H-Cet in 10 organs 24 hours after intraperitoneal injection of ^{3}H-Cet–HDL into hyperthyroid (T_3) and control (saline) CETP Tg mice. Data represent the mean ± SE of 4 to 7 mice. *P < .02 for muscle and P < .01 for intestine by Mann-Whitney test. LIV, liver; ADI, adipose; MUS, skeletal muscle; INT, small intestine; SPL, spleen; HEA, heart; LUN, lungs; KID, kidneys; TES, testis; ADR, adrenals. Adipose tissue mass was estimated as 7.8% and muscle mass as 45% of the total body weight.

DISCUSSION

The present study shows that an excess of thyroid hormone but not its deficiency can significantly modify CETP and HL activities and hence HDL metabolism in CETP Tg mice.

The sole alteration in the plasma lipoprotein profile observed in the animals made hypothyroid was an increase in the LDL fraction (Figs 1 and 2). This could be due to an increase of the precursor VLDL production and/or decrease of the LDL receptor number. Since no effect was observed in the levels of VLDL-cholesterol in hypothyroidism, we conclude that the elevation of the LDL was due to the previously reported reduction in the LDL receptor number.[8-10]

Hyperthyroid Tg mice exhibited an elevation of VLDL-cholesterol levels (Fig 2). In theory, VLDL levels could be influenced by the rate of hepatic secretion, by the LPL activity and by the number of the recently identified VLDL receptors. The physiologic role of VLDL receptors has not as yet been fully understood. Jokinen et al[44] have shown that VLDL receptor number is upregulated in the muscle of hyperthyroid rats. Therefore, it is unlikely that these receptors could be involved in the increased plasma VLDL-cholesterol levels found in the T_3-treated Tg mice. In spite of not directly measuring plasma LPL activity, the increased fasting plasma triglyceride levels (Table 2) and previous studies[36,44] suggest that LPL activity may be hampered in experimental hyperthyroidism. Thus, a reduced LPL could contribute to an increase in the VLDL-cholesterol fraction.[45,46] However, the approximate 2-fold increase in CETP activity is sufficient to produce the observed shift of cholesterol from HDL to the VLDL fraction.

Additionally, the well-documented stimulation of the adipose tissue lipolysis rate that occurs in hyperthyroidism[40,47] brings on elevation of nonesterified fatty acid plasma concentration (Table 2), which by itself may stimulate the hepatic triacylglycerol synthesis and VLDL secretion.[47]

The reduction of HDL-cholesterol level found in T_3-treated CETP Tg mice is in agreement with previous studies in human subjects.[5,6,23] However, hyperthyroid rats, which do not express CETP, exhibited an increase in HDL (apolipoprotein AI and cholesterol) levels.[17-19] We demonstrated a 2-fold faster HDL-CE plasma removal rate (FRR) in hyperthyroid as compared with euthyroid CETP Tg mice. The increase in CETP activity verified in hyperthyroid CETP Tg mice (Table 1) could account for the entire effect of the thyroid hormone on the HDL-CE FRR in these animals. Thus, it is conceivable that the CETP expression may explain the differences in the response to the hormonal treatment in rats and humans, making CETP Tg mice respond to hyperthyroidism like humans (decreased HDL-cholesterol) and opposite to CETP-nonexpressing rats (increased HDL-cholesterol). To test this possibility, nTg wild-type C57Bl6 mice received the same T_3 treatment. There was a severe reduction in the total cholesterol concentration of T_3-treated nTg mice. However, contrary to what happened in the CETP Tg mice, there were no significant differences in the distribution of cholesterol among the plasma lipoprotein fractions of both nTg groups, control and hyperthyroid mice. Therefore, these results confirm the role of CETP in determining the HDL response to hyperthyroidism in CETP Tg mice.

Considering that the liver is the main site of all lipoprotein-CE catabolism and that CETP expression increases liver clearance of HDL-CE,[48,49] it is surprising that we did not find an increased liver uptake of HDL-derived Cet in hyperthyroid CETP Tg mice that presented higher HDL-Cet FRR and plasma CETP activity as compared with euthyroid CETP Tg mice. Measuring ^{3}H-Cet liver uptake 24 hours after the tracer injection was likely inappropriate because this long period of time would have allowed accumulation of ^{3}H-Cet in the liver of euthyroid mice that masked the differences that otherwise would have been observed during an earlier time period such as 4 to 8 hours after HDL injection. In fact, by comparing CETP Tg and nonexpressing mice, we have recently shown that CETP expression significantly increases HDL-CE FRR and liver uptake 6 hours after injection of the labeled HDL.[49]

HDL-derived ^{3}H-Cet muscle uptake was significantly increased in hyperthyroid CETP Tg mice. This finding seems unrelated to the number of scavenger receptor type B-I (SRBI) or to the muscle CETP activity since the expression of both proteins is not detectable in muscle.[30,50] It is probably related to the higher expression of LDL receptors in the muscle of hyperthyroid mice, which then would take up the ^{3}H-Cet transferred by CETP from HDL to apolipoprotein B-lipoproteins. However, there is no clear reason to think that the muscle uptake could explain the faster FRR of the HDL-CE measured at an earlier time (2 to 8 hours).

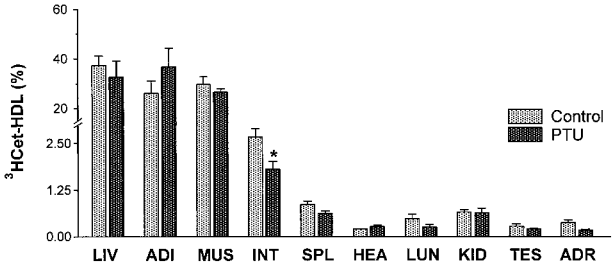

Fig 5. Percent distribution of ^{3}H-Cet in 10 organs 24 hours after intraperitoneal injection of ^{3}H-Cet–HDL into hypothyroid (PTU) and control (H_2O) CETP Tg mice. Data represent the mean ± SE of 4 to 7 mice. *P < .05 for intestine by Mann-Whitney test.

Interestingly, small intestine uptake of plasma ^{3}H-Cet was reduced in both hyperthyroid and hypothyroid states. It has previously been described that the intestinal acyl cholesterol acytyltransferase (ACAT) reaction is elevated in hyperthyroid rats.[51] Thus, we could speculate that in this state, an increased CE content and a consequent downregulation of LDL receptors in this tissue would impair plasma ^{3}H-Cet uptake. We have no explanation for the reduced intestinal ^{3}H-Cet uptake observed also in hypothyroid Tg mice.

Tan et al[28] showed that there was a strong correlation between thyroid hormone concentration and plasma CETP activity, which was increased in hyperthyroid and decreased in hypothyroid subjects. In their study and in ours, the CETP activity assay employed is indicative of CETP mass.[43] Thyroid hormone mediates several of its effects by modifying the expression of target genes.[40] For example, LDL receptor mRNA is increased by 50% in hyperthyroid rats.[9] Thyroid hormone also stimulates the transcription of apolipoprotein AI in rat liver.[52] Whether CETP activity elevation induced by thyroid hormone represents an upregulation of the CETP gene expression remains to be determined. Several thyroid response element-like sequences are found in the CETP transgene used for preparing this CETP transgenic line (Oliveira HCF, Bruce C, Tall AR, unpublished data), suggesting that a direct regulation of CETP expression by thyroid hormones may occur.

Tan et al[28] also showed that hyperthyroid subjects exhibited higher levels of HL activity and attributed the variation of HDL-cholesterol levels to modification in both CETP and HL activities. Others also have shown an increase in HL in hyperthyroid subjects and a decrease in hypothyroid subjects.[1,9] In CETP Tg mice, the HL activity was significantly reduced in hyperthyroidism, strongly indicating this enzyme is not involved in the reduction of the HDL-cholesterol levels. In spite of having the same HDL-cholesterol lowering effect in mice and humans, HL could somehow be differently regulated in these two species. Although the mechanisms that lead to alteration of HL activity in hyperthyroidism are not known, Staels et al[9] reported that thyroid status does not modify the HL gene expression (mRNA) in rats.

In summary, we have shown that the excess of thyroid hormone induces elevation of plasma CETP activity, which can explain the increase of the plasma HDL-CE FRR and decrease of HDL-cholesterol level.

Stimulated CETP action overwhelmed the effect of low HL activity in modifying the plasma levels of HDL in hyperthyroid CETP Tg mice.

ACKNOWLEDGMENT

The authors are indebted to Dr Eder C.R. Quintão for laboratory facilities and helpful suggestions, and to Dr Alan R. Tall for kindly providing the CETP transgenic founders. We are grateful to Dr Eliana C. de Faria for the plasma analysis of hepatic and renal function. We also thank Daniela C. Vidotti and Andre L. Cavallaro for excellent technical assistance and animal care.

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