

# Effects of dietary cholesterol and hypothyroidism on rat apolipoprotein mRNA metabolism

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**Abstract** The effects of dietary cholesterol and hypothyroidism on the mRNA levels of rat apolipoproteins A-I, A-IV, and E were measured in extracts of rat liver and rat intestine by hybridization to specific cDNA. Four groups, each comprised of six rats, were fed diets consisting of normal laboratory rat chow and either i) no supplements (control); ii) 5% lard, 1% cholesterol, and 0.3% taurocholic acid (CF); iii) 5% lard, 1% cholesterol, 0.3% taurocholic acid, and 0.1% propylthiouracil (CF-PTU); or iv) 0.1% propylthiouracil (PTU) for 32 days. At the conclusion of the diets, serum cholesterol, triiodothyronine, and thyroxine levels were measured. The average serum cholesterol concentrations for the four groups were  $50.4 \pm 3.7$ ,  $75.6 \pm 15.3$ ,  $135.3 \pm 41.5$ , and  $73.3 \pm 16.4$  mg/dl, respectively. The presence of propylthiouracil in the diets significantly lowered triiodothyronine and thyroxine levels in the serum. The mRNA levels for apolipoproteins A-I and A-IV in rat liver decreased significantly after the feeding of the CF-PTU diet ( $31 \pm 4\%$  and  $32 \pm 3\%$  of normal, respectively) and the PTU diet ( $34 \pm 8\%$  and  $43 \pm 12\%$  of normal, respectively), but showed little change after the CF diet ( $88 \pm 16\%$  and  $108 \pm 15\%$  of normal, respectively). The effects of dietary propylthiouracil on the hepatic mRNA levels for apolipoproteins A-I and A-IV imply a role for thyroid hormones in regulating the mRNA levels for these apolipoproteins in rat liver. ApoE mRNA levels in the rat liver decreased slightly after the CF-PTU diet ( $74 \pm 12\%$  of normal) and after the PTU diet ( $73 \pm 10\%$  of normal). These changes are similar to those observed for rat serum albumin ( $64 \pm 7\%$  and  $66 \pm 8\%$  of normal, respectively). A small increase in apoE mRNA levels was observed following the CF diet ( $114 \pm 13\%$  of normal). The intestinal mRNA levels of apolipoproteins A-I, A-IV, and E, and the serum concentrations of apolipoproteins A-I and A-IV did not change significantly for any of the dietary groups. — **Apostolopoulos, J. J., G. J. Howlett, and N. Fidge.** Effects of dietary cholesterol and hypothyroidism on rat apolipoprotein mRNA metabolism. *J. Lipid Res.* 1987. **28**: 642–648.

**Supplementary key words** apolipoproteins • hypothyroidism • cholesterol • thyroxine

Although used extensively as an animal model for investigating lipoprotein metabolism, the rat is particularly refractive to atherogenic diets which produce hypercholesterolemia and varying degrees of atherosclerosis when administered to other species. Earlier studies (1–4)

have found that the natural resistance to fat-induced hypercholesterolemia, which is displayed by rats, can be overcome by the addition of propylthiouracil to a diet rich in fat and cholesterol. The subsequent elevation in serum cholesterol levels is accompanied by increased lipoprotein concentrations and by changes in lipoprotein composition, not the least of which is the accumulation of apoE-rich very low density lipoprotein and cholesterol-rich high density lipoprotein (1).

The suggestion that these changes may be attributable to altered apolipoprotein metabolism, brought about by one or several components in the atherogenic diet, has been investigated in this study by feeding different elements of the diet to different groups of animals. We have used cytoplasmic dot hybridization (5–7) to determine the amount of specific mRNA for apolipoproteins in different rat tissues. It was found that hypothyroidism decreased the mRNA levels of apoA-I and apoA-IV in the liver, while apoE mRNA levels remained relatively constant. Cholesterol diets (without propylthiouracil) had little effect on any of the apolipoprotein mRNA levels. The results suggest that thyroid hormones may play an important role in regulating the mRNA levels of specific apolipoproteins.

## EXPERIMENTAL PROCEDURES

### Materials

Nitrocellulose membrane filters (BA85,  $0.45 \mu\text{m}$ ) were purchased from Schleicher and Schuell (West Germany) and [ $\alpha$ - $^{32}\text{P}$ ]dATP and [ $\alpha$ - $^{32}\text{P}$ ]dCTP (specific activity

Abbreviations: apo, apolipoprotein; CF, cholesterol-fed; CF-PTU, cholesterol-fed + propylthiouracil; PTU, propylthiouracil; SSC, standard saline citrate.

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> 1700 Ci/mmol) were from Bresa (South Australia). Taurocholic acid and propylthiouracil were purchased from Sigma (St. Louis, MO); cholesterol and EDTA were from Ajax Chemicals (Sydney, Australia). Oligo (dT)<sub>30</sub> was kindly provided by Peter Roche (Howard Florey Institute).

#### Identification of cDNA clones

The isolation of a cDNA clone for apolipoprotein E has been reported previously (8). cDNA clones for human apoA-I and apoA-IV were initially isolated by antibody screening of a human cDNA library constructed in the expression vector  $\lambda$ gt11-Amp3 (9). The cDNA inserts from these clones were isolated and used to produce radioactive probes to identify the corresponding cDNA clones from a rat liver cDNA library (8). Restriction mapping (10) indicated that the rat apoA-I cDNA clone coded for amino acids 1–260 of the mature protein. Nucleotide sequencing and restriction mapping showed that the rat apoA-IV cDNA coded for amino acids 110–380 of the mature protein (11). Cloned cDNA for rat serum albumin and rat transferrin have been reported previously (12, 13). Experiments were carried out under the C1 containment conditions of the Committee on Recombinant DNA Molecules of the Australian Academy of Science.

#### Preparation of <sup>32</sup>P-labeled DNA

<sup>32</sup>P-labeled cDNA probes were prepared by nick translation (14) of approximately 50 ng of cDNA. Specific activities of approximately  $2 \times 10^8$  cpm/ $\mu$ g of DNA were routinely obtained. <sup>32</sup>P-labeled oligo (dT)<sub>30</sub> was end-labeled using T4 polynucleotide kinase (14).

#### Animals and diets

Male Buffalo rats approximately 10 weeks old and weighing between 210 and 290 g were divided into four groups, each with six rats, and fed separate diets ad libitum consisting of normal laboratory rat chow and either i) no supplements (control); ii) 5% lard, 1% cholesterol, and 0.3% taurocholic acid (CF); iii) 5% lard, 1% cholesterol, 0.3% taurocholic acid, and 0.1% propylthiouracil (CF-PTU); or iv) 0.1% propylthiouracil (PTU). After a period of 32 days on the diets, the animals were fasted overnight and the required tissues were removed while the rats were under ether anaesthesia. Serum samples were kept at 4°C for subsequent assays. In addition to these studies, a group of eight male Buffalo rats (ca. 200 g) were given daily subcutaneous injections of thyroxine (40  $\mu$ g/100 g body weight) for 21 days while being fed a normal laboratory rat chow. The animals were killed and tissues were collected as described above.

#### Preparation of extracts from liver using the Tris/EDTA method

Tissues were suspended in 10 mM Tris-HCl, pH 7.0, 1 mM EDTA, 0.5% Nonidet P-40 (10 ml/g wet tissue weight) and homogenized in a 30-ml Potter Elvehjem homogenizer (7, 15). Homogenates were centrifuged at 4°C (15,000 g, 15 min) and aliquots of the supernatants were taken. To 1 ml of supernatant, 600  $\mu$ l of  $20 \times$  standard saline citrate (SSC) (3 M NaCl, 0.3 M trisodium citrate) and 400  $\mu$ l of 37% (w/v) formaldehyde were added. The mixture was incubated at 60°C for 15 min, and centrifuged at 4°C (15,000 g, 15 min). Aliquots of the supernatants (Tris/EDTA extracts) were stored at -70°C. Control samples were treated with 100  $\mu$ l of ribonuclease A (10 mg/ml) and with 400  $\mu$ l of cold 10 mM Tris-HCl, pH 7.0, 1 mM EDTA, 0.5% Nonidet. The mixture was incubated at 37°C for 1 hr and centrifuged at 4°C (15,000 g, 15 min). To 1 ml of supernatant, 600  $\mu$ l of  $20 \times$  SSC and 400  $\mu$ l of 37% (w/v) formaldehyde were added. The mixture was centrifuged at 4°C (15,000 g, 15 min). Aliquots of all supernatants were stored at -70°C.

#### Preparation of extracts from liver and small intestine using the guanidine thiocyanate method

Samples of the small intestine were removed by taking a 3.5-cm segment immediately distal to the stomach (duodenum), a 20-cm segment 10 cm distal to the duodenum (jejunum), and a 20-cm segment 10 cm distal to the middle of the small intestine (ileum). The intestinal lumen was flushed with a solution of 0.9% NaCl to wash out the contents. The tissue (liver or intestine) was suspended in guanidine thiocyanate buffer (10 ml/g wet tissue) (4 M guanidine thiocyanate, 5 mM trisodium citrate, pH 7.0, 0.1 M 2-mercaptoethanol, 0.5% N-laurylsarcosine, and 0.335 ml antifoam A emulsion (Sigma)) and homogenized in sterile 20-ml glass tubes for 30 sec using a Polytron Ystral homogenizer (6). Homogenates were then treated with formaldehyde and centrifuged as described above for the Tris/EDTA extracts. Aliquots of the supernatants were stored at -70°C.

#### Detection of the mRNA in tissue extracts

Tris/EDTA extracts were diluted 1:50 with  $15 \times$  SSC; guanidine thiocyanate extracts were diluted 1:75 with  $15 \times$  SSC and guanidine thiocyanate dilution buffer (0.1 M guanidine thiocyanate, 0.1 M 2-mercaptoethanol, 2.25 M NaCl, 0.22 M trisodium citrate) prior to spotting onto nitrocellulose membrane filters in a 96-well Bio-Rad Bio-Dot apparatus. The nitrocellulose sheets, after baking under vacuum at 80°C for 2 hr, were prehybridized in a

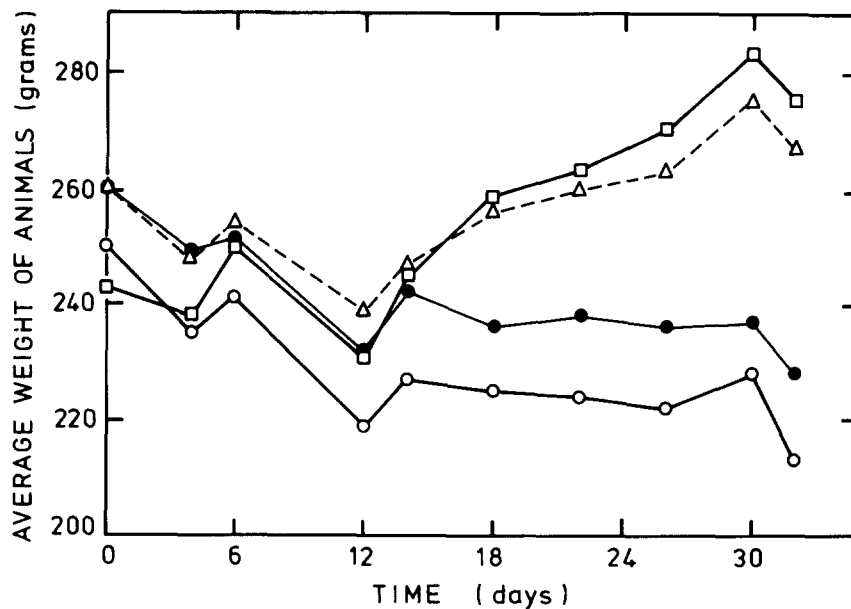


Fig. 1. Changes in the average body weight of animals during the 32-day diet period. Each of the four groups contained six animals. The individual groups are: (Δ) control, (●) CF-PTU, (□) CF, and (○) PTU.

buffer containing 50% formamide, 0.75 M trisodium citrate, 0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin, 0.1% Ficoll, 50 mM  $\text{NaH}_2\text{PO}_4$ , pH 6.5, and 250  $\mu\text{g/ml}$  sonicated denatured sperm DNA for 4 hr at  $42^\circ\text{C}$ . Radiolabeled cDNA probes were denatured at  $100^\circ\text{C}$  for 10 min, chilled, and added to the hybridization buffer (50% formamide, 0.75 M NaCl, 0.075 M trisodium citrate, 0.01% Ficoll, 0.01% polyvinylpyrrolidone, 0.01% bovine serum albumin, 20 mM  $\text{NaH}_2\text{PO}_4$ , pH 6.5, 100  $\mu\text{g/ml}$  sonicated denatured herring sperm DNA). The filters were hybridized for 16 hr at  $42^\circ\text{C}$  and then washed three times at room temperature in  $2 \times \text{SSC}$ , 0.1% sodium dodecyl sulfate, for a total of 15 min. This was followed by two washes at  $50^\circ\text{C}$  in 15 mM NaCl,  $0.1 \times \text{SSC}$ , 0.1% sodium dodecyl sulfate for a total of 30 min. The filters were air-dried, and autoradiographed at  $-70^\circ\text{C}$  using Kodak XAR film and an intensifying screen. After autoradiography, areas of the nitrocellulose with hybridized cDNA were excised and the bound radioactivity was determined by liquid scintillation spectrometry. Total poly A<sup>+</sup> mRNA was detected as described above using  $^{32}\text{P}$ -labeled oligo (dT)<sub>30</sub> with the exception that the  $50^\circ\text{C}$ -wash steps were omitted.

#### Estimation of the serum concentrations of cholesterol, triglyceride, triiodothyronine, and thyroxine

Triglyceride and cholesterol concentrations were determined enzymatically by the methods of Bucolo and David (16) and Allain et al. (17), respectively, using a Technicon AutoAnalyzer (Baker Medical Research Institute, Mel-

bourne). Serum triiodothyronine and thyroxine were measured by double antibody radioimmunoassay of unextracted serum (18). Assay coefficients of variation ( $n = 10$ ) were 5.8% and 5.2% for  $\text{T}_3$  and  $\text{T}_4$ , respectively, for rat serum.

## RESULTS

### Effects of diet on animal body weight

Fig. 1 illustrates the changes seen in the average body weight of the four dietary groups over a period of 32 days. After 32 days, the control and CF groups showed increases in the average mean weights of 12% and 3%, respectively, whereas the CF-PTU and PTU groups lost 12% and 15% weight, respectively.

TABLE 1. Serum concentrations of cholesterol and triglyceride in control, CF-PTU-, PTU-, and CF-treated rats<sup>a</sup>

Diet	Cholesterol	Triglyceride
Control	50.4 ± 3.7	82.0 ± 44.3
CF-PTU	135.3 ± 41.5 <sup>b</sup>	55.0 ± 20.0
PTU	73.3 ± 16.4 <sup>b</sup>	59.6 ± 35.4
CF	75.6 ± 15.3 <sup>b</sup>	134.4 ± 50.1

<sup>a</sup>Values are expressed as the mean values ± standard deviation obtained using six animals per group, fed diets for 32 days.

<sup>b</sup>Significantly different from controls ( $P < 0.005$ ).

**Effects of diet on the serum concentrations of cholesterol, triglyceride, triiodothyronine, and thyroxine**

The serum concentrations of cholesterol and triglyceride in the different dietary groups are shown in **Table 1**. The largest change in the serum cholesterol levels occurred in the CF-PTU group where there was a 2.7-fold average increase. An average increase of 1.5-fold and 1.45-fold in the serum cholesterol levels occurred in the CF and PTU groups, respectively. Triglyceride concentrations (**Table 1**) in CF animals increased to an average of 134.4 mg/dl, while the concentrations in both CF-PTU and PTU-treated animals decreased from an average of 82.0 mg/dl, for control animals, to 55.0 and 59.6 mg/dl, respectively. The decrease in triglyceride levels in hypothyroidism has been attributed to an overall increase in the peripheral lipoprotein lipase activity (19). The effectiveness of propylthiouracil in inducing hypothyroidism was assessed by determining the serum concentrations of the thyroid hormones triiodothyronine (T<sub>3</sub>) and thyroxine (T<sub>4</sub>). The results in **Table 2** show that the levels of T<sub>3</sub> and T<sub>4</sub> decreased by 33% and 87%, respectively, in the PTU group, and 56% and 91%, respectively, in the CF-PTU group.

**Effects of diet on mRNA levels for rat apolipoproteins A-I, A-IV, and E**

The effects of diet on the mRNA levels for rat apolipoproteins A-I, A-IV, and E were determined by cytoplasmic dot hybridization (5-7). **Fig. 2** shows the

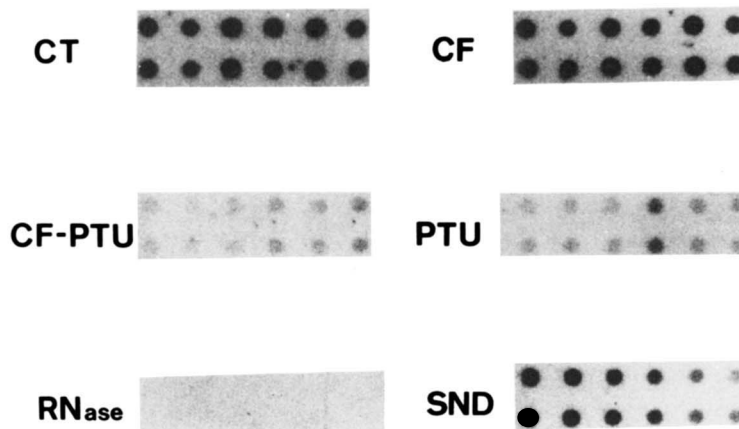
**TABLE 2.** Serum concentrations of triiodothyronine and thyroxine in control, CF-PTU-, PTU-, and CF-treated rats<sup>a</sup>

Diet	Triiodothyronine	Thyroxine
	ng/dl	µg/dl
Control	59.0 ± 2.6	5.4 ± 0.3
CF-PTU	26.2 ± 1.3 <sup>b</sup>	0.5 ± 0.05 <sup>b</sup>
PTU	39.3 ± 6.2 <sup>b</sup>	0.7 ± 0.2 <sup>b</sup>
CF	59.0 ± 2.6	5.0 ± 0.1

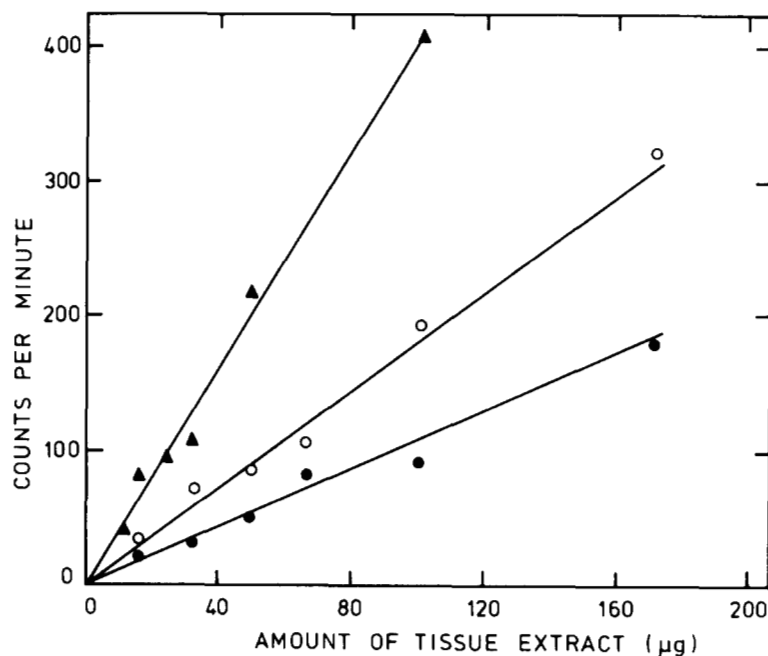
<sup>a</sup>Values are expressed as the mean values ± standard error obtained using six animals per group, fed diets for 32 days.

<sup>b</sup>Significantly different from controls (*P* < 0.001).

autoradiograph obtained for extracts of liver probed with <sup>32</sup>P-labeled apoA-I cDNA. To show that the signals on the autoradiograph were due to hybridization of the probe to RNA, ribonuclease-treated samples were applied to the filter (RNase panel). The absence of a signal in the ribonuclease-treated samples indicates that the hybridization observed in the other panels was due to the presence of RNA. The radioactivity of the nitrocellulose filters corresponding to the autoradiographic signals from the standard (SND) panel was analyzed by liquid scintillation spectrometry. The results (**Fig. 3**) show that a linear relationship exists between the bound radioactivity and the amount of tissue extract applied to the filter. The spots corresponding to the liver samples for the CF-PTU and PTU groups (**Fig. 2**) show a significant decrease in intensity, compared to the control group. The intensity of the spots in the panel for the CF group remained relatively



**Fig. 2.** Determination of the effect of diet on mRNA levels of rat apoA-I in liver extracts by hybridization to specific <sup>32</sup>P-labeled cDNA. The SND panel corresponds to cytoplasmic extracts spotted in serial dilutions for a standard curve; 167, 100, 67, 50, 33, and 25 µg of liver, wet weight, per spot (from left to right). The CT panel corresponds to the control group while the CF, CF-PTU, and PTU panels correspond to the respective diet groups. Each spot in these panels corresponds to 100 µg of liver per spot. Autoradiography at -70°C, using Kodak XAR film and an intensifying screen, was for 5 days. Extracts were prepared from six animals per group, fed the particular diet for 32 days, using the guanidine thiocyanate method (as described in Experimental Procedures). The RNase panel represents cytoplasmic extracts incubated with ribonuclease prior to processing for hybridization.



**Fig. 3.** A standard curve obtained from a dot hybridization experiment using  $^{32}\text{P}$ -labeled rat apoA-I cDNA. The results were obtained by adding different amounts of extracts to the nitrocellulose filters and determining the amount of radioactivity (counts per minute) following hybridization. The amount of liver extract applied was 167, 100, 67, 50, 33, and 25  $\mu\text{g}$ . The amounts of intestinal extract applied were 100, 50, 33, 25, 17, and 12.5  $\mu\text{g}$ . Liver ( $\circ$ ) and intestinal ( $\blacktriangle$ ) extracts prepared by the guanidinium thiocyanate method; liver ( $\bullet$ ) extracts prepared by the Tris/EDTA method. Extracts were prepared from one animal in the control group.

similar to the control group. The decreases in the liver mRNA levels of apoA-I (as measured by liquid scintillation spectrometry) were to 31% and 34% of normal for the CF-PTU and PTU groups, respectively (Table 3). The decreases compared to the normal group were significantly different ( $P < 0.001$ ). The mRNA levels of apoA-IV (Table 3) also decreased significantly in the CF-PTU (32% of normal) and PTU (43% of normal) groups. The hepatic mRNA levels of the CF group for both apoA-I and A-IV remained relatively constant. The level of total poly A<sup>+</sup> mRNA and the mRNA levels for apoE in the CF-PTU and PTU groups (Table 3)

decreased slightly. These changes were similar to those observed for transferrin and albumin (Table 4).

The values observed for rat serum albumin in the CF-PTU and PTU groups are consistent with the observations of Williams (20). The intestinal mRNA levels (Table 4) show that apoA-I, A-IV, and E mRNA levels for the CF-PTU, PTU, and CF groups remained relatively constant.

#### Effects of diet on the serum concentrations of rat apolipoproteins A-I and A-IV

The serum concentrations of apolipoproteins A-I and A-IV were determined by the method of rocket immunoelectrophoresis (21). The results (Table 5) show that the diets produced little change in the serum concentrations of apoA-I and A-IV.

#### Effects of thyroxine injection on the mRNA levels for rat apolipoprotein A-I

Hyperthyroidism was induced in eight rats by daily subcutaneous injections of thyroxine for a total of 21 days. Thyroxine levels in the serum increased from  $2.01 \pm 0.29$   $\mu\text{g}/\text{dl}$  in controls to  $3.83 \pm 0.82$   $\mu\text{g}/\text{dl}$  in injected animals. The hepatic apolipoprotein A-I mRNA levels for the thyroxine-treated group was  $352 \pm 55\%$  of normal ( $P < 0.001$ ). A small, but not significant, decrease was seen in the intestinal mRNA levels for apoA-I ( $84 \pm 5\%$  of normal).

**TABLE 3.** Effect of diet on liver mRNA levels of rat apolipoproteins A-I, A-IV, and E<sup>a</sup>

Diet	Relative mRNA Levels <sup>b</sup>			
	ApoA-I	ApoA-IV	ApoE	Poly A <sup>+</sup> RNA
Control	100 $\pm$ 9%	100 $\pm$ 7%	100 $\pm$ 6%	100 $\pm$ 5%
CF-PTU	31 $\pm$ 4% <sup>c</sup>	32 $\pm$ 3% <sup>c</sup>	74 $\pm$ 12%	65 $\pm$ 8%
PTU	34 $\pm$ 8% <sup>c</sup>	43 $\pm$ 12% <sup>c</sup>	73 $\pm$ 10%	72 $\pm$ 6%
CF	88 $\pm$ 16%	108 $\pm$ 15%	114 $\pm$ 13%	131 $\pm$ 22%

<sup>a</sup>Extracts were prepared using the guanidinium thiocyanate method as described in Experimental Procedures.

<sup>b</sup>Values are expressed as a percentage of the mRNA levels  $\pm$  standard error obtained using extracts from control animals, using six animals per group, fed diets for 32 days.

<sup>c</sup>Significantly different from control group ( $P < 0.001$ ).

## DISCUSSION

The results in Table 3 indicate that there is a significant decrease in liver mRNA levels of apoA-I and A-IV after the hypothyroid (CF-PTU and PTU) diets, while mRNA levels after the CF diet remain relatively constant. The liver mRNA levels of apoE (Table 3) decrease slightly for the hypothyroid diets. The observation that apoE mRNA levels decrease slightly in the liver after the CF-PTU diet may be compared to the findings of Lin-Lee et al. (3). These authors used the method of *in vitro* translation to show that there was a twofold increase in the levels of translatable apoE mRNA. Their observation may reflect a modification in the translational efficiency of pre-existing mRNA, but further studies would be needed to determine this. Studies of apoE serum levels show that rats fed diets supplemented with either propylthiouracil or a combination of propylthiouracil and cholesterol have increased serum levels of apoE, while rats fed with cholesterol only show a decrease (3, 4). During hypothyroidism in the rat there is a decreased removal of lipoproteins from the circulation (19). This may account for increased apoE serum levels rather than any major change in apoE output.

The changes in liver mRNA levels for apoA-I and apoA-IV (Table 3) do not parallel those found in the intestine (Table 4). The intestinal mRNA levels remain relatively constant. This may imply that the mechanisms for regulating the mRNA levels of apoA-I and A-IV in the liver act independently of the mechanisms in the intestine.

The results in Fig. 1 show that the average weight of the CF-PTU and PTU groups decreased by 12% and 15%, respectively. This weight loss is consistent with previous observations that hypothyroid animals decrease their food intake (22, 23). This could mean that a decrease in food

TABLE 5. Serum concentrations of rat apolipoproteins A-I and A-IV in control, CF-PTU-, PTU-, and CF-treated rats

Diet	Serum Concentrations of Apolipoproteins <sup>a</sup>	
	A-I	A-IV
	mg/dl	
Control	99.0 ± 13.0	16.7 ± 1.0
CF-PTU	93.0 ± 18.0	17.6 ± 2.9
PTU	80.0 ± 8.5	16.3 ± 1.0
CF	93.8 ± 5.0	18.4 ± 4.0

<sup>a</sup>Values are expressed as mean values ± standard error obtained from six animals per group, after feeding diets for 32 days.

intake may be responsible for the decrease seen in the liver mRNA levels of apoA-I and A-IV. However, our recent studies show no significant changes in the mRNA levels of apoA-IV in the liver or intestine for rats that had been deprived of food for 48 hr (deJong, F. A., G. J. Howlett, and G. Schreiber, unpublished data).

A conclusion that can be made from our results is that the addition of the hypothyroid drug propylthiouracil has an effect on the liver mRNA levels for apoA-I and A-IV. These results imply a role for the thyroid hormones in regulating the synthesis of these apolipoproteins in rat liver. This conclusion is supported by the studies (Results section) with hyperthyroid rats where the hepatic apoA-I mRNA levels increased almost fourfold after daily injections with thyroxine. The thyroid hormones can act at a pretranslational level to affect gene expression (24). This action is thought to be mediated by the binding of T<sub>3</sub> to a chromatin-associated receptor found in the responsive tissue. It is believed that the nuclear receptor-T<sub>3</sub> complex causes specific alterations in the rates of synthesis of

TABLE 4. Effect of diet on liver mRNA levels for rat serum albumin (RSA) and transferrin and intestinal mRNA levels for apolipoproteins A-I, A-IV, and E

Plasma Protein	Relative mRNA Levels in the Various Diet Groups <sup>a</sup>			
	Control	CF-PTU	PTU	CF
Intestinal mRNA levels				
ApoA-I	100 ± 9%	100 ± 23%	89 ± 15%	81 ± 17%
ApoA-IV	100 ± 7%	94 ± 27%	78 ± 15%	122 ± 42%
ApoE	100 ± 11%	92 ± 12%	84 ± 8%	119 ± 7%
Poly A <sup>+</sup> RNA	100 ± 4%	94 ± 4%	91 ± 6%	136 ± 14%
Liver mRNA levels				
RSA <sup>b</sup>	100 ± 8%	64 ± 7%	66 ± 8%	123 ± 6%
Transferrin <sup>b</sup>	100 ± 6%	88 ± 11%	59 ± 19%	80 ± 25%

<sup>a</sup>Values are expressed as in Table 3, using six animals per group, fed diets for 32 days.

<sup>b</sup>Extracts were prepared by the Tris/EDTA method, as described in Experimental Procedures.

nuclear RNA (25). Subsequently, there are changes in specific cytoplasmic mRNA levels. ■■

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