Role of thyroid hormone in the expression of apolipoprotein A-IV and C-III genes in rat liver

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Abstract The genes coding for apolipoproteins A-I, C-III, and A-IV are closely linked to one another in the rat genome. Thyroid hormone stimulates apoA-I expression in rat liver by an unusual mechanism that enhances the maturation of mRNA. This hormone also increases apoA-IV mRNA abundance by a mechanism not yet studied, and its role in the expression of apoC-III has not been defined but may be of relevance to the metabolism of triglyceride-rich lipoproteins. We therefore measured the transcriptional activity of the apoA-IV and apoC-III genes and the abundance of their nuclear RNA and total cellular mRNA in livers of control rats and rats made hyper- and hypothyroid. After a single receptor-saturating dose of triiodothyronine (3 mg/100 g body weight), apoA-IV gene transcription increased at 20 min and reached a maximum of 260% of control at 6 h. Increases of transcription were reflected in increases of nuclear and total apoA-IV mRNA levels. ApoC-III gene transcription was temporarily increased to 160% at 2 h without changes in the abundance of its nuclear or total mRNA over 24 h. Lower hormone doses (20–500 μg/100 g body weight) stimulated apoA-IV mRNA transcription as well, but tended to reduce transcription from the apoC-III gene. Upon chronic administration of thyroid hormone, apoA-IV transcription decreased to 55% and nuclear apoA-IV RNA levels to 87% of control. However, total cellular apoA-IV mRNA levels increased to 279% of control, implying stabilization of mRNA in the cytoplasm. ApoC-III transcription decreased to 28% of control, but abundance of nuclear and total cellular apoC-III mRNA was reduced to a lesser extent. In hypothyroid rats, apoA-IV gene expression was decreased fourfold at the transcriptional level. In contrast, apoC-III gene transcription increased to 178% of control, but the abundance of nuclear and total cellular apoC-III mRNA did not differ from control rats. Thus, thyroid hormone affects the abundance of apoA-IV mRNA by changing its synthesis and its rate of degradation and enhances the efficiency of apoC-III mRNA maturation, thereby blunting the net effect of altered mRNA synthesis on mRNA abundance.—**Lin-Lee, Y-C., W. Strobl, S. Soyal, M. Radosavljevic, M. Song, A. M. Gotto, Jr., and W. Patsch.** Role of thyroid hormone in the expression of apolipoprotein A-IV and C-III genes in rat liver. *J. Lipid Res.* 1993. **34:** 249–259.

Supplementary key words hypothyroidism • hyperthyroidism • apoA-I gene

Apolipoproteins are structural components of plasma lipoproteins that serve as recognition sites for cellular uptake of lipoproteins and as activators of enzymes involved in lipoprotein metabolism. A number of physiological conditions have been described as altering net production of specific apolipoproteins by the intestine and liver through effects at the posttranslational (1–3), translational (4–6), posttranscriptional (7–9), or transcriptional level (9–12).

Apolipoproteins make up a multigene family that is believed to have arisen from one primordial gene (13, 14). Apolipoproteins A-I, C-III, and A-IV are of particular interest, not only because of their structural homologies, but also because the genes encoding them are closely linked within a 20-kb DNA segment in the human, rat, and mouse genome (15–17). ApoA-I and apoA-IV share several functions in lipoprotein metabolism. Both are activators of LCAT (18, 19) and have been reported to mediate the uptake of high-density lipoproteins by hepatocytes (20–22). ApoA-IV may facilitate the transfer of apoC-II to triglyceride-rich lipoproteins and thereby enhance their catabolism (23). In contrast, apoC-III is thought to reduce the catabolic rate of triglyceride-rich lipoproteins as it inhibits the activity of lipoprotein lipase (24, 25) and apoE-mediated remnant removal (26, 27). Indeed, in transgenic mice overexpression of apoC-III is associated with hypertriglyceridemia (28). Hence, the relationship between apoA-IV expression and apoC-III expression may be critical to triglyceride metabolism.

Changes in thyroid hormone status have profound effects on plasma lipid transport (29, 30) and on the

Abbreviations: PTU, propylthiouracil; T_3, triiodothyronine; VLDL, very low density lipoproteins; apo, apolipoprotein; SDS, sodium dodecyl sulfate; SSC, standard saline citrate (0.15 M NaCl, 0.015 M sodium citrate, pH 7.0); HEPES, 4-8(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PIPES, 1,4-piperazine-diethanesulfonic acid; EGTA, ethyleneglycol-bis-(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid.

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hepatic expression of the apoA-I and apoA-IV genes in the rat (30–34). However, little is known about the effects of T_3 on apoC-III gene expression. In acute hyperthyroidism, apoA-I gene transcription is enhanced (9, 12). In chronic hyperthyroidism, apoA-I gene transcription is reduced but apoA-I mRNA abundance is increased threefold by an unusual mechanism that involves stabilization and/or more efficient processing of nuclear apoA-I mRNA precursors (9). Also in chronic hyperthyroidism, apoA-IV production is augmented, which can be accounted for by increased apoA-IV mRNA abundance (30, 33). The relationship among apoA-IV transcription, abundance of nuclear precursors, and abundance of cytoplasmic mRNA in chronic hyperthyroidism has not been studied. Conceivably, similar mechanisms could be involved in the hormonal stimulation of the apoA-I and apoA-IV genes.

To gain insight into the role of T_3 in the hepatic expression of the apoA-IV and apoC-III genes, we measured the transcriptional activities and the abundance of nuclear and of total cellular mRNA of these two apolipoproteins in various settings of hormone excess and deficit. We report that perturbations of thyroid hormone status are associated with complex but distinct changes in the expression of the apoA-IV and apoC-III genes. The principal mechanism for enhanced apoA-IV gene expression in chronic hyperthyroidism is decreased catabolism of mature mRNA, as opposed to the mechanism of more efficient mRNA maturation previously described for apoA-I. ApoC-III mRNA abundance is less affected than apoA-IV mRNA abundance by changes in T_3 status, since the pronounced hormonal effects on transcription are counteracted by posttranscriptional events.

MATERIALS AND METHODS

[5' α-^{32}P]dCTP (3000 Ci/mM), [5' γ ^{32}P]dATP (4500 Ci/mM), and [5' α-^{32}P]UTP (3000 Ci/mM) were purchased from ICN Radiochemicals (Irvine, CA). [α-^{35}S]dATP (3000 Ci/mM) was from Amersham Corp. (Arlington Heights, IL). T4 DNA ligase, T4 DNA polynucleotide kinase, RNase-free DNase I, and calf intestine alkaline phosphatase were obtained from Boehringer Mannheim (Indianapolis, IN). Amplitaq® was from Perkin-Elmer Cetus (Norwalk, CT), and Quiaex was from Quiagen Inc. (Chatsworth, CA). The Sequenase dideoxy-sequencing kit was purchased from United States Biochemical Corp. (Cleveland, OH). The nick-translation kit was from Bethesda Research Laboratories (Gaithersburg, MD). Nitrocellulose and S&S Nytran membranes were obtained from Schleicher & Schuell, Inc. (Keene, NH).

Adult male Sprague-Dawley rats (Texas Animal Specialties, Humble, TX) weighing about 250 g were housed in a room with a 12-h light cycle (light between 0700–1900). Animals were fed normal rat chow ad libitum. T_3 was dissolved in 0.15 M NaCl, pH 11. Animals were injected subcutaneously or intraperitoneally as indicated under Results. Rats serving as injection controls received the alkaline 0.15 M NaCl only. Hypothyroidism was induced by feeding rat chow containing 0.1% PTU (ICN Nutritional Biochemicals, Cleveland, OH) for 3 weeks. Food was removed at 0900, and rats were anesthetized with pentobarbital (5 mg/100 g body weight) 1–7 h later. Free T_3 was measured by radioimmunoassay (Quantimune® RIA, Bio-Rad Laboratories). Daily weight changes (mean ± SEM) of control rats, hypothyroid rats, and rats rendered hyperthyroid by daily subcutaneous injections of T_3 (50 ug/100 g body weight) for 7 days were 3.4 ± 0.6, 0.1 ± 0.1, and −5.1 ± 1.6 g, respectively, all differences at $P < 0.01$ (analysis of variance).

The recombinant plasmids used in this study included plasmids pGEM-3Z containing full-length rat apoA-IV or apoC-III cDNA inserts isolated in this laboratory (35). Double-stranded sequencing by the dideoxy-chain-termination method (36) showed complete homology to the published sequence of rat apoA-IV and rat apoC-III cDNA (16). Plasmid pGEM-3Z containing apoA-I cDNA, kindly provided by L. Chan, was used in previous studies (9, 11).

Total RNA was extracted from 1 g of liver tissue by the guanidine hydrochloride method (37). Abundance of apoA-I, apoA-IV, and apoC-III mRNA was determined by quantitative slot blotting (37). Increasing amounts of RNA were applied to S&S Nytran membranes and hybridized to cDNA inserts labeled with [^{32}P]dCTP by nick translation (38). Hybridization was quantified by liquid scintillation counting of radioactivity or by soft laser densitometry of X-ray films. Relative abundance of apoA-I, apoA-IV, and apoC-III mRNA was calculated from the slopes of the hybridization signals. Northern transfer of RNA was performed as outlined by Thomas (39). Total liver RNA was denatured with 1 M glyoxal, 50% DMSO and separated by electrophoresis in 1.2% agarose. The RNA was transferred to S&S Nytran membranes by diffusion blotting and hybridized to ^{32}P-labeled cDNA inserts. The relative abundance of mRNA was determined from the intensities of the bands, which were quantified by soft laser densitometry. Probe stripping and rehybridization of membranes was carried out according to the manufacturer's recommendations.

Liver cell nuclei were prepared by the method of Northemann et al. (40) as previously described (9, 11). The DNA content in the nuclei was determined by a fluorimetric assay (41) using salmon sperm DNA as standard.

Cell-free transcription was performed by the method of Birch and Schreiber (42) as previously described (9, 11, 35). Under our experimental conditions, total [^{32}P]UTP

incorporation ranged from 0.15 to 40 pmol/mg DNA per min, transcription was DNA-dependent, and RNA polymerase activity amounted to 55% of total transcription (11). Transcription from the albumin, apoE, and apoA-I genes was completely abolished by 2.5 μg/ml amanitin (9, 11). The newly synthesized ^{32}P-labeled transcripts of the apoA-I, apoA-IV, and apoC-III genes were quantified by dot-blot hybridization to an excess (6–8 μg) of plasmid containing full-length apoA-I, apoA-IV, or apoC-III cDNA inserts as previously described (9, 11, 35). Nonrecombinant plasmid pGEM-3Z was used to determine nonspecific hybridization. Hybridization was carried out using 3–16 × 10^6 cpm of extracted nuclear ^{32}P-labeled RNA under conditions identical to those described previously (11). To monitor hybridization efficiency, ^{3}H-labeled cRNAs of apoA-I, apoA-IV, and apoC-III were synthesized from the cDNA templates using SP6 or T7 RNA polymerase (43) and included in hybridizations. The relative rates of apoA-I, apoA-IV, and apoC-III mRNA synthesis were calculated by subtracting the counts per minute of ^{32}P bound to the filters containing nonrecombinant pGEM-3Z (1–3 ppm) from the counts per minute of ^{32}P bound to the filters with plasmids containing the apoA-I, apoA-IV, and apoC-III cDNA inserts, and dividing by counts per minute of ^{32}P bound by the ^{32}P-labeled RNA input. Values were corrected for efficiency of hybridization and are given as parts per million of ^{32}P-labeled RNA input.

Nuclear RNA was extracted from isolated nuclei by the method of Lamers, Hanson, and Meisner (44) as previously described (9). Nuclear RNA was analyzed by Northern blotting and quantitative slot blotting using the ^{32}P-labeled cDNA inserts as hybridization probes. Soft laser densitometry was used to quantify signal intensity on autoradiograms.

ApoA-I and apoC-III mRNA precursors were identified by intron-specific hybridization. Probes specific for intron 3 of the rat apoA-I or apoC-III gene were obtained by amplification of genomic DNA using the polymerase chain reaction (45). Primers were synthesized by using a Cyclone plus synthesizer and reagents from Milligen Biosearch Division (Burlington, MA). For amplification of intron 3 sequences of the apoA-I gene, the following oligonucleotides were used (the location of 5' and 3' ends relative to the major transcription start site [16] are given in parentheses): 5'-GCCTTGCAACTGGCACCAAC-3' (+898, +907); 5'-CTAGAGGGGAAGAGAGCAGCAG-CTGAGAGATGA-3' (+1139, +1110). The primers for amplifiation of intron 3 sequences of the apoC-III gene were: 5'-CACCCCTCCCTTGATCCAT-3' (+1484, +1502) and 5'-CTCCTGTGGGCAGCTAGACCC-3' (+1793, +1773). PCR assays contained 1 μg of genomic DNA isolated from rat liver (38), 0.2 μM each upstream and downstream primer, 200 μM each dNTP, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 2.5 U of Amplitaq in a 100-μl reaction volume that was overlaid with mineral oil. Samples were processed through initial denaturation for 5 min at 94°C, 30 cycles of amplification, which consisted of 1 min at 60°C (annealing), 1 min at 72°C (extension), and 1 min at 94°C (denaturation) with a final extension at 60°C for 10 min. PCR products were separated by electrophoresis in 1.2% agarose, purified using Quiaex, and labeled with [^{32}P]dCTP by nick translation.

To evaluate differences in transcriptional activities and nuclear or total mRNA abundance between euthyroid and hyperthyroid or hypothyroid rats, one-way analysis of variance and planned comparison were used. For comparison of two group means, Student's *t*-test was applied.

RESULTS

Effects of acute hyperthyroidism

To elucidate acute effects of thyroid hormone on hepatic apolipoprotein gene expression, a single dose of

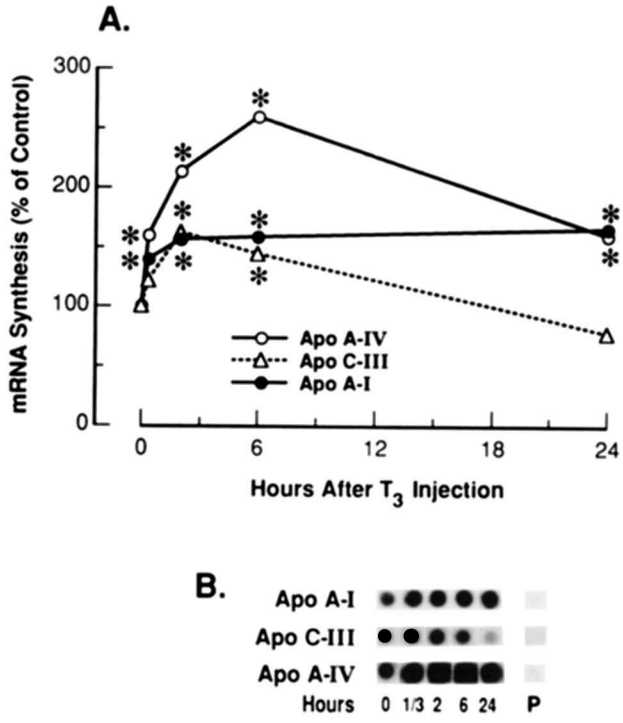

Fig. 1. A: Time course of transcription rates of the apoA-IV, apoC-III, and apoA-I genes in livers from rats after a single dose (3 mg/100 g body weight i.p.) of T_3. Transcription rates were determined by run-on assays using two pools of two rats per time point, except for the 20-min timepoint at which nuclei from a single rat and a pool of two rats were used. Hybridization of the newly synthesized nuclear RNA to plasmids containing apoA-I, apoC-III, or apoA-IV cDNA inserts was carried out in duplicate or triplicate. Results are expressed in percent of the 0-h time point; $^*P < 0.05$, analysis of variance. B: representative dot blots illustrating the relative transcription rates of the apoA-IV, apoC-III, and apoA-I genes shown in A. Background hybridization to nonrecombinant plasmid pGEM-3Z is labeled P. Data on apoA-I transcription rates measured in the same nuclear preparations have been published previously (9) and are shown for comparison.

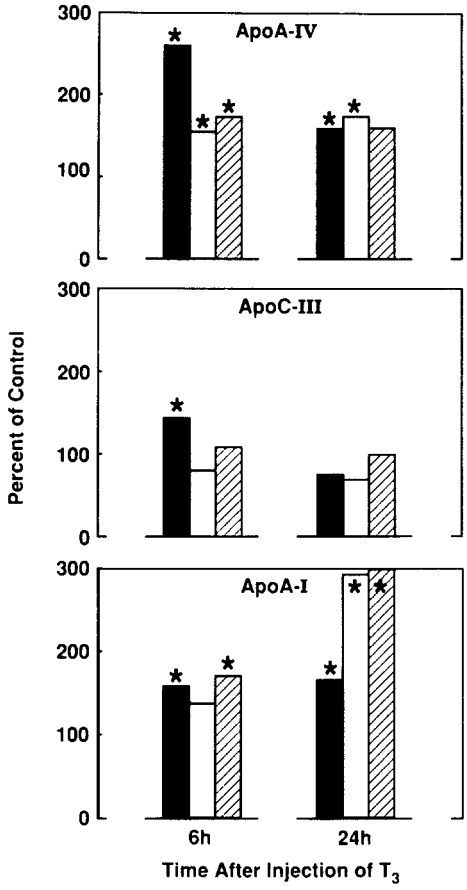

Fig. 2. Relationship of transcription rates (solid bars), abundance of nuclear (open bars) and total cellular mRNA (dashed bars) of apoA-IV (top), apoC-III (middle), and apoA-I (bottom) in rat liver 6 and 24 h after a single dose of 3 mg/100 g of T_3. Results are expressed as percent of control and are the means of two pools of two rats; $^*P < 0.05$ from control, analysis of variance.

T_3 was injected into rats intraperitoneally, and the gene transcription rates as well as the abundance of nuclear and of total cellular mRNA of apoA-IV, apoC-III, and apoA-I were followed for 24 h. A dose of 3 mg T_3/100 g body weight was chosen to saturate nuclear T_3 receptors over the experimental period (46).

ApoA-IV gene transcription increased to 160% of control ($P < 0.05$) at 20 min after the injection, the earliest timepoint studied (**Fig. 1**). ApoA-IV gene transcription reached a maximum of 260% at 6 h and remained elevated for 24 h. Total transcription did not change significantly during the experiment (minimum 85% maximum 95%; ns by analysis of variance). Nuclear apoA-IV mRNA abundance increased to 160% of control at 6 h (**Fig. 2**). At 24 h the increase of nuclear apoA-IV mRNA was commensurate with the increase in gene transcription. Total cellular apoA-IV mRNA increased to 173% of control at 6 h after injection and its abundance was similar to that of nuclear apoA-IV RNA at 24 h (Fig. 2).

ApoC-III gene transcription increased transiently to a maximum of 160% of control at 2 h ($P < 0.05$) but returned to baseline levels by 24 h. Neither nuclear nor total cellular apoC-III mRNA abundance changed significantly at the 6- and 24-h timepoints (Figs. 1 and 2).

ApoA-I gene transcription increased rapidly to 158% of control levels and remained elevated for the experimental period. As previously shown (9), nuclear apoA-I RNA precursors and total cellular apoA-I mRNA increased to about three times the control value 24 h after the injection (Figs. 1 and 2), thus exceeding by twofold the levels expected from the increase in transcription.

To determine the acute effects of lower, more physiological doses of T_3, transcription rates were measured 6 h after single doses of 20, 50, and 500 μg T_3/100 g body weight (**Table 1**). Like apoA-I gene transcription (9), apoA-IV gene transcription increased at these lower hormone doses, although to a significant degree only at the highest dose, while apoC-III transcription rates decreased significantly only at the lowest dose (20 μg/100 g). These results were obtained using liver cell nuclei of individual animals and were confirmed using nuclei pooled from three animals per group. In the latter confirmatory experiment, apoA-IV gene transcription increased to 119% ± 2%, 144% ± 4%, and 141% ± 2% (mean ± SEM of triplicate hybridizations) in rats injected with 20, 50, and 500 μg T_3/100 g body weight, respectively. For the

TABLE 1. Effect of acute T_3 administration on the transcriptional activity of the apoA-IV and apoC-III genes in isolated rat liver nuclei

Treatment	ApoA-IV Transcription		ApoC-III Transcription	
	ppm	*% of control*		
Saline	268 ± 8	100 ± 3	44 ± 6	100 ± 14
T_3 (20 μg/100 g)	446 ± 47	166 ± 17	20 ± 3	45 ± 6[a]
T_3 (50 μg/100 g)	370 ± 74	138 ± 28	32 ± 8	73 ± 18
T_3 (500 μg/100 g)	440 ± 35	164 ± 13[a]	34 ± 3	77 ± 8

Liver nuclei were isolated 5 h after injection of saline or various doses of T_3. Transcription rates, measured by run-on assays using nuclei of three individual animals per group, are expressed as parts per million (ppm) of ^{32}P-labeled RNA input (3×10^6 and 6×10^6 cpm for apoA-IV and apoC-III, respectively) and are corrected for efficiency of hybridization. Results are means ± SEM.

[a] $P < 0.05$, analysis of variance.

TABLE 2. Effect of chronic T_3 administration on the transcriptional activity of the apoA-IV, apoC-III, and apoA-I genes in isolated rat liver nuclei

Animal Set	^{32}P-Labeled RNA Input	Transcription Rate		
		Control	T_3 (50 μg/100 g, 7 d)	
	cpm × 10^{-6}	*ppm*	*ppm*	*% of control*
ApoA-IV				
I	16	168, 143	87, 80	
II	11	176, 203	105, 123	55 ± 3[a]
III	6	180, 164, 170	80, 78, 102	
ApoC-III				
I	16	29, 39	7, 5	
II	11	78, 40	6, 7	28 ± 13[a]
III	6	48, 28, 50	22, 16, 32	
ApoA-I				
I	8	52	19	37[b]
II	8	52	16	30[b]

Transcription rates were measured in three sets of animals each consisting of six control and six T_3-injected rats by nuclear run-on assays using nuclei pooled from four to six rats/group. Transcription rates are expressed as parts per million (ppm) of ^{32}P-labeled RNA input for individual hybridizations and are corrected for hybridization efficiency.

[a]Mean ± SEM, $P < 0.01$, *t*-test.

[a]For confirmation of previous results (9), only single hybridizations were performed.

same doses of T_3, apoC-III gene transcription was 49% ± 7%, 63% ± 5%, and 98% ± 2% of control levels, respectively.

Effects of chronic hyperthyroidism

Four sets of rats (referred to as sets I–IV in figures and tables) were injected subcutaneously with a nearly receptor-saturating dose of 50 μg T_3/100 g body weight per day for 7 days. Sets III and IV also contained a group of animals made hypothyroid through use of PTU (see below).

Chronic thyroid hormone administration decreased the transcription rates of the three genes in all experiments (**Table 2**). Mean apoA-IV, apoC-III, and apoA-I mRNA gene transcription rates were 55, 28, and 34% of control ($P < 0.01$), respectively.

Despite these coordinate decreases in transcription, the three apolipoproteins varied widely from one another in changes in nuclear and total cellular mRNA abundance on T_3 administration (**Table 3**). Abundance of nuclear apoA-IV RNA increased slightly above the level expected from transcription and total cellular apoA-IV mRNA abundance increased 3.2-fold ($P < 0.01$) above the abundance level of nuclear A-IV RNA, implying stabilization of mature mRNA. Nuclear and total cellular apoC-III mRNA abundance levels increased when compared with the rate of transcription, but remained well below the abundance of euthyroid control. Against the reduced level of apoA-I gene transcription, the abundance of nuclear and cellular apoA-I mRNA increased 6.7-fold and 8.5-fold, respectively, results consistent with our previous findings and, as discussed previously, with more effective

TABLE 3. Transcriptional activity and abundance of nuclear and cellular apoA-IV, apoC-III, and apoA-I mRNA in livers of euthyroid and chronic hyperthyroid rats

Variable	ApoA-IV		ApoC-III		ApoA-I	
	Control	T_3	Control	T_3	Control	T_3
Transcription	100 ± 5	55 ± 3[a]	100 ± 16	28 ± 13[a]	100	34
Nuclear RNA abundance	100 ± 4	87 ± 5[b]	100 ± 4	53 ± 8[a]	100 ± 3	227 ± 12[a]
Cellular mRNA abundance	100 ± 8	279 ± 22[a]	100 ± 5	63 ± 3[a]	100 ± 7	289 ± 9[a]

Nuclei, nuclear RNA, and total cellular RNA were isolated from livers of control rats and rats injected with T_3 (50 μg/100 g/d s.c. for 7 d). Transcription data are from Table 2. Nuclear and cellular mRNA abundance was determined by quantitative slot blotting using 6–12 pools per group, two rats per pool. Results are expressed in percent of control and are given as means ± SEM.

[a]$P < 0.01$, *t*-test.

[b]$P < 0.05$.

processing of apoA-I mRNA precursors (9). Northern blotting of total cellular RNA showed mRNA species of expected size and confirmed the quantitative differences obtained by slot blotting (**Fig. 3**).

Northern blots of nuclear RNA showed at least three nuclear apoA-I RNA species (**Fig. 4**). The most abundant apoA-I RNA was similar in size to mature cytoplasmic RNA. At least two larger, additional species, not well separated from each other but clearly separated from the smallest nuclear apoA-I mRNA, could be identified on blots probed with apoA-I cDNA. These two larger RNA species hybridized to an intron 3-specific genomic apoA-I fragment and were thus apoA-I mRNA precursors (**Fig. 5A**). In chronic hyperthyroidism, the smallest nuclear apoA-I RNA species became relatively more abundant (83 ± 3% of total nuclear apoA-I RNA in T_3 vs. 61 ± 3% in control; n = 6 pools/group, 1–2 rats/pool; mean ± SEM; $P < 0.01$). The pattern of apoA-IV nuclear RNA species did not change in response to chronic thyroid hormone administration, while the proportion of the smallest nuclear apoC-III RNA increased from 86 ± 2% in control to 95 ± 2% in hyperthyroid rats (mean ± SEM; n = 6 pools/group; 1–2 rats/pool; $P < 0.02$).

Effects of hypothyroidism

Two groups of rats (contained in animal sets III and IV) were made hypothyroid by feeding a PTU-containing diet for 3 weeks. The control groups were fed rat chow. Plasma free T_3 levels were 0.5 ± 0.1 pg/ml in the hypothyroid and 2.1 ± 0.3 pg/ml in the euthyroid rats (mean ± SEM; $P < 0.01$).

Chronic hypothyroidism greatly reduced apoA-IV gene transcription as well as nuclear and total cellular abundance of apoA-IV mRNA in the liver (**Table 4,** Figs. 3 and 4). In contrast, the rate of apoC-III gene transcription increased to 178 ± 24% of euthyroid controls (Table 4). Despite the increase in transcription, chronic hypothyroidism did not significantly alter the abundance of nuclear or of cellular apoC-III mRNA (Table 4). The decrease in the abundance of nuclear apoC-III RNA relative to transcription was associated with a change in the pattern of nuclear mRNA precursors. The proportion of the smallest nuclear apoC-III, which showed the same migration as mature apoC-III mRNA, decreased from 86 ± 2% in control to 68 ± 4% in hypothyroid rats (mean ± SEM; n = 4 pools/group, 1–2 rats/pool, $P < 0.01$); the proportion of higher-molecular-weight precursors, as identified by intron 3-specific hybridization (Figs. 4 and 5B), increased in hypothyroid animals. As expected from our previous experiments (9), nuclear and total cellular apoA-I mRNA abundance also decreased in hypothyroid rats (Figs. 3 and 4); this result is consistent with some (30, 32), but not all (34) studies from other laboratories. In our previous experiments, apoA-I gene transcrip-

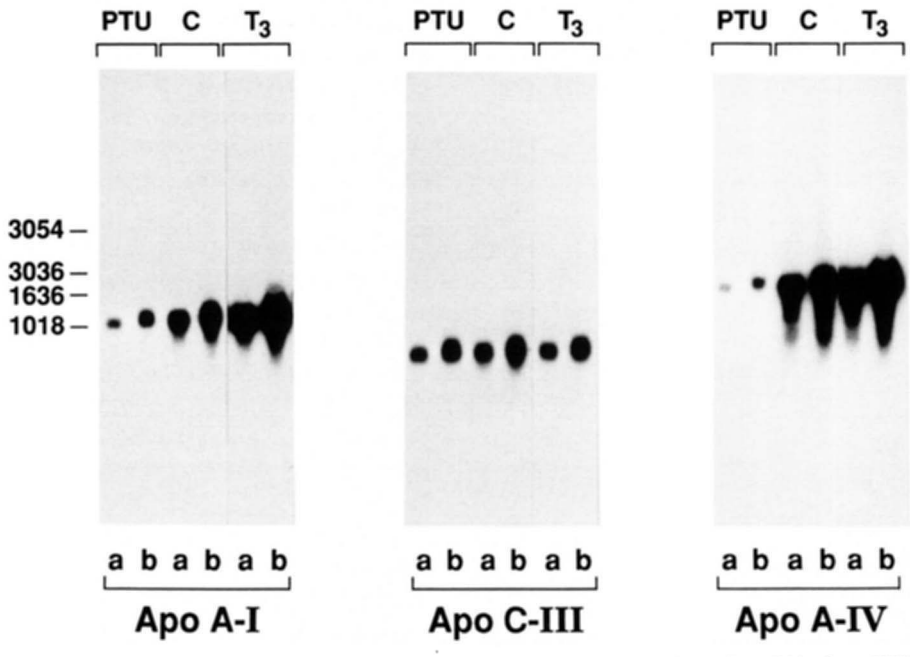

Fig. 3. Representative autoradiograms of Northern blots of pools (six rats/pool) of total liver RNA from PTU-fed animals (PTU), control animals (C), and rats fed the control diet and injected with 50 μg/100 g of T_3 for 7 d (T_3). Ten and 20 μg of total RNA were subjected to electrophoresis in lanes a and b, respectively. The same blots were hybridized to ^{32}P-labeled apoA-I, apoC-III, and apoA-IV cDNA inserts. Molecular weight standards are shown on the left.

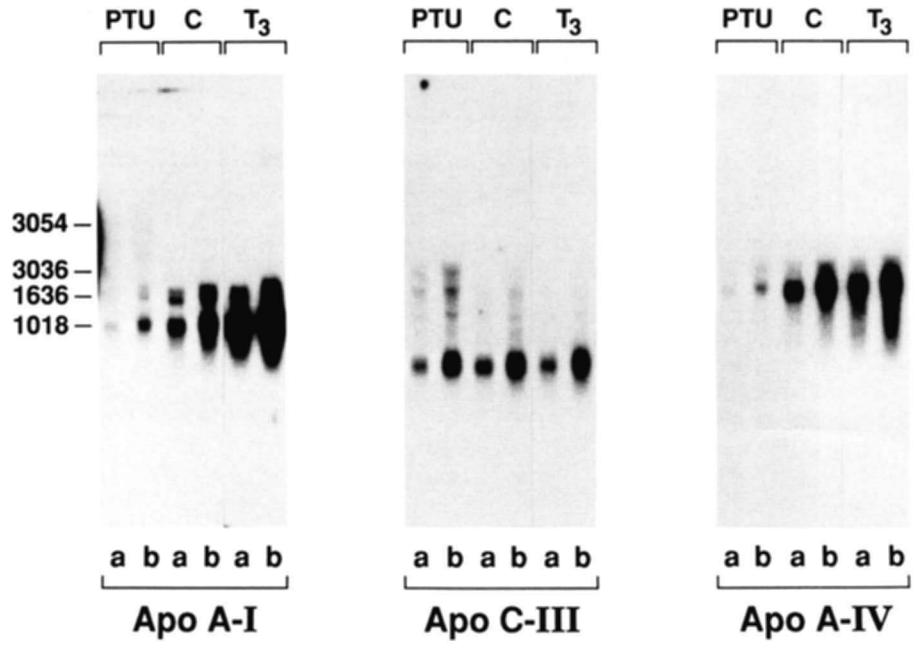

Fig. 4. Representative autoradiogram of Northern blots of pools (six rats/pool) of nuclear RNA from PTU-fed animals (PTU), control rats (C), and rats fed the control chow and injected daily with 50 μg/100 g of T_3 for 7 d (T_3). Fifteen and 30 μg of nuclear RNA were subjected to electrophoresis in lanes a and b, respectively. The same blots were hybridized to ^{32}P-labeled apoA-I, apoC-III, and apoA-IV cDNA inserts. Molecular weight standards are shown on the left.

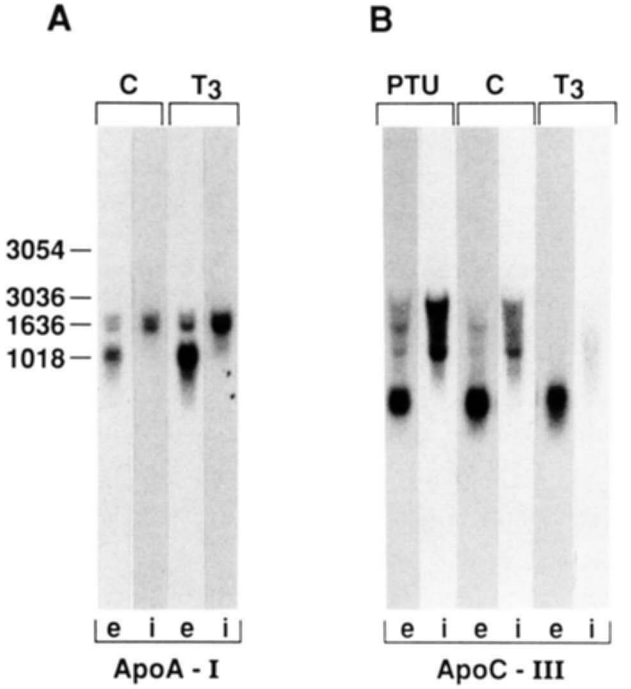

Fig. 5. Autoradiograms of Northern blots of pools (six rats/pool) of nuclear RNA from control rats (C), rats fed the control chow and injected daily with 50 μg/100 g of T_3 (T_3), rats fed the PTU-diet (PTU). In A, the membrane was hybridized with ^{32}P-labeled apoA-I cDNA insert (e) or with a ^{32}P-labeled probe specific for apoA-I intron-3 sequences (i). In B, the membrane was hybridized with ^{32}P-labeled apoC-III cDNA (e) or with a ^{32}P-labeled probe specific for apoC-III intron-3 sequences (i).

tion was decreased to 60% of control (9), which would account at least in part for the reduced total cellular apoA-I mRNA abundance. In our current studies, the hybridization signals of nuclear RNA of hypothyroid rats with labeled apoA-I cDNA or labeled intron 3 sequences were too weak for reliable determination of the proportion of nuclear apoA-I RNA.

DISCUSSION

These studies confirm and extend previous reports that alterations of thyroid status have major effects on the expression of the apoA-IV and apoA-I genes in rat liver (30–34). The main new findings are: *i)* Transcription rates of the apoA-IV gene exhibit the largest changes in response to thyroid hormone excess and deficit among the three genes studied. *ii)* The mechanism for the increased abundance of apoA-IV mRNA in chronic hyperthyroidism involves decreased catabolism of mRNA in the cytoplasm and is therefore clearly distinct from the mechanism by which apoA-I mRNA levels are increased in this condition. *iii)* Transcription rates of the apoC-III gene differ severalfold between chronic hypothyroidism and chronic hyperthyroidism; however, the impact of these transcription differences on apoC-III mRNA abundance is blunted by counteracting changes in mRNA maturation.

Our findings that mRNA abundance increased for

TABLE 4. Effect of chronic hypothyroidism on apoA-IV and apo-CIII gene transcription, nuclear and cellular mRNA abundance in rat liver

	ApoA-IV		ApoC-III	
Variable	Control	PTU	Control	PTU
Transcription	100 ± 13	24 ± 7[a]	100 ± 20	178 ± 24[b]
Nuclear RNA abundance	100 ± 6	9 ± 2[a]	100 ± 8	115 ± 9
Cellular mRNA abundance	100 ± 23	6 ± 2[a]	100 ± 30	83 ± 4

Nuclei, nuclear RNA, and total cellular RNA were isolated from livers of control rats and rats fed a PTU-containing diet for 3 weeks. Transcription rates were measured by nuclear run-on assays using nuclei from four individual rats/group. Nuclear and total cellular RNA abundance was measured by quantitative slot blotting in three to six pools of two rats each per group. Transcription rates and mRNA abundance data are mean ± SEM and expressed as percent of control.

[a] $P < 0.01$, *t*-test.

[b] $P < 0.05$.

apoA-IV and apoA-I and decreased for apoC-III after chronic T_3 administration suggest that the hepatic abundance was altered in a fashion resembling the expression of the apoA-IV, apoA-I, and apoC-III genes in the intestine (16, 47). This notion is supported by an increased hepatic production of apoB-48 at the expense of apoB-100 that has been described as a response to T_3 administration (48, 49). Because apoC-III may inhibit and apoA-IV may enhance the catabolism of triglyceride-rich lipoproteins, triglyceride-rich lipoproteins should be cleared faster from the circulation in chronic hyperthyroidism. This hypothesis assumes that apoC-III protein production is commensurate with changes in apoC-III mRNA abundance, as has been described for apoA-IV (30).

Our studies show that the mechanisms whereby thyroid hormone alters the expression of the apoA-IV, apoC-III, and apoA-I genes operate at least at three levels, i.e., transcription, mRNA maturation, and mRNA stability. The transcriptional activities of all three genes changed in response to altered hormone status. After a single receptor-saturating dose of T_3, transcription of the three genes increased, albeit to a different extent. Whether the rapid induction of transcriptional activity results from interaction of *cis*-acting sequences of these genes with the T_3 receptor complex (50), or with other T_3-regulated transcription factors (51) is not known. Stimulation of apoC-III transcription was short-lived and of only moderate intensity, resembling that of the apoE (9) and apoA-II (W. Strobl, L. Chan, and W. Patsch, unpublished observations) genes. Furthermore, lower, more physiological doses of T_3 inhibited apoC-III transcription, while stimulation of apoA-IV transcription and apoA-I transcription was observed (Table 1, ref. 9). The mechanism for the biphasic effect of T_3 on apoC-III transcription is not known. Upon repeated administration of T_3, transcription of all three genes was inhibited. Transcriptional activities of the albumin (9), apoE (9), and apoA-II (W. Strobl, L. Chan, and W. Patsch, unpublished observations) genes are not influenced in these experimental conditions, implying specificity of transcriptional effects on the apoA-IV, apoC-III, and apoA-I genes. The transcriptional activities of the three genes were comparable in ranking between chronic hyperthyroidism and acute thyroid hormone stimulation. The synthetic rate of mRNA was highest for apoA-IV and lowest for apoC-III. Thus, the interaction of *trans* factors with a *cis*-regulatory element that resulted in stimulation of transcription after a single dose may have been maintained, but transcription of the entire gene cluster may have been repressed by a more effective mechanism.

Common and unique *cis*- and *trans*-regulatory elements have been identified for the apoA-I and apoC-III genes (52, 53). Several members of the steroid receptor superfamily that bind with high affinity to palindromic thyroid hormone-response elements (54) have been shown to converge at sites in the proximal promoter of the apoA-I and apoC-III genes. Hepatocyte nuclear factor 4 stimulates transcription from the apoC-III promoter (55), while retinoic acid-responsive factor α activates transcription from basal apoA-I promoters (56). Apolipoprotein A-I regulatory protein 1 and Ear3/COUP-TF suppress transcriptional activity from both promoters (54). Thus, the intracellular balance of these factors may be important for apoA-I and apoC-III transcription and may be altered by perturbations of thyroid status (57, 58). Much less is known about *cis*- and *trans*-regulatory elements determining apoA-IV gene transcription (52). However, the sensitivity of apoA-IV gene transcription to thyroid status established in this study (24% of control vs. 260% of control, Fig. 1 and Table 4) may be exploited to delineate the hierarchy of *cis* and *trans* factors determining the transcriptional activity of the apoA-IV gene.

Besides effects on transcription, thyroid status affects the expression of the apoA-I and apoC-III genes by influencing nuclear maturation of mRNA. Despite reduced transcription, nuclear apoA-I RNA abundance is increased in chronic hyperthyroidism (9). Northern blots probed with apoA-I cDNA and/or apoA-I intron 3 sequences showed an increase in the level of apoA-I mRNA

precursors and an even greater increase in the level of mature nuclear RNA (Figs 4 and 5A). Considering the decrease in transcription, such changes in the abundance and proportion of nuclear RNA species are most consistent with protection of a nuclear apoA-I mRNA precursor from degradation or with enhancement of intron removal (C. Seelos, S. Soyal, Y. C. Lin-Lee, A. M. Gotto, Jr., and W. Patsch, unpublished observations).

In hypothyroid rats, the abundance of nuclear apoC-III RNA decreased relative to the level of transcription, and the proportion of high-molecular-weight precursors increased. In chronic hyperthyroid rats, the abundance of nuclear apoC-III RNA increased relative to transcription, and the proportion of mature nuclear RNA increased (Table 4, Figs. 4 and 5B). Thus, thyroid hormone increases the rate of both apoA-I and apoC-III mRNA maturation. While the factors governing the efficiency of mRNA maturation are not fully understood, some mechanistic insight has been presented. Changes in the stabilization of nuclear mRNA precursors may help to explain the different expression of the human liver/bone/kidney alkaline phosphatase gene in osteoblast-derived cells and HepG2 cells despite similar rates of transcription (59). Furthermore, the efficiency of splicing may indirectly control the stability of pre-mRNA. Splicing blocks may lead to degradation of mRNA precursors by permitting the attack of introns by nucleolytic enzymes. Such a mechanism may control the expression of the L1 ribosomal protein gene in *Xenopus laevis* (60).

Changes in the stability of cytoplasmic mRNA played little, if any, role in T_3 effects on apoA-I or apoC-III gene expression (Figs. 2–4, Tables 3 and 4). However, apoA-IV gene expression is enhanced by decreased catabolism of mature RNA in chronic hyperthyroidism (Fig. 3, Table 3). The molecular mechanisms that change the turnover of selective mRNA are only partially understood (61). Sequences in the 3'-noncoding region of mRNA have been shown to determine stability of transiently expressed genes, and AU sequences may be recognition signals for mRNA degradation pathways (62, 63). However, the half-life of other mRNA is determined by sequences close to their 5' end (64, 65), and studies with chimeric mRNA of β-globin and c-fos suggest that sequences at both the 5' and 3' ends contribute to the stability of mRNA (66). As chimeras with reciprocally exchanged sequences do not always show reciprocal half-lives, involvement of secondary structure is thought to have a significant effect on the degradation signal conferred by specific sequences (66). Indeed, the initial cleavage site for histone mRNA may be in stem-loop structures (67). The nucleases responsible for mRNA degradation have not been identified, but an association of such enzymes with ribosomes has been suggested (68).

Hormones such as estrogen and prolactin reduce the turnover of cytoplasmic mRNA of hormone-dependent proteins such as vitellogenin and casein (69, 70). Estrogen alters as well the stability of apoVLDL-II, a chicken apolipoprotein (7), but little is known about how the degradation pathway of these mRNA is influenced by hormones. ApoA-IV mRNA abundance increases twofold in rat intestinal epithelial cells 4 h after a fat bolus, but tends to decrease towards baseline levels over the next 4 h (71). For enterocytes to respond rapidly and repeatedly to fat intake with changes in apoA-IV mRNA concentrations, their apoA-IV mRNA turnover must be high, so that changes in transcription rates are effectively converted into changes in mRNA levels. Alternatively, nutrient-induced modulations in the stability of apoA-IV mRNA could be involved in the rapid changes in mRNA concentrations. Studies in strains of inbred mice showed wide variation in the abundance of apoA-IV mRNA in liver that resulted from different rates in apoA-IV mRNA turnover. Heterogeneity in mRNA structure and *trans*-acting proteins was suggested to explain the variation of mRNA stability in these strains (72). These data together with our results indicate that mRNA stability is a determinant of apoA-IV gene expression that is of physiological relevance. Thus, elucidation of apoA-IV mRNA degradation pathways may enhance our understanding of lipid transport.

This work was supported by U.S. National Institutes of Health Grant HL-34457 and SCOR Contract HL-27341, and by Austrian Science Foundation Grant P-7944MED. The technical assistance of I. Y. Chen and Christian Seelos and the assistance of S. Simpson in editing the manuscript are greatly appreciated.

Manuscript received 5 March 1992 and in revised form 13 August 1992.

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