

Apolipoprotein gene expression in the rat is regulated in a tissue-specific manner by thyroid hormone

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Abstract We have studied the regulation of rat intestinal and hepatic apolipoprotein gene expression, *in vivo*, after alterations in thyroid hormone status. When compared to those of chow-fed controls, rates of synthesis of intestinal apoA-I and apoB-48 decreased 60–66% in hypothyroid animals and increased three- to fourfold after triiodothyronine (T₃) administration. These changes were not accompanied by changes in mRNA abundance. By contrast, intestinal apoA-IV synthesis rates and mRNA abundance were both unaltered over the range of thyroid hormone manipulations tested. Hepatic apoA-I and apoA-IV synthesis rates decreased by 70–80% in hypothyroid animals, while synthesis rates and mRNA abundance increased coordinately six- to eightfold when hypothyroid rats were made hyperthyroid. Hepatic apoE synthesis rates increased twofold in hypothyroid rats and decreased sevenfold in hyperthyroid animals. ApoE mRNA abundance, however, was comparable in all groups. Hypothyroid animals had reduced synthesis rates of hepatic apoB-100 and apoB-48. After induction of hyperthyroidism, apoB-100 synthesis (studied from 5 to 60 min) was undetectable (< 0.01%) without further change in apoB-48 synthesis and without alterations in either apoB mRNA abundance or transcript size. Despite undetectable hepatic apoB-100 synthesis rates in hyperthyroid animals, total plasma triglyceride secretion rates (after Triton WR-1339 injection) were normalized compared to a 50% decrease in hypothyroid rats. Taken together, the data provide evidence for tissue-specific, independent regulation of apolipoprotein gene expression *in vivo*. Furthermore, the data suggest that aspects of hepatic triglyceride assembly and secretion and apolipoprotein gene expression may be coordinately responsive to alterations in thyroid hormone status.—Davidson, N. O., R. C. Carlos, M. J. Drewek, and T. G. Parmer. Apolipoprotein gene expression in the rat is regulated in a tissue-specific manner by thyroid hormone. *J. Lipid Res.* 1988. **29**: 1511–1522.

Supplementary key words apoB-100 • apoB-48 • triglyceride assembly

Alterations in thyroid hormone status produce a wide variety of effects on lipoprotein homeostasis in human subjects and experimental animals (reviewed in reference 1). Many of these alterations have been

exploited as a means of manipulating aspects of lipoprotein assembly and secretion. In particular, a combination of cholesterol feeding plus hypothyroidism has been widely used by investigators examining the mechanisms of dietary-induced atherogenic hypercholesterolemia in the rat (2). Observations emerging from studies using this model suggest that hypothyroidism exerts widespread effects on hepatic triglyceride assembly and secretion (3–5), hepatic (5–7) and intestinal cholesteryl ester accumulation (8, 9), and aspects of apolipoprotein metabolism (10, 11). Many of these effects appear to be unique to the hypothyroid state (12) and need to be distinguished from the combined effects resulting from hypothyroidism and cholesterol feeding (9, 12). Additionally, hyperthyroidism has been demonstrated in animals to be associated with alterations in hepatic glycerolipid assembly (13, 14), while hyperthyroid human subjects have been shown to have decreased serum low density lipoprotein (LDL) concentrations with alterations in cholesterol synthesis and LDL catabolism (15, 16).

We now report the results of studies in which the effect of altered thyroid hormone status was examined on the tissue-specific accumulation of several rat apolipoprotein mRNAs and *in vivo* synthesis rates of their primary translation products. Studies were additionally conducted to examine the effects of these altera-

Abbreviations: apo, apolipoprotein; CM, chylomicron; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; PBS, phosphate-buffered saline; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; PMSF, phenylmethylsulfonylfluoride; TCA, trichloroacetic acid; SSC (1X), 0.15 M NaCl/0.015 M Na citrate, pH 7; EDTA, ethylenediaminetetraacetic acid, Na salt; TLC, thin-layer chromatography; GLC, gas-liquid chromatography; PTU, (2-thio-4-hydroxy-6-n-propylpyrimidine); T₃, 3,3',5-triiodo-L-thyronine.

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tions on aspects of hepatic triglyceride assembly and secretion. The results suggest that thyroid hormone exerts independent, tissue-specific effects on intestinal and hepatic apolipoprotein gene expression. Furthermore, the combination of quantitative approaches suggests that regulation may involve both pre- and posttranslational mechanisms. Finally, evidence is presented that links several of these alterations to aspects of hepatic triglyceride assembly and secretion.

MATERIALS AND METHODS

Animals and treatment protocol

Male Sprague-Dawley rats were obtained in the weight range of 150 g from Charles River, Wilmington, MA. Animals were housed four per cage and segregated according to specific treatment protocol. Control animals were fed Purina rat chow (Ralston-Purina, St. Louis, MO) ad libitum for 21–28 days prior to study. The remaining animals were randomly assigned to one of three experimental groups and received chow supplemented with 0.1% (w/w) propylthiouracil (2-thio-4-hydroxy-6-n-propylpyrimidine, Sigma, St. Louis, MO) as previously described (9) for 21 days. All treated animals were thereby rendered initially hypothyroid, as confirmed by radioimmunoassay (performed by the University of Michigan Veterinary Laboratory) of T_4 , T_3 , free T_4 , and free T_3 levels in serum (hypothyroid: T_4 , 0.10 ± 0.08 ng/ml; T_3 , 0.43 ± 0.01 ng/ml; free T_4 , 0.17 ± 0.29 pg/ml; free T_3 , 0.52 ± 0.36 pg/ml vs. control: T_4 , 16.7 ± 2.5 ng/ml; T_3 , 0.63 ± 0.06 ng/ml; free T_4 , 15.1 ± 1.6 pg/ml; free T_3 , 2.2 ± 0.47 pg/ml). Groups of hypothyroid rats were made chemically euthyroid by seven daily intraperitoneal injections of 3,3',5'-triiodo-L-thyronine (T_3), $0.5 \mu\text{g}/100$ g body weight. Other groups were made hyperthyroid by alternate-day injections of $50 \mu\text{g } T_3/100$ g body weight for 7 days. Both groups of T_3 -treated animals continued to consume the chow-PTU diet. Untreated hypothyroid rats were fed chow-0.1% PTU for 28 days. Animals were studied, as detailed below, following a 16–20-hr fast. This period, in the T_3 -treated euthyroid and hyperthyroid groups, began following the last injection of T_3 . At the time of killing, control rats weighed 280–300 g while hypothyroid, T_3 -treated euthyroid and hyperthyroid rats weighed 200–230 g with no differences noted among the three experimental groups.

Determination of intestinal apolipoprotein synthesis rates

Animals were anesthetized with sodium pentobarbital and a 10-cm loop of jejunum (proximal portion

5 cm from ligament of Treitz) was isolated and pulse-labeled with L-[4,5- ^3H]leucine (500 μCi , sp act 120 Ci/mmol, Amersham, Arlington Heights, IL) for 9 min. This time point effectively precludes any apparent hepatic contribution to the newly synthesized intestinal apolipoprotein pool (17). Following exsanguination, the loop was removed and enterocytes were isolated using citrate-EDTA chelation buffers containing sequentially 20, 10, and 5 mM leucine as detailed (17) to prevent isotope reutilization. The final washed cell pellet was homogenized on ice in buffer H (PBS-1% Triton-2 mM leucine-1 mM PMSF-1 mM benzamidine, pH 7.4) and a 105,000 g supernatant was prepared. These conditions have been previously shown to optimize apolipoprotein recovery from intestinal cells (17). Aliquots of homogenate were saved for measurement of total protein concentration (18) and trichloroacetic acid (TCA)-insoluble radioactivity. Samples of the final 105,000 g supernatant were frozen at -80°C prior to immunoprecipitation with specific antisera as described below.

Determination of hepatic apolipoprotein synthesis rates

Animals were anesthetized with sodium pentobarbital and received 1.0 mCi L-[4,5- ^3H]leucine via intra-portal vein injection. At the time intervals detailed in the legends to the figures, animals were exsanguinated via the abdominal aorta, the liver was perfused in situ for 10 min with 75 ml iced PBS-20 mM leucine, and pieces were taken from all lobes for homogenization in five volumes of buffer H. A 225,000 g supernatant was prepared and stored at -80°C prior to immunoprecipitation. Aliquots of liver homogenate were processed as described above.

Quantitative immunoprecipitation of intestinal and hepatic apolipoproteins was performed as described (17) using monospecific polyclonal antisera directed against rat apoA-I (19), apoA-IV (20), and apoB (17). Anti-albumin antibodies were obtained commercially (ICN, Costa Mesa, CA), and anti-apoE antibodies were a gift from Dr. R. Hay, University of Chicago. Aliquots of cytosolic supernatant were reacted with excess antiserum (as defined below), and the immune complexes were precipitated by addition of washed *S. aureus* cells (IgG-sorb Enzyme Center, Boston, MA). After extensive washing, the immune complexes were characterized by denaturing SDS-PAGE and radioactivity incorporation into the specific apolipoproteins was determined by liquid scintillation spectrometry following addition of 3% Protosol-Econofluor (NEN, Boston, MA) to gel slices. In all instances, antibody excess stoichiometry was established by exhaustive reprecipitation.

Apolipoprotein synthesis rates are expressed as a fraction of total protein synthesis (9, 17). Each value represents the mean of two to six separate assays corrected for nonspecific and background radioactivity.

RNA extraction and analysis

Total cellular RNA was extracted and pooled (up to four animals per pool) from the proximal (jejunum) small intestine using 8 M guanidine-HCl as previously described (21). Yields of total cellular RNA averaged 3–6 mg/g wet weight mucosa. Hepatic RNA was similarly extracted, with comparable yield. All preparations of RNA were determined to be intact following analytical methylmercury agarose gel electrophoresis (22).

For quantitation of apolipoprotein mRNA abundance, serially diluted aliquots of total cellular RNA (0.5–3.0 μ g) were applied to nitrocellulose filters using a commercial template (23). In addition, samples of intestinal and hepatic total cellular RNA (30 ng–3 μ g) were run as internal standards for each filter. Filters were probed with various cDNAs (as detailed below) labeled with 32 P to a specific activity of 10^8 – 10^9 cpm/ μ g (24). Hybridization solutions (pH 7.0) contained 50% (vol/vol) formamide, 6 \times SSC, 50 mM sodium phosphate, 1 mM EDTA, 1 \times Denhardt's solution, 50 μ g/ml sheared, single-stranded salmon sperm DNA, and 10% (wt/vol) dextran sulfate. Following a 24-hr incubation at 42°C, filters were washed twice in 0.1% (wt/vol) NaDodSo₄/1 \times SSC at 25°C and four times (15 min each) in 0.1 \times SSC at 50°C prior to autoradiography. mRNA abundance was calculated by quantitative scanning densitometry using an LKB Laser Densitometer (Ultrascan LX, LKB, Gaithersburg, MD). Data are expressed as absorbance units per μ g RNA, each value representing the mean \pm SD of three or four representative pools. Relative abundance was calculated by reference to a standard curve constructed from the signals of hepatic and intestinal RNA standards, thereby allowing comparison between different films. Only signals in the linear range of film sensitivity were used (23).

Northern blots of 20 μ g total hepatic RNA were prepared as described (25) following fractionation through 6% formaldehyde/0.75% agarose gels. These blots were hybridized as described above with a rat 3' apoB cDNA (26).

cDNAs used in these studies include a 2.9 Kb 3' rat apoB fragment (26) (a gift from Drs. J. Elovson and A. Lusis, UCLA Medical Center, CA); rat apoA-I (27), apoA-IV (28), and rat liver fatty acid binding protein (29), (gifts from Dr. J. Gordon, Washington University, St. Louis); rat apoE (30), (a gift from Dr. J. Taylor, Gladstone Foundation Laboratories, San Francisco, CA); and human beta actin (31) (a gift from Dr. P.

Gunning, VA Medical Center, Stanford University, CA).

Hepatic microsome preparation and analysis

After the rats were exsanguinated, portions of the liver were removed and finely minced prior to homogenization in five volumes of buffer I (0.25 M sucrose–10 mM Tris–1 mM EDTA, pH 7.4) using a loose-fitting Teflon–glass homogenizer. The homogenate was centrifuged at 2000 *g* for 10 min and the resulting supernatant was centrifuged at 25,000 *g* for 10 min, both at 4°C. This supernatant was then centrifuged at 100,000 *g* for 60 min at 4°C and the pellet was suspended in 1 ml 0.5 M KCl–0.25 M sucrose. Following recentrifugation at 100,000 *g*, the final, washed microsome pellet was resuspended in 2 ml buffer I, aliquoted, and frozen at –80°C. Once thawed, samples were not reused.

Samples were submitted to lipid extraction according to the method of Folch et al. (32). Total lipid classes were separated by thin-layer chromatography (TLC) using silica gel G in a solvent of petroleum ether–ethyl ether–glacial acetic acid 80:20:1 (v/v/v). Triglyceride and free fatty acid bands were identified by comparison to standards, scraped into Teflon-lined screw-capped tubes, and transmethylated directly using 14% BF₃ in methanol, following addition of heptadecanoic acid as an internal standard. The derivatized fatty acids were assayed using a Perkin Elmer model 8410 gas–liquid chromatograph equipped with a 6 ft \times 2 mm ID column packed with 10% SP-2330 on 100/120 mesh Chromasorb (Supelco, Bellefonte, PA). Authentic fatty acid methyl esters (Nu-Chek-Prep, Elysian, MN) were used to identify peaks based on their relative retention times. Values are presented as μ g fatty acid, normalized to protein content.

Enzyme assays

Glycerol-3-phosphate acyltransferase was assayed using 75 μ M [14 C]palmitoyl CoA ([1- 14 C]palmitoyl coenzyme A, New England Nuclear, Boston, MA) and 30 μ M glycerol-3-phosphate as described (33, 34). Greater than 92% of the reaction product migrated as lysophosphatidic acid. Diacylglycerol acyltransferase activity was assayed using 30 μ M [14 C]palmitoyl CoA and 125 μ M 1,2-*sn*-diacylglycerol (Serdary, London, Ontario) dispersed in ethanol (35, 36). Reaction products (86 \pm 7%, *n* = 41, migrating as authentic triglyceride) were resolved by TLC using silica G in petroleum ether–ethyl ether–acetic acid 80:20:1 (v/v/v). The triglyceride band was scraped and counted in Budget-Solv (RPI, Mount Prospect, IL). Monoacylglycerol acyltransferase activity was assayed using 25 μ M [14 C]palmitoyl CoA and 50 μ M *sn*-2-monooleoylglycerol (Serdary, London, Ontario) dispersed in acetone (37, 38). Reaction products (diglyceride and triglycer-

eride) were resolved by TLC using silica G in heptane–isopropyl ether–acetic acid 60:40:4 (v/v/v). Di- and triglyceride bands were scraped and counted as above. All assays were performed at 23°C and were found to be proportional to both time and microsomal protein concentration. Assays were performed in triplicate, at two protein concentrations, and the results are expressed as nmol product formed/min per mg protein.

Plasma triglyceride secretion rates

Total triglyceride secretion was determined in groups of control, hypothyroid, and hyperthyroid animals as described (39). Animals were fasted for 20 hr prior to study. Under light ether anesthesia, each animal received an intravenous bolus of 600 mg/kg body weight Triton WR-1339 (Sigma) as a sterile-filtered solution made 15% (w/v) in 0.15 M NaCl. Animals were bled from the tail vein (100 μ l) at zero time and at 2 and 4 hr. Plasma triglyceride concentration was determined using a commercial enzymatic kit (Sigma) and the results are expressed as mg triglyceride secreted/hr per kg body weight (39), assuming the plasma volume to be 4.5% body weight.

Miscellaneous assays

Serum apoA-I and apoB concentrations were determined by radioimmunoassay (9, 17). The relative distribution of serum apoB molecular forms was assessed following electrophoresis of whole serum through 2% acrylamide–0.5% agarose mini slab gels (40) and electroblotting to nitrocellulose membranes. Membranes were blocked for 2 hr with 5% Blotto and immunostained using 1:500 dilutions of apoB antiserum and 1:3000 goat anti-rabbit horseradish peroxidase-linked IgG. Color development with 4-chloro-1-naphthol followed the Bio-Rad (Richmond, CA) protocol. Protein determinations (18) used bovine serum albumin as a standard.

Statistical comparisons were made using independent *t*-tests and employed both methods for pooled and separate variance where appropriate. Data, unless otherwise stated, are expressed as mean \pm SD.

RESULTS

Intestinal apolipoprotein gene expression

ApoA-I and apoB-48 synthesis rates (% total protein) were suppressed in hypothyroid animals by 66% and 60%, respectively (Table 1). There was a three- to fourfold range of apoA-I and apoB-48 synthesis rates encountered over the range (Table 1) of thyroid hormone perturbations that were examined. Total protein synthesis, as evidenced by [³H]leucine incorporation into TCA-insoluble material per μ g protein, was comparable between control, euthyroid, and hyperthyroid animals (1110 \pm 380 cpm/ μ g protein (n=4), 1063 \pm 291 cpm/ μ g (n=5), 918 \pm 140 cpm/ μ g (n=4), respectively). Hypothyroid animals, however, were noted to have increased incorporation of [³H]leucine into total protein, (2707 \pm 1042 (n=10), *P* < 0.05). The basis for this increase and consequently elevated specific activity of total protein is unknown. However, despite the apparent increase in [³H]leucine incorporation in hypothyroid animals, intestinal apoA-IV synthesis rates were comparable in all experimental groups (Table 1). Taken together, the data provide evidence for regulation of intestinal apolipoprotein synthesis distinct from a general alteration in intestinal protein synthesis. Additionally, the changes described in apolipoprotein synthesis rates were not accompanied by changes in mRNA abundance (Table 2), suggesting that thyroid hormone may exert independent regulation of intestinal apoA-I and apoB-48 gene expression at a translational or posttranslational level.

TABLE 1. In vivo intestinal apolipoprotein synthesis: effects of thyroid hormone

Experimental Group	Apolipoprotein Synthesis		
	ApoA-I	ApoA-IV % total protein	ApoB-48
1. Control	2.24 \pm 0.41	2.67 \pm 0.23	1.16 \pm 0.11
2. Hypothyroid	0.77 \pm 0.12*	2.44 \pm 0.47	0.41 \pm 0.08*
3. Euthyroid	1.45 \pm 0.47**	3.47 \pm 1.06	0.80 \pm 0.22**
4. Hyperthyroid	2.27 \pm 0.62**	2.76 \pm 0.59	1.53 \pm 0.32**
Hyper/hypo	2.95**	1.13 (NS)	3.73**

Animals were maintained on control rat chow (control) or made hypothyroid (groups 2,3,4) after consuming chow supplemented with 0.1% PTU for 21–28 days. Animals were administered T₃ by intraperitoneal injection (groups 3 and 4) to produce euthyroid or hyperthyroid states, respectively, (Methods). Apolipoprotein synthesis rates were determined by quantitative immunoprecipitation following in vivo pulse radiolabeling (Methods). Data are expressed as percent of total (trichloroacetic acid-insoluble) radioactivity, each value representing the mean \pm SD of two to six separate assays on three to nine animals per group. *, Indicates statistically significant difference from control animals (*P* < 0.01); **, indicates statistically significant difference from hypothyroid animals (*P* < 0.01); NS, not statistically significant. (*P* > 0.05).

TABLE 2. Intestinal apolipoprotein mRNA abundance: effects of thyroid hormone

Experimental Group	mRNA Abundance			
	ApoA-I	ApoA-IV	ApoB	Actin
	<i>absorbance units per µg RNA</i>			
1. Control	0.81 ± 0.11 (100)	0.68 ± 0.35 (100)	1.44 ± 0.11 (100)	0.81 ± 0.18 (100)
2. Hypothyroid	0.93 ± 0.30 (115)	0.64 ± 0.17 (94)	1.98 ± 0.38 (137)	0.68 ± 0.21 (84)
3. Euthyroid	1.67 ± 0.63 (206)	0.63 ± 0.18 (91)	2.47 ± 0.48 (171)	1.05 ± 0.42 (130)
4. Hyperthyroid	1.33 ± 0.28 (165)	0.59 ± 0.24 (87)	2.08 ± 0.38 (144)	0.63 ± 0.13 (78)
Hyper/hypo	1.43	0.93	1.05	0.93

Animals were maintained on control rat chow (control) or made hypothyroid (groups 2,3,4) after consuming chow supplemented with 0.1% PTU for 21–28 days. Animals were administered T₃ by intraperitoneal injection (groups 3 and 4) to produce euthyroid or hyperthyroid states, respectively (Methods). Total intestinal RNA was prepared from groups of rats and subjected to dot-blot hybridization analysis of mRNA abundance (Methods). Data are presented as arbitrary absorbance units per µg RNA, determined by scanning laser densitometry of autoradiograms (Methods). Values in parentheses refer to percent of control animals, the latter having been normalized to 100%. There were no significant differences for any mRNA examined.

Hepatic apolipoprotein gene expression

Preliminary studies confirmed that peak incorporation rates of [³H]leucine into total, TCA-insoluble material (Methods), were found 15 min after an intraportal vein bolus of radiolabel [5 min, 126 ± 30 cpm/µg protein (n=4); 15 min, 169 ± 61 cpm/µg (n=7); 30 min, 121 ± 37 cpm/µg (n=4)]. Determination of albumin synthesis by quantitative immunoprecipitation revealed comparable results [5 min, 2.43 ± 1.02% (n=4); 15 min, 2.63 ± 0.92% (n=3); 30 min, 2.07 ± 1.33% (n=4)]. Although neither set of values demonstrated significant differences between 5 and 30 min

labeling intervals, apoB synthesis required 15 min labeling for completion of both molecular forms (described below). A 15-min time point was therefore used in all comparisons of hepatic apolipoprotein synthesis rates.

There was a concordant response of hepatic apoA-I and apoA-IV synthesis rates to changes in thyroid hormone status, there being a sixfold range encountered when hypo- and hyperthyroid animals were compared (Table 3). The observation that hepatic apoA-IV synthesis rates were suppressed by 80% in hypothyroid animals contrasts with the absence of change in intestinal apoA-IV synthesis, suggesting that thyroid

TABLE 3. In vivo hepatic apolipoprotein synthesis: effects of thyroid hormone

Experimental Group	Apolipoprotein Synthesis				
	ApoA-I	ApoA-IV	ApoE	ApoB- (100 + 95)	ApoB-48
	<i>% total protein</i>				
1. Control	0.24 ± 0.03	0.12 ± 0.04	0.21 ± 0.07	0.24 ± 0.13	0.37 ± 0.08
2. Hypothyroid	0.066 ± 0.03*	0.024 ± 0.01*	0.49 ± 0.21*	0.13 ± 0.09	0.22 ± 0.06*
3. Euthyroid	0.13 ± 0.09	0.20 ± 0.04**	0.26 ± 0.05**	0.14 ± 0.09	0.33 ± 0.19
4. Hyperthyroid	0.38 ± 0.11***	0.15 ± 0.07***	0.067 ± 0.001***	ND (<0.01)	0.23 ± 0.14
Hyper/hypo	5.8***	6.2***	0.14**	0	1.05 (NS)

Animals were maintained on control rat chow (control) or made hypothyroid (groups 2,3,4) after consuming chow supplemented with 0.1% PTU for 21–28 days. Animals were administered T₃ by intraperitoneal injection (groups 3 and 4) to produce euthyroid or hyperthyroid states, respectively (Methods). Animals received an intraportal vein bolus of [³H] leucine and were exsanguinated 15 min later (Methods). Livers were removed and homogenized, and a 225,000 g supernatant was prepared. Apolipoprotein synthesis rates were determined by quantitative immunoprecipitation (Methods). Data are expressed as percent of total (trichloroacetic acid-insoluble) radioactivity, each value representing the mean ± SD of two to six separate assays on four to eight animals per group. Total protein synthesis, as evidenced by [³H] leucine incorporation into total TCA-insoluble material per µg protein was comparable in all groups: control, 169 ± 61 cpm/µg (n = 7); hypothyroid, 175 ± 45 cpm/µg (n = 10); euthyroid, 214 ± 53.5 cpm/µg (n = 5); hyperthyroid, 122 ± 42 cpm/µg (n = 8); P > 0.05. *, Indicates statistically significant difference from control animals (P < 0.05 or greater); **, indicates statistically significant difference between euthyroid and hypothyroid animals (P < 0.05 or greater); ***, indicates statistically significant difference between hyperthyroid and hypothyroid animals (P < 0.01 or greater); NS, not statistically significant, P > 0.05; ND, none detected.

TABLE 4. Hepatic apolipoprotein mRNA abundance: effects of thyroid hormone

Experimental Group	mRNA Abundance					
	ApoA-I	ApoA-IV	ApoE	ApoB	Liver FABP ^a	B-Actin
	<i>absorbance units/μg RNA</i>					
1. Control	0.54 ± 0.08 (100)	1.05 ± 0.24 (100)	0.66 ± 0.15 (100)	1.51 ± 0.17 (100)	0.90 ± 0.28 (100)	0.53 ± 0.25 (100)
2. Hypothyroid	0.39 ± 0.09* (72)	0.21 ± 0.05* (20)	0.72 ± 0.10 (109)	1.97 ± 0.26* (130)	0.72 ± 0.25 (80)	0.49 ± 0.12 (92)
3. Euthyroid	0.57 ± 0.23 (105)	1.14 ± 0.37** (109)	0.69 ± 0.15 (105)	2.05 ± 0.37 (136)	1.29 ± 0.49 (143)	0.60 ± 0.20 (113)
4. Hyperthyroid	2.47 ± 1.24*** (457)	1.69 ± 0.79*** (169)	0.82 ± 0.11 (124)	1.70 ± 0.26 (113)	1.06 ± 0.30 (117)	0.75 ± 0.32 (141)
Hyper/hypo	6.3***	8.4***	1.14 (NS)	0.87 (NS)	1.47 (NS)	1.53 (NS)

Animals were maintained on control rat chow (control) or made hypothyroid (groups 2,3,4) after consuming chow supplemented with 0.1% PTU for 21–28 days. Animals were administered T₃ by intraperitoneal injection (groups 3 and 4) to produce euthyroid or hyperthyroid states, respectively (Methods). Total hepatic RNA was prepared from groups of rats and subjected to dot-blot hybridization analysis of mRNA abundance (Methods). Data are presented as arbitrary absorbance units per μg RNA, determined by scanning laser densitometry of autoradiograms (Methods). Values in parentheses refer to percent of control animals, the latter having been normalized to 100%. *, Indicates statistically significant difference from control values ($P < 0.05$ or greater); **, indicates statistically significant difference between euthyroid and hypothyroid animals ($P < 0.005$); ***, indicates statistically significant difference between hyperthyroid and hypothyroid animals ($P < 0.01$ or greater); NS, not statistically significant, $P > 0.05$.

^aFatty acid-binding protein.

hormone exerts a tissue-specific effect on apoA-IV synthesis in the rat. Furthermore, the changes in hepatic apoA-I and apoA-IV synthesis rates were accompanied by parallel changes in mRNA abundance (Table 4), suggesting that thyroid hormone-mediated regulation of these hepatic apolipoprotein genes (in adult male rats) occurs at a pretranslational level. These changes in mRNA abundance were found in the setting of comparable levels of mRNA for B-actin and liver fatty acid-binding protein (Table 4). Furthermore, the significance of these changes in HDL apolipoprotein gene expression is strengthened by the demonstration that serum levels of apoA-I rose from 60 ± 14 mg/dl ($n = 5$) in hypothyroid animals to 94 ± 27 mg/dl ($n = 4$) in T₃-treated euthyroid animals, and 167 ± 37 mg/dl ($n = 4$) in hyperthyroid animals, ($P < 0.05$).

Regulation of apoE synthesis by thyroid hormone demonstrated the opposite pattern to that described above for apoA-I and apoA-IV. Hypothyroid animals were found to have a twofold elevation in synthesis rates (Table 3). Furthermore, administration of T₃ to produce a chemically euthyroid state restored apoE synthesis rates back to control levels, while administration of pharmacologic doses of T₃ produced 86% suppression of apoE synthesis rates. Despite a sevenfold range of apoE synthesis rates, apoE mRNA abundance was strictly comparable in all four groups irrespective of thyroid hormone status (Table 4). This suggests that apoE gene regulation by thyroid hormone is exerted at a translational or posttranslational level. A striking effect of thyroid hormone administration was encountered in relation to both the rates

of total hepatic apoB synthesis and the forms of apoB elaborated. Synthesis rates of apoB gene products, namely B-100 and B-48, were separately quantitated as detailed in Methods. This revealed a time-dependent synthesis of the molecular species of hepatic apoB as illustrated in Fig. 1A. Control animals demonstrated only apoB-48 following immunoprecipitation of hepatic cytosolic supernatants labeled *in vivo* for 5 min, while at 15 and 30 min, both major forms of apoB were detected.²

Hypothyroid animals were noted to have modest reductions in hepatic synthesis rates of both apoB-(100 + 95) and apoB-48 (Table 3). However, hyperthyroid animals failed to produce detectable (< .01%) apoB-100 at any time from 5 to 60 min following intraportal vein administration of [³H]leucine (Fig. 1B). Despite the absence of detectable newly synthesized apoB-100, hepatic synthesis rates of apoB-48 in hyperthyroid animals were unchanged from those found in untreated hypothyroid animals either when studied after 15 or 30 min *in vivo* radiolabeling, (Table 3, Fig. 1C). ApoB-(100 + 95) synthesis rates in T₃-treated euthyroid animals were similar to those of hypothyroid animals while apoB-48 synthesis rates were restored to control levels (Table 3), suggesting a dose-

²A consistent finding was the presence of a peak or "shoulder" of specifically immunoprecipitable material migrating immediately below the apoB-100 peak; this has been identified previously (5, 41) and referred to as apoB-95. For the purposes of quantitating immunoprecipitable apoB products from hepatocytes, apoB-100 and apoB-95 peaks have been summed and the data are presented as the combined B-(100 + 95) synthesis of the large molecular form of intracellular apoB.

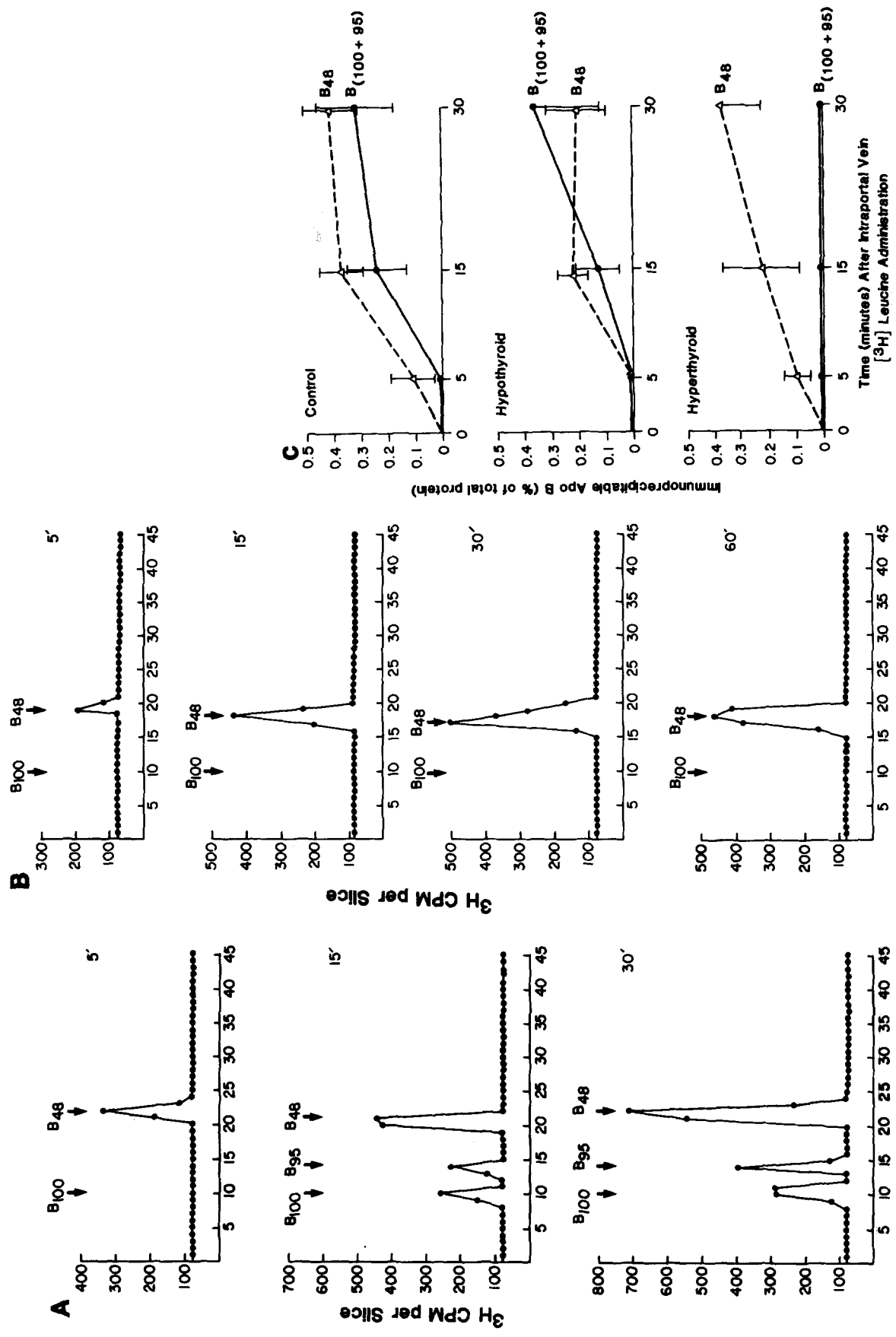


Fig. 1. Hepatic apoB synthesis: effects of thyroid hormone. Animals were maintained on control rat chow (control) or made hypothyroid after consuming chow supplemented with 0.1% PTU for 21–28 days. Animals were administered T₃ by intraperitoneal injection to produce euthyroid or hyperthyroid states, respectively (Methods). Animals received an intraportal vein bolus of [³H]leucine and were exsanguinated at various times as indicated in the figures. Livers were removed and homogenized, and apoB synthesis was determined by immunoprecipitation and SDS-PAGE using 4% acrylamide disc gels (Methods). The migration of apoB-100 and B-48 from serum apoVLDL is indicated by the arrows. A: Control animals; B: T₃-treated hypothyroid animals; C: immunoprecipitable apoB (% of total protein). Methods following in vivo hepatic radiolabeling in control, hypothyroid, and hyperthyroid animals. Data represent the mean ± SD for three to seven animals per time point. Total protein synthesis, as evidenced by [³H]leucine incorporation into TCA-insoluble material, was indistinguishable between control, hypothyroid, and hyperthyroid animals at all time points (P>0.05).

dependent effect of thyroid hormone repletion on apoB gene expression. In a final group of experiments, control chow-fed rats were injected with 50 $\mu\text{g}/100$ g body weight T_3 to induce hyperthyroidism, without antecedent hypothyroidism, and hepatic apoB synthesis was characterized as described above. In four animals so studied, apoB-(100 + 95) synthesis was reduced by 80% to $0.05 \pm 0.02\%$ (control $0.24 \pm 0.13\%$) while apoB-48 synthesis was unchanged from control values ($0.33 \pm 0.03\%$ vs. $0.37 \pm 0.08\%$). Thus, from a procedural standpoint, antecedent hypothyroidism is a requisite for the apparent abolition of apoB-(100 + 95) synthesis following induction of hyperthyroidism, while there appears to be little effect on hepatic apoB-48 synthesis of thyroid hormone administration to previously euthyroid animals.

These changes in apoB synthesis were observed despite no detectable alteration in either total apoB mRNA abundance (Table 4) or the size distribution of hepatic apoB transcript(s) (Fig. 2).

Serum levels of apoB (from animals fasted 16–20 hr) fell from 105 ± 16 mg/dl ($n=5$) to 23 ± 8 mg/dl ($n=4$, $P<0.001$) when hypothyroid rats were made chemically hyperthyroid, changes undoubtedly reflecting both altered synthesis and catabolism. Analysis of the molecular forms of serum apoB revealed reduced amounts of apoB-100 in hyperthyroid rat

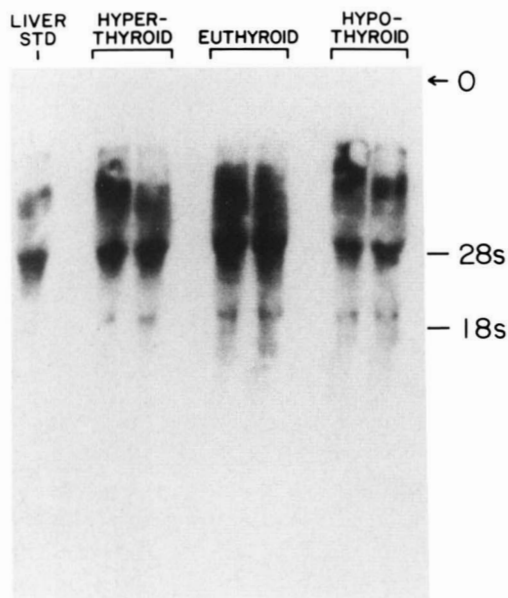


Fig. 2. Northern blot of hepatic RNA from control, hypothyroid, euthyroid, and hyperthyroid animals. Animals were subjected to the experimental manipulations detailed in the legend to Fig. 1. Total hepatic RNA (20 μg) was denatured, electrophoresed through a 0.75% agarose–6% formaldehyde gel, and transferred to nitrocellulose. The blots were probed with a rat 3' apoB cDNA (24). The migration of 28, and 18, ribosomal RNA was determined by ethidium bromide staining of a parallel hepatic RNA sample.

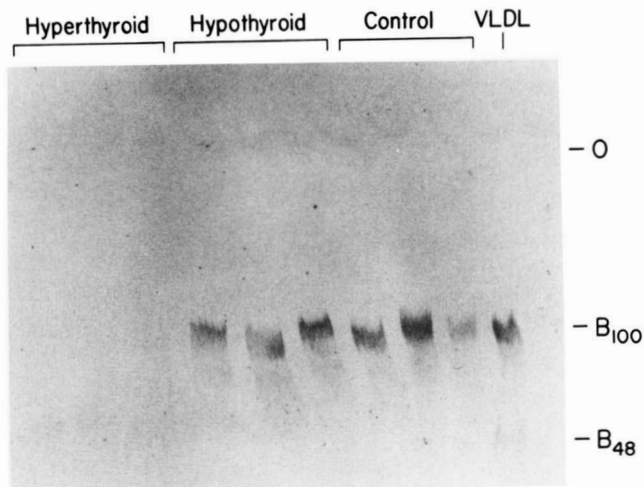


Fig. 3. Immunoblot analysis of molecular forms of serum apoB. One-microliter aliquots of serum from three fasting control, hypothyroid, or hyperthyroid rats (Methods) were electrophoresed through 2% acrylamide–0.5% agarose minislab gels and transferred to nitrocellulose. The membranes were immunostained with rabbit anti-rat apoB-100 antiserum and horseradish peroxidase-linked anti-rabbit IgG, as detailed in Methods, prior to color development with 4-chloro-1-naphthol. The migration of apoB-100 and B-48 is indicated using a coelectrophoresed sample of control serum VLDL ($d<1.020$).

serum compared to control and hypothyroid animals (Fig. 3).

Hepatic triglyceride assembly and secretion

Hepatic microsomes were prepared to examine the extent of fatty acid and triglyceride accumulation and the relative activities of the major enzymes of glycerolipid biosynthesis following alterations in thyroid hormone status and particularly in regard to the apparent absence of apoB-(100 + 95) synthesis in hyperthyroid animals. As illustrated in Table 5, there was a twofold increase in microsomal free fatty acid (FFA) mass in hypothyroid animals compared to control animals. This increase was partially reversed in euthyroid animals and completely reversed in hyperthyroid animals (Table 5). Microsomal triglyceride fatty acid (TG-FA) content, by contrast, was comparable between the four groups. No changes in distribution between different fatty acid species were observed in either FFA or TG-FA classes despite overall changes in the total free fatty acid mass (data not shown).

The specific activities of three enzymes involved in hepatic glycerolipid assembly were examined in relation to the hormonal changes described above. Monoacylglycerol acyltransferase activity was comparable in the four groups (Table 5). Glycerol-3-phosphate acyltransferase specific activity showed a 40% decrease in hypothyroid animals ($P>0.05$) with a twofold increase from these levels observed in hyperthyroid animals

TABLE 5. Hepatic triglyceride assembly and secretion: effects of thyroid hormone

Experimental Group	Microsome Lipid Content		Microsome Enzyme Activity			Plasma Triglyceride Secretion
	FFA	TG-FA	MGAT	G3PAT	DGAT	
	$\mu\text{g FA/mg protein}$			nmol/min per mg		mg/hr per kg
1. Control	153 \pm 16	319 \pm 66	1.59 \pm 0.17	1.37 \pm 0.35	2.03 \pm 0.06	138 \pm 64
2. Hypothyroid	313 \pm 52*	243 \pm 36	1.32 \pm 0.36	0.88 \pm 0.61	2.84 \pm 0.77	77 \pm 34*
3. Euthyroid	258 \pm 52	298 \pm 105	1.55 \pm 0.25	1.40 \pm 0.61	5.68 \pm 1.29**	ND
4. Hyperthyroid	121 \pm 23**	302 \pm 94	1.83 \pm 0.39	2.01 \pm 0.38**	5.54 \pm 0.76**	153 \pm 39**

Animals were maintained on control rat chow (control) or made hypothyroid (groups 2,3,4) after consuming chow supplemented with 0.1% PTU for 21–28 days. Animals were administered T_3 by intraperitoneal injection (groups 3 and 4) to produce euthyroid or hyperthyroid states, respectively (Methods). Hepatic microsomes were prepared from fasting rats ($n = 3$ or 4 per group) and lipid was extracted for assay of both free fatty acid (FFA) and triglyceride fatty acid (TG-FA) mass by GLC (Methods). Microsomal enzymes of hepatic glycerolipid biosynthesis, monoacylglycerol acyltransferase (MGAT), glycerol-3-phosphate acyltransferase (G3PAT), and diacylglycerol acyltransferase (DGAT), activities were assayed in triplicate at two protein concentrations as detailed in Methods, data representing four to eight animals per group. Separate groups of animals ($n = 4$ –8 per group) subjected to identical treatment protocol were administered Triton WR-1339 and plasma triglyceride secretion rates were determined at 2 and 4 hr (Methods). Data are expressed as mean \pm SD, differences detected by independent *t*-test. *, Indicates statistically significant difference from control animals ($P < 0.05$ or greater); **, indicates statistically significant difference from hypothyroid animals ($P < 0.01$ or greater); ND, not determined.

($P < 0.01$), suggesting that aspects of hepatic glycerolipid assembly may be regulated by thyroid hormone. Additionally, the specific activity of diacylglycerol acyltransferase, the only microsomal enzyme exclusive to triglyceride biosynthesis (42), showed a significant increase upon repletion of hypothyroid animals with either euthyroid or hyperthyroid doses of T_3 (Table 5). Finally, direct analysis of total plasma triglyceride secretion rates demonstrated approximately 50% suppression in hypothyroid rats with restoration to control levels in hyperthyroid animals (Table 5).

DISCUSSION

These studies provide evidence of tissue-specific, independent regulation of apolipoprotein gene expression in the rat after changes in thyroid hormone status.

The data confirm and extend our recent observations in regard to alterations in intestinal apoA-I and apoB-48 synthesis by hypothyroid animals (9). The present demonstration of a three- to fourfold range of intestinal apoA-I and apoB-48 synthesis rates despite no alterations in apoA-IV synthesis rates provides a further example of independent regulation of intestinal apolipoprotein gene expression. Previous studies (17, 19, 20) had demonstrated that intestinal apoA-IV synthesis rates were acutely modulated by dietary triglyceride flux while apoA-I and apoB-48 synthesis rates were unchanged. The proximate mechanism(s) whereby thyroid hormone regulates intestinal apoA-I and apoB-48 synthesis remain speculative. It was recently proposed that intestinal apoB-48 synthesis may be related to aspects of mucosal cholesterol flux (9) and in this regard both hypo- and hyperthyroidism

have been demonstrated to affect cholesterol absorption (9, 43). However, in the absence of conclusive evidence concerning the tissue distribution of the putative nuclear thyroid hormone receptor (44, 45), a direct effect of thyroid hormone cannot be excluded.

The data demonstrate important differences in the tissue-specific regulation of apolipoprotein gene expression following alterations in thyroid hormone status. Tissue-specific regulation by thyroid hormone has been described for a number of genes including malic enzyme (46, 47) and myosin heavy chain genes (48, 49), and appears to involve both transcriptional and posttranscriptional events. The effector(s) mediating pretranslational regulation of hepatic apoA-I and apoA-IV gene expression and, in particular, the possibility that the apoC-III gene is regulated by mechanisms operating through chromosomal linkage (50) are currently under investigation.

The data suggest that translational control mechanisms may be of major importance in the regulation of hepatic apoE gene expression. Previous studies (51) had documented a twofold increase in both (in vitro) hepatic apoE biosynthesis and translatable mRNA activity in rats fed an atherogenic diet. More recently, however, other investigators have demonstrated only minimal changes in hepatic apoE mRNA abundance following exposure to an atherogenic diet (52). We have found that rats made hypercholesterolemic in addition to being made hypothyroid (9) manifested a significant (50%) increase in hepatic apoE mRNA abundance (Davidson, N. O., unpublished observations), suggesting that there may be distinct effects of altered sterol flux and thyroid hormone perturbations.

A particularly significant effect of thyroid hormone was encountered in relation to hepatic apoB gene expression. Although a modest decrease was found in

both hepatic apoB-(100 + 95) and apoB-48 synthesis in hypothyroid rats, the most striking effect was encountered in hyperthyroid animals where no newly synthesized apoB-100 could be detected. Rat liver is distinct from many higher species in elaborating both molecular forms of apoB and the current studies represent the first demonstration of a hormonally mediated, regulatory event in apoB transcript processing. The recent demonstration (53, 54) that intestinal apoB-48 arises by co- or posttranscriptional insertion of an in-frame stop codon at nucleotide position 6666 may have important implications in this regard. Hormonal regulation of adult rat hepatic apoB transcript processing may be analogous to the recent report by Glickman, Rogers, and Glickman (55) suggesting that a developmentally regulated switch in apoB synthesis occurs in human fetal gut.

The observations concerning hepatic apoB-100 synthesis additionally offer insight into the role of the two molecular forms of apoB as indispensable adjuncts in the process of normal triglyceride-rich lipoprotein assembly and secretion. Descriptions of at least two syndromes in human subjects (56, 57) have demonstrated that expression of apoB-100 and apoB-48 may be under separate control and that incomplete or abnormal expression of one or other forms may be accompanied by varying degrees of cellular triglyceride accumulation. Previous observations in rats subjected to prolonged fasting also demonstrated divergent regulation of hepatic apoB synthesis (58), specifically a 50% decrease in apoB-48 synthesis, without changes in apoB-100 synthesis, accompanied by a two- to fourfold elevation in apoE synthesis. These findings are similar to the current observations in hypothyroid animals. Furthermore, both this previous description (58) and the present findings were demonstrated in the setting of suppressed hepatic triglyceride assembly and secretion. Taken together, the evidence suggests that aspects of hepatic apolipoprotein gene expression and hepatic glycerolipid assembly may be coordinately regulated. Whether apoB-48 assumes similar intracellular distribution and functions as effectively as apoB-100 in facilitating hepatic VLDL assembly and secretion is unknown at present. These and other questions concerning the molecular basis for the regulatory events described above are the focus of active investigation. ■

Note added in proof: Studies completed after submission of this manuscript indicate that thyroid hormone modulates insertion of a stop codon in rat liver apoB mRNA in a position analogous to that previously identified (53, 54) in human and rabbit small intestine (Davidson, N. O., L. M. Powell, S. C. Wallis, and J. Scott. 1988. *J. Biol. Chem.* **263**: 13482–13485).

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REFERENCES

1. Heimberg, M., J. O. Olubadewo, and H. G. Wilcox. 1985. Plasma lipoproteins and regulation of hepatic metabolism of fatty acids in altered thyroid states. *Endocr. Rev.* **6**: 590–607.
2. Mahley, R. W. 1982. Atherogenic hyperlipidemia. The cellular and molecular biology of plasma lipoproteins altered by dietary fat and cholesterol. *Med. Clin. N. Am.* **66**: 375–402.
3. Keyes, W. G., H. G. Wilcox, and M. Heimberg. 1981. Formation of the very low density lipoprotein and metabolism of [^{14}C]oleate by perfused livers from rats treated with triiodothyronine or propylthiouracil. *Metabolism.* **30**: 135–146.
4. Wilcox, H. G., W. G. Keyes, T. A. Hale, R. Frank, D. W. Morgan, and M. Heimberg. 1982. Effects of triiodothyronine and propylthiouracil on plasma lipoproteins in male rats. *J. Lipid Res.* **23**: 1159–1166.
5. Dolphin, P. J., and S. J. Forsyth. 1983. Nascent hepatic lipoproteins in hypothyroid rats. *J. Lipid Res.* **24**: 541–551.
6. Dolphin, P. J. 1981. Serum and hepatic nascent lipoproteins in normal and hypercholesterolemic rats. *J. Lipid Res.* **22**: 971–989.
7. Swift, L. L., N. R. Manowitz, G. D. Dunn, and V. S. LeQuire. 1980. Isolation and characterization of hepatic Golgi lipoproteins from hypercholesterolemic rats. *J. Clin. Invest.* **66**: 415–425.
8. Swift, L. L., P. D. Soulé, M. E. Gray, and V. S. LeQuire. 1984. Intestinal lipoprotein synthesis. Comparison of nascent Golgi lipoproteins from chow-fed and hypercholesterolemic rats. *J. Lipid Res.* **25**: 1–13.
9. Davidson, N. O., A. M. Magun, T. A. Brasitus and R. M. Glickman. 1987. Intestinal apoA-I and B-48 metabolism: effects of sustained alterations in dietary triglyceride and mucosal cholesterol flux. *J. Lipid Res.* **28**: 388–402.
10. Dory, L., and P. S. Roheim. 1981. Rat plasma lipoproteins and apolipoproteins in experimental hypothyroidism. *J. Lipid Res.* **22**: 287–296.
11. DeLamatre, J. G., and P. S. Roheim. 1981. Effect of cholesterol feeding on apoB and apoE concentrations and distribution in euthyroid and hypothyroid rats. *J. Lipid Res.* **22**: 297–306.
12. Ridgeway, N. D. and P. J. Dolphin. 1985. Serum activity and hepatic secretion of lecithin:cholesterol acyltransferase in experimental hypothyroidism and hypercholesterolemia. *J. Lipid Res.* **26**: 1300–1313.
13. Laker, M. E., and P. A. Mayes. 1981. Effect of hyperthyroidism and hypothyroidism on lipid and carbohydrate metabolism of the perfused rat liver. *Biochem. J.* **196**: 247–255.

14. Lund, H., J. A. Stakkestad, and S. Skrede. 1986. Effects of thyroid state and fasting on the concentrations of CoA and malonyl-CoA in rat liver. *Biochim. Biophys. Acta.* **876**: 685–687.
15. Thompson, G. R., A. K. Soutar, F. A. Spengel, A. Jadhav, S. J. P. Garigan, and N. B. Myant. 1981. Defects of receptor-mediated low density lipoprotein catabolism in homozygous familial hypercholesterolemia and hypothyroidism in vivo. *Proc. Natl. Acad. Sci. USA.* **78**: 2591–2595.
16. Abrams, J. J., and S. M. Grundy. 1981. Cholesterol metabolism in hypothyroidism and hyperthyroidism in man. *J. Lipid Res.* **22**: 323–338.
17. Davidson, N. O., M. E. Kollmer, and R. M. Glickman. 1986. Apolipoprotein B synthesis in rat small intestine: regulation by dietary triglyceride and biliary lipid. *J. Lipid Res.* **27**: 30–39.
18. Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265–275.
19. Davidson, N. O., and R. M. Glickman. 1985. Apolipoprotein A-I synthesis in rat small intestine: regulation by dietary triglyceride and biliary lipid. *J. Lipid Res.* **26**: 368–379.
20. Apfelbaum, T. F., N. O. Davidson, and R. M. Glickman. 1987. Apolipoprotein A-IV synthesis in rat intestine: regulation by dietary triglyceride. *Am. J. Physiol.* **252**: G662–G666.
21. Gordon, J. I., D. P. Smith, R. Andy, D. H. Alpers, G. Schonfeld, and A. W. Strauss. 1982. The primary translation product of rat intestinal apolipoprotein A-I mRNA is an unusual preprotein. *J. Biol. Chem.* **257**: 971–978.
22. Bailey, J. M., and N. Davidson. 1976. Methylmercury as a reversible denaturing agent for agarose gel electrophoresis. *Anal. Biochem.* **70**: 75–85.
23. Demmer, L. A., M. S. Levin, J. Elovson, M. A. Reuben, A. J. Lusis and J. I. Gordon. 1986. Tissue-specific expression and developmental regulation of the rat apolipoprotein B gene. *Proc. Natl. Acad. Sci. USA.* **83**: 8102–8106.
24. Feinberg, A. P., and B. Vogelstein. 1973. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **132**: 6–13.
25. Thomas, P. S. 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. *Proc. Natl. Acad. Sci. USA.* **77**: 5201–5205.
26. Lusis, A. J., R. West, M. Mehrabian, M. A. Reuben, R. C. LeBoeuf, J. A. Kaptein, D. F. Johnson, V. N. Schumaker, M. P. Yahasz, M. C. Schotz, and J. Elovson. 1985. Cloning and expression of apolipoprotein B, the major protein of low and very low density lipoproteins. *Proc. Natl. Acad. Sci. USA.* **82**: 4597–4601.
27. Boguski, M. S., N. Elshourbagy, J. M. Taylor, and J. I. Gordon. 1985. Comparative analysis of repeated sequences in rat apolipoproteins A-I, A-IV and E. *Proc. Natl. Acad. Sci. USA.* **82**: 992–996.
28. Boguski, M. S., N. Elshourbagy, J. M. Taylor, and J. I. Gordon. 1984. Rat apolipoprotein AIV contains 13 tandem repetitions of a 22-amino acid segment with amphipathic helical potential. *Proc. Natl. Acad. Sci. USA.* **81**: 5021–5025.
29. Gordon, J. I., D. H. Alpers, R. K. Ockner, and A. W. Strauss. 1983. The nucleotide sequence of rat liver fatty acid binding protein mRNA. *J. Biol. Chem.* **258**: 3356–3363.
30. McLean, J. W., C. Fukazawa, and J. M. Taylor. 1983. Rat apolipoprotein E mRNA. Cloning and sequencing of double-stranded cDNA. *J. Biol. Chem.* **258**: 8993–9000.
31. Gunning, P. P. Ponte, H. Okayama, J. Engel, H. Blau, and L. Kedes. 1983. Isolation and characterization of full-length cDNA clones for human α -, β -, and γ -actin mRNAs: skeletal but not cytoplasmic actins have an amino-terminal cysteine that is subsequently removed. *Mol. Cell Biol.* **3**: 787–795.
32. Folch, J. L., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226**: 497–509.
33. Schlossman, D. M., and R. M. Bell. 1976. Triacylglycerol synthesis in isolated fat cells. Evidence that the *sn*-glycerol-3-phosphate and dihydroxyacetone phosphate acyltransferase activities are dual catalytic functions of a single microsomal enzyme. *J. Biol. Chem.* **251**: 5738–5744.
34. Coleman, R. A., and E. B. Haynes. 1983. Selective changes in microsomal enzymes of triacylglycerol and phosphatidylcholine synthesis in fetal and postnatal rat liver. *J. Biol. Chem.* **258**: 450–456.
35. Coleman, R. A., and E. B. Haynes. 1986. Monoacylglycerol acyltransferase. Evidence that activities from rat intestine and suckling liver are tissue-specific isoenzymes. *J. Biol. Chem.* **261**: 224–228.
36. Coleman, R. A., J. P. Walsh, D. S. Millington, and D. A. Maltby. 1986. Stereospecificity of monoacylglycerol acyltransferase activity from rat intestine and suckling rat liver. *J. Lipid Res.* **27**: 158–165.
37. Coleman, R. A., and R. M. Bell. 1976. Triacylglycerol synthesis in isolated fat cells. Studies on the microsomal diacylglycerol acyltransferase activity using ethanol-dispersed diacylglycerols. *J. Biol. Chem.* **251**: 4537–4543.
38. Coleman, R. A., E. B. Haynes, and C. D. Coats. 1987. Ontogeny of microsomal activities of triacylglycerol synthesis in guinea pig liver. *J. Lipid Res.* **28**: 320–325.
39. Marsh, J. B. 1986. Hepatic lipoprotein biosynthesis. *Methods Enzymol.* **129**: 498–519.
40. Gabelli, C., D. G. Stark, R. E. Gregg, and H. B. Brewer, Jr. 1986. Separation of apolipoprotein B species by agarose-acrylamide gel electrophoresis. *J. Lipid Res.* **27**: 457–460.
41. Windmueller, H. G., and A. E. Spaeth. 1985. Regulated biosynthesis and divergent metabolism of three forms of hepatic apolipoprotein B in the rat. *J. Lipid Res.* **26**: 70–81.
42. Bell, R. M., and R. A. Coleman. 1980. Enzymes of glycerolipid synthesis in eukaryotes. *Annu. Rev. Biochem.* **49**: 459–487.
43. Mathe, D., and F. Chevallier. 1976. Effects of the thyroid state on cholesterol metabolism in the rat. *Biochim. Biophys. Acta.* **441**: 155–164.
44. Weinberger, C., C. C. Thompson, E. S. Ong, R. Lebo, D. J. Gruol, and R. M. Evans. 1986. The *c-erb-A* gene encodes a thyroid hormone receptor. *Nature.* **324**: 641–646.
45. Glass, C. K., R. Franco, C. Weinberger, V. R. Albert, R. M. Evans, and M. G. Rosenfeld. 1987. A *c-erb-A* binding site in rat growth hormone gene mediates trans-activation by thyroid hormone. *Nature.* **329**: 738–741.
46. Dozin, B., M. A. Magnuson, and V. M. Nikodem. 1986. Thyroid hormone regulation of malic enzyme synthesis.

- Dual tissue-specific control. *J. Biol. Chem.* **261**: 10290–10292.
47. Back, D. W., S. B. Wilson, S. M. Morris, Jr., and A. G. Goodridge. 1986. Hormonal regulation of lipogenic enzymes in chick embryo hepatocytes in culture. *J. Biol. Chem.* **261**: 12555–12561.
48. Izumo, S., B. Nadal-Ginard, and V. Mahdavi. 1986. All members of the MHC multigene family respond to thyroid hormone in a highly tissue-specific manner. *Science*. **231**: 597–600.
49. Gustafson, T., B. E. Markham, J. J. Bahl, and E. Morkin. 1987. Thyroid hormone regulates expression of a transfected myosin heavy-chain fusion gene in fetal heart cells. *Proc. Natl. Acad. Sci. USA*. **84**: 3122–3126.
50. Haddad, I. A., J. M. Ordovas, T. Fitzpatrick, and S. K. Karathanasis. 1986. Linkage, evolution and expression of the rat apolipoprotein A-I, C-III and A-IV genes. *J. Biol. Chem.* **261**: 13268–13277.
51. Lin-Lee, Y.-C., Y. Tanaka, C.-T. Lin, and L. Chan. 1981. Effects of an atherogenic diet on apolipoprotein E biosynthesis in the rat. *Biochemistry*. **20**: 6474–6480.
52. Apostolopoulos, J. J., G. J. Howlett, and N. Fidge. 1987. Effects of dietary cholesterol and hypothyroidism on rat apolipoprotein mRNA metabolism. *J. Lipid Res.* **28**: 642–648.
53. Powell, L. M., S. C. Wallis, R. J. Pease, Y. H. Edwards, T. J. Knott, and J. Scott. 1987. A novel form of tissue-specific RNA processing produces apolipoprotein B-48 in intestine. *Cell*. **50**: 831–840.
54. Chen, S.-H., G. Habib, C.-Y. Yang, Z.-W. Gu, B. R. Lee, S.-A. Weng, S. R. Silberman, S.-J. Cai, J. P. Deslypere, M. Rosseneu, A. M. Gotto, Jr., W.-H. Li, and L. Chan. 1987. Apolipoprotein B-48 is the product of a messenger RNA with an organ-specific in-frame stop codon. *Science*. **238**: 363–366.
55. Glickman, R. M., M. Rogers, and J. N. Glickman. 1986. Apolipoprotein B synthesis by human liver and intestine in vitro. *Proc. Natl. Acad. Sci. USA*. **83**: 5296–5300.
56. Malloy, M. J., J. P. Kane, A. Hardman, R. L. Hamilton, and K. B. Dalal. 1981. Normotriglyceridemic abetalipoproteinemia. *J. Clin. Invest.* **67**: 1441–1450.
57. Herbert, P. N., J. S. Hyams, D. N. Bernier, M. M. Beriman, A. L. Saritelli, K. M. Lynch, A. V. Nichols, and T. M. Forte. 1985. Apolipoprotein B-100 deficiency. Intestinal steatosis despite apolipoprotein B-48 synthesis. *J. Clin. Invest.* **76**: 403–412.
58. Davis, R. A., J. R. Boogaerts, R. A. Borchardt, M. Malone-McNeal, and J. Archambault-Schexnayder. 1985. Intrahepatic assembly of very low density lipoproteins. Varied synthetic response of individual apolipoproteins to fasting. *J. Biol. Chem.* **260**: 14137–14144.