ELSEVIER

Vitamin A deficiency increases hepatic apolipoprotein A-I mRNA expression in both euthyroid and hypothyroid rats

Reza Zolfaghari*† and A. Catharine Ross*†

**The Department of Biochemistry, The Medical College of Pennsylvania, Philadelphia, PA, and †the Department of Nutrition, Pennsylvania State University, University Park, PA, USA*

We have examined the effects of chronic thyroid hormone deficiency, vitamin A deficiency, and subsequent acute repletion with thyroid hormone (T_3) and/or retinoic acid (RA) on the expression of hepatic apolipoprotein (apo) A-I mRNA. Chronically hypothyroid and/or retinoid-deficient male Lewis rats were treated once or three times with T_3, all-trans-RA, or both hormones. The hypothyroid state significantly reduced ($P < 0.001$) and the vitamin A-deficient state significantly elevated ($P < 0.0001$) the expression of apo A-I mRNA. A single injection of T_3 (10 μg/100 gm body weight) significantly elevated apo A-I mRNA in hypothyroid rats ($P < 0.001$). A single injection of RA (20 μg/rat) reduced apo A-I mRNA by about 20 to 25% and daily injection of RA for three days reduced apo A-I mRNA abundance further in hypothyroid ($P < 0.05$), but not euthyroid, rats. There was a significant interaction between T_3 hormone treatment and vitamin A status of the rats, as well as between RA and T_3 treatment and the thyroid status of the rats. These in vivo results may indicate that nuclear retinoid receptors and thyroid hormone receptors interact in the regulation of the apo A-I gene in liver. (J. Nutr. Biochem. 8:451–455, 1997) © *Elsevier Science Inc. 1997*

Keywords: apolipoprotein A-I; vitamin A deficiency; thyroid hormone; retinoic acid; rat

Introduction

Apolipoprotein A-I (apo A-I), the major protein of high density lipoprotein (HDL), has been implicated in the process of "reverse cholesterol transport" [see Ref. 1 for review]. HDL transports cholesterol from peripheral tissues to the liver where it may be metabolized and excreted as bile acids. Apo A-I is primarily expressed in the small intestine and liver[2] and its expression has been shown to be regulated by both dietary factors and hormones.[3–16] Two such hormones are RA and thyroid hormone (T_3). Previous studies have shown that the expression of apo A-I mRNA is increased in the liver of intact, retinoid-deficient rats, and is reduced toward normal after retinoid-deficient rats are treated with all-*trans*-RA.[14–17]

It is well known that RA can activate retinoid-responsive genes through two classes of nuclear receptors which function as ligand-activated transcription factors.[18,19] All-*trans*-RA and 9-*cis*-RA both may serve as a high-affinity ligand for the RA receptors (RAR alpha, beta, and gamma), whereas only 9-*cis*-RA is a high-affinity ligand for the retinoid X receptors (RXR alpha, beta, and gamma). Both the RAR and RXR are members of the thyroid/steroid hormone receptor superfamily. The retinoid receptors regulate the expression of target genes by binding as dimers to specific regulatory regions of DNA (retinoid response elements).[19] In addition to forming dimers with RAR, the RXR proteins form heterodimeric partners with some of the other members of the steroid/thyroid hormone family, including the thyroid hormone receptors (TRs), vitamin D receptor, peroxisome-proliferator activated receptors, and others.[18] The RARs and TRs share considerable structural homology, for example, more than 60% of the amino acid residues in the DNA binding domain of RAR-alpha are identical to those in the DNA binding domain of TR-beta.[18] There also may be functional interactions between these receptors. The RAR and TR may interact to activate specific thyroid hormone response elements,[20] or the TR may function antagonistically to reduce the induction of RAR or RXR-mediated gene expression.[18,21,22] Moreover, members

Address reprint requests and correspondence to A. Catharine Ross, Ph.D., Department of Nutrition, S-126 Henderson Building, Pennsylvania State University, University Park, PA 16802, USA.
Received February 19, 1997; accepted April 30, 1997.

655 Avenue of the Americas, New York, NY 10010

0955-2863/97/$17.00
PII S0955-2863(97)00055-7

of the RXR subfamily may activate the expression of certain retinoid-responsive and thyroid hormone-responsive genes directly through the formation of RAR-RXR or TR-RXR heterodimers, or they may function as negative regulators by binding with and sequestering the RAR or TR proteins.[18] Finally, RXR gene expression itself may be regulated in vivo by thyroid status as well as the administration of T_3 hormone.[23]

The regulatory region of the hepatic apo A-I gene, located immediately upstream of the gene's promoter, contains nucleotides sequences that are recognized by RXR and RAR.[24–26] Additionally, in vivo studies have shown that thyroid status can regulate hepatic apo A-I gene expression through both transcriptional and post-transcriptional mechanisms.[4–6,10,12,27] With this background, the present experiments were designed to evaluate the effects and possible interaction of thyroid status and retinoid status on the hepatic expression of apo A-I mRNA in intact adult rats. We also have examined the effects of acute in vivo treatment with RA and T_3, either alone or in combination, on the level of apo A-I mRNA in retinoid-deficient rats.

Methods and materials

Animals and diets

The experimental animals and diets used in this study have been reported elsewhere.[28,29] Briefly, lactating dams of the Lewis strain with litters of ten 2-to-5-day-old male pups were purchased from Charles River Breeding Laboratory (Wilmington, DE USA) and fed a vitamin A-free diet on arrival. When the pups were 20 to 21 days old, 42 of them were weaned onto the same vitamin A-free diet and 12 were given diet of the same composition except that it contained 4 mg of retinol, as retinyl palmitate, per kilogram of diet. Half of the rats undergoing retinoid depletion and nine of the vitamin A-sufficient rats were made hypothyroid by the inclusion of 0.05% of propylthiouracil (PTU) in their drinking water. When the rats were 55 to 61 days old, they were treated either once or three times with 20 μg RA and/or 10 μg/100 g of T_3, or with vehicle only. Rats treated once were killed 18 hr post-treatment and rats treated three times received a daily injection of hormone(s) and were killed approximately 18 hr after the final treatment (72 hr from the beginning of hormone treatment). Rats were euthanized by CO_2 inhalation and blood was drawn from the inferior vena cava. The liver was removed, cut into small pieces, and frozen immediately in liquid nitrogen before storage at −70°C. The body weights, plasma and liver retinol concentration, and plasma T_3 hormone concentration of these rats have been reported previously[28,29] and are summarized in the accompanying manuscript.[30]

RNA extraction and dot-blot analysis

Total RNA was extracted with guanidine salts[31] from approximately 1-gm portions of individual livers. Each sample of RNA was dissolved in diethyl pyrocarbonate-treated autoclaved water, quantified spectrophotomerically, and subjected to electrophoresis in formaldehyde-containing agarose to evaluate its integrity. In previous studies[14] we had observed excellent specificity of the apo A-I and beta-actin probes by Northern blot analysis and had obtained comparable quantitative results using Northern blot and dot-blot techniques. We chose the dot-blot method for the present studies because the sample RNA is applied directly to the Nytran membrane (Schleicher & Schuell, Inc., Keene, NH USA) used for hybridization to avoid the transfer step necessary in Northern blot analysis. For this, 5 μgm of each total RNA sample was applied to two separate Nytran membranes using a dot-blot apparatus (Bio-Rad Laboratories, Inc., Hercules, CA USA), then cross-linked by UV light (Stratalinker, Stratagene, San Diego, CA). As probes we used a full-length rat apo A-I cDNA (kindly provided by Dr. Lawrence Chan, Baylor University, Houston, TX USA) and, as a control, a rat beta-actin cDNA probe (kindly provided by Dr. Laurence Kedes, USC, Los Angeles, CA USA). The probes were labeled by the random primer method (Prime-A-Gene labeling kit, Promega, Madison, WI USA) using [alpha-^{32}P]-dCTP (specific activity of 3,000 Ci/mmol, New England Nuclear, Boston, MA USA). Each probe was purified before use by passage through a Sephadex G-50 DNA mini-column (Boehringer Mannheim Biochemicals Co., Indianapolis, IN USA). After prehybridization, the membranes were hybridized overnight at 42°C in a solution of 50% formamide with the purified, denatured radiolabeled apo A-I probe (10^6 cpm/mL),[31] then washed twice for 10 min at room temperature in 2× SSC solution (0.3 M NaCl + 0.03 M sodium citrate, pH 7.0) and 0.1% SDS, and twice for 40 min at 55°C in 0.1× SSC and 1% SDS. The membranes were then exposed to x-ray film (XAR-5, Eastman Kodak Co., Rochester, NY USA) in a cassette with an intensifying screen at −70°C. After exposure, films were developed and the intensities of the radiographic signals were quantified using a scanning laser densitometer (Hoefer Scientific Instruments, San Francisco, CA USA). The Nytran membranes were then stripped of the apo A-I probe by boiling in 0.1% SDS solution for 20 min; stripping was confirmed by re-exposure of the membranes to x-ray film. Thereafter, the membranes were rehybridized with the radiolabeled beta-actin probe, re-exposed to x-ray film, and subjected again to scanning densitometry. The relative abundance of each mRNA was determined and the two duplicates for each sample were averaged. The ratio of apo A-I mRNA:beta-actin mRNA was calculated for each rat liver. For comparison among groups, the mean ratio of hepatic apo A-I:beta-actin for euthyroid vitamin A-sufficient rats was set to 1.00, and all other ratios were calculated as a fraction of this ratio. It is important to note that the differences reported for the ratio of apo A-I mRNA:beta-actin mRNA reflect true differences in the numerator of this ratio.

Statistical analysis

Data are reported as the mean ± SD of the ratio of apo A-I mRNA to beta-actin mRNA for the number of rats indicated. The data for apo A-I mRNA alone, beta-actin mRNA alone and the ratio were analyzed statistically by two-way analysis of variance (ANOVA). The statistically significant differences that are reported for the ratio of apo A-I mRNA to beta-actin mRNA were confirmed to exist for uncorrected values of apo A-I mRNA alone; indeed, the treatment effects are represented more conservatively by the ratio of apo A-I mRNA to beta-actin mRNA than by uncorrected apo A-I mRNA. Statistically significant differences between treatments were determined by a least significant difference test using the program SuperANOVA (Abacus Concepts Inc., Berkeley, CA USA).

Results

Vitamin A deficiency was confirmed by the near absence of liver total retinol and plasma retinol concentrations <0.05 μmol/L and the hypothyroid state was confirmed by a significant reduction in plasma T_4 hormone.[29]

Effects of chronic thyroid hormone and retinoid deficiencies

The mRNA levels of apo A-I and beta-actin were determined in liver of individual rats and expressed as the ratio

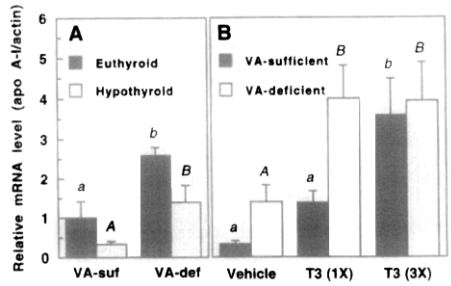

Figure 1 Expression of apo A-I mRNA in vitamin A-sufficient and -deficient euthyroid and hypothyroid rats, and the effect of acute administration of T_3 in vitamin A-sufficient and -deficient hypothyroid rats. The expression of both apo A-I and beta-actin were determined for individual livers and the signal intensity ratio of apo A-I was divided by that for beta-actin; the mean ratio for euthyroid vitamin A-sufficient rats was then set at 1.00 and all other ratios were expressed as a fraction of this ratio. Each bar represents the mean ± SD. *Panel A*: By two-way ANOVA there were significant main effects of vitamin A status ($P < 0.0001$) and thyroid status ($P < 0.001$) on apo A-I mRNA expression. There was no significant interaction between the vitamin A status and the thyroid status of the animals ($P > 0.05$). Significant differences ($P < 0.05$) due to vitamin A status are shown by different lower case letters in euthyroid groups and by different capital letters in hypothyroid groups. *Panel B*: T_3 hormone was administered either once 18 hr or three times beginning 72 hr before the end of the experiment. By two-way ANOVA there was a significant effect of treatment with T_3 on apo A-I mRNA expression in both vitamin A-sufficient and vitamin A-deficient hypothyroid rats ($P < 0.0001$). There also was a significant interaction between T_3 hormone treatment and the vitamin A status of the animals ($P < 0.05$). Vitamin A-*sufficient* groups that differed significantly ($P < 0.05$) from one another are indicated by different lower case letters, whereas vitamin A-*deficient* groups that differed significantly from one another are indicated by different capital letters.

of apo A-I mRNA:beta-actin mRNA. For group comparison, the ratio for euthyroid, vitamin A-sufficient rats was set at 1.00. Hepatic apo A-I mRNA was affected significantly by both the thyroid hormone status and the vitamin A status of the rats (*Figure 1A*). Regardless of vitamin A status, hepatic apo A-I mRNA was reduced significantly by hypothyroidism ($P < 0.001$ by two-way ANOVA), whereas regardless of thyroid status, vitamin A deficiency resulted in higher levels of apo A-I mRNA ($P < 0.0001$). However, there was no significant interaction ($P > 0.05$) between retinoid status and chronic thyroid hormone status. The results in *Figure 1* show that hypothyroidism reduced apo A-I mRNA proportionately in vitamin A-sufficient and vitamin A-deficient rats.

Acute treatment with T_3 and/or RA

The administration of thyroid hormone, T_3, to hypothyroid rats restored the level of hepatic apo A-I mRNA in both vitamin A-sufficient and -deficient rats (*Figure 1B*). After one injection of T_3, apo A-I mRNA was elevated four fold higher in vitamin A-deficient rats compared with euthyroid controls (set at 1.00, see *Figure 1A*). There was no further increase in apo A-I mRNA when vitamin A-deficient rats were treated for 3 days with T_3. When vitamin A-sufficient rats were injected once with T_3, there was a nonsignificant increase in apo A-I mRNA. This increase was statistically significant after three treatments and the apo A-I mRNA levels were then equivalent in treated vitamin A-deficient rats and vitamin A-sufficient rats. The greater efficiency of

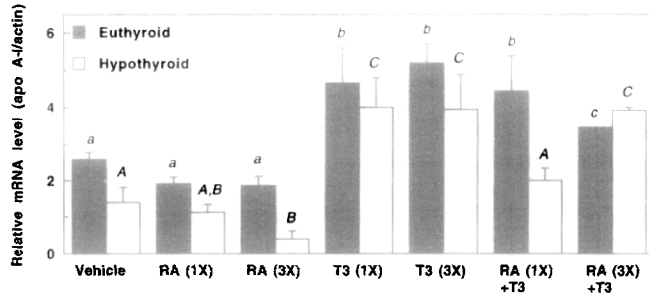

Figure 2 The effect of acute administration of RA or T_3 alone or in combination on the hepatic apo A-I mRNA in euthyroid and hypothyroid vitamin A-deficient rats. Rats were treated i.p. with RA or T_3 (or vehicle) alone, or with both hormones in combination, either once 18 hr or three times beginning 72 hr before the end of the experiment. Apo A-I RNA was analyzed for apo A-I mRNA and beta-actin mRNA as described in *Figure 1*. Each bar in the graph shows the mean ± SD. By two-way ANOVA, there was a significant interaction between T_3 hormone and RA treatment and the thyroid status of the animals ($P = 0.005$). *Euthyroid* groups that differed significantly ($P < 0.05$) by the least significant difference test are shown by different lower case letters; *hypothyroid* groups that differed significantly are shown by different capital letters.

T_3 treatment in inducing apo A-I mRNA in vitamin A-sufficient rats than in vitamin A-deficient rats was apparent as a statistically significant interaction ($P < 0.05$) between T_3 treatment as one factor, and vitamin A-status, as the other factor, in hypothyroid rats.

The effects of RA treatment in euthyroid and hypothyroid vitamin A-deficient rats are shown in *Figure 2*. After a single injection of 20 μg of RA in euthyroid rats, apo A-I mRNA was reduced by about 25%. A similar decrease of about 20% was observed for hypothyroid rats. However, neither of these reductions was statistically significant ($P > 0.05$). In euthyroid rats, three treatments with RA caused no further decrease in apo A-I mRNA. However, in hypothyroid rats there was a significant further reduction in apo A-I mRNA ($P < 0.05$ vs. vehicle-treated hypothyroid rats).

The effects of combined, concomitant administration of RA and T_3 also are shown in *Figure 2*. In euthyroid vitamin A-deficient rats, a single treatment with RA plus T_3 resulted in the same increase in expression as was observed with T_3 alone. In hypothyroid rats treated once with RA plus T_3, apo A-I mRNA was lower than in rats treated with T_3 alone ($P < 0.0001$). In euthyroid rats treated three times with RA, there was a reduction in the increase in apo A-I mRNA caused by T_3 alone ($P < 0.01$ for three treatments with RA plus T_3 versus T_3 alone). There was a significant interaction ($P < 0.01$) between treatment with T_3 and RA as one factor, and thyroid status as the other factor, in vitamin A-deficient rats. However, the inductive effect of T_3 was generally predominant.

Discussion

This study was designed to examine the effect of vitamin A deficiency and hypothyroidism in combination, and of subsequent repletion with RA and/or T_3, on the expression of apo A-I mRNA in the liver of intact adult rats. In agreement with previous in vivo results which examined either vitamin A status or thyroid hormone status as single

factors,[28–30] we observed that vitamin A deficiency increased and the hypothyroid state decreased the level of hepatic apo A-I mRNA. In a previous study of vitamin A-deficient rats, hepatic apo A-I mRNA was increased by two to six fold in both male and female rats compared with vitamin A-sufficient controls.[14] This increased expression was mostly attributable to an increase in apo A-I gene transcription.[14] In the present study, vitamin A deficiency caused an increase of 2.5-fold in euthyroid and more than fourfold in hypothyroid rats as compared to the respective vitamin A-sufficient groups. With respect to thyroid hormone status, Apostolopoulos et al.[6] reported previously that apo A-I mRNA was significantly reduced in hypothyroid (vitamin A-sufficient) rats and that this reduction was attributable to a significant decrease in the rate of apo A-I gene transcription.

The i.p. administration of RA, once or twice, to vitamin A-deficient rats was shown previously to reduce the apo A-I mRNA levels by about 40% and 60%, respectively,[14] whereas the intragastric administration of RA in higher amounts to vitamin A-sufficient rats for 7 days was reported to lower hepatic apo A-I mRNA significantly.[15] The results of the present study are consistent with these results[14,15] in showing that RA reduces hepatic apo A-I mRNA expression, but differ from those of Nagasaki et al.[16] who reported an elevation, rather than reduction, in hepatic apo A-I mRNA after the administration of RA in vitamin A-deficient rats. Contrasting results between in vivo studied and experiments in isolated hepatocytes have also been reported previously;[15] the latter could be attributable to a lack of heterotypic cell-cell communication involved in the normal regulation of gene expression in the intact liver.

A main objective of this work was to determine whether there is an interaction between retinoid status and thyroid hormone status on the regulation of apo A-I mRNA. There was a significant interaction ($P < 0.01$) between treatment (with T_3 and RA) as one factor, and thyroid status (euthyroid versus hypothyroid) as the other factor, in vitamin A-deficient rats. However, the inductive effect of T_3 was generally predominant. A combined effect of T_3 and RA has been reported in the regulation of other genes.[32]

Although we[14] demonstrated previously that the increase in hepatic apo A-I mRNA in vitamin A-deficient rats is due almost exclusively to an increase in the rate of apo A-I gene transcription, it should be noted that, in various thyroid hormones states, the hepatic apo A-I mRNA level is regulated by complex processes that includes both transcriptional and posttranscriptional mechanisms.[6,12,27,33] Whereas in hypothyroid rats, a decrease in the apo A-I mRNA level was found to be caused by a reduction in transcription rate,[6] in rats made hyperthyroid by chronic administration of T_3 hormone, the transcription rate was shown to decrease, but the stability of nuclear RNA increased so that, overall, the level of apo A-I mRNA was increased.[12,33] As noted above, we observed significant interactions during *acute* hormone treatment between T_3 and RA treatment and the chronic vitamin A and thyroid status of the animals. If control of apo A-I mRNA expression is exerted at the level of transcription, these data may indicate an interaction between the RXR and RAR, or of either RAR or RXR with TR, in regulating apo A-I mRNA transcription. A DNA response element that binds RXR and RAR (RARE) has been located in the hormone response element region of the regulatory region of the apo A-I gene.[24–26] However, no perfect thyroid hormone response element (TRE) is located in this region. Recently Tzameli and Zannis[34] demonstrated that the hormone response element of the apo A-I gene is recognized not only by the RXR-alpha homodimer and RXR-alpha/RAR-alpha heterodimer but also by heterodimers composed of RXR-alpha/TR-beta. The activity of the apo A-I promoter/enhancer has been shown to be regulated in vitro by RXR-alpha/TR-beta in the presence of T_3 hormone.[34] These interesting in vitro results seem consistent with the in vivo results of the present study. From these data we postulate the existance of an interaction between RXRs/or RARs and TRs, all of which are expressed in liver, in the regulation of the apo A-I gene in intact rats. Further in vivo studies are warranted to confirm that such interactions occur not only under the conditions used in this study for acute treatment with T_3 hormone and RA, but also after the long-term administration of retinoids and/or thyroid hormone.

Acknowledgments

This research was supported in part by National Institutes of Health grant DK-46869 and funds from the Howard Heinz Endowment.

References

1 Karathanasis, S. (1992) Lipoprotein metabolism: high-density lipoprotein. *Monogr. Hum. Genet.* **14,** 140–171

2 Lenich, C., Brecher, P., Makrides, S., Chobanian, A., and Zannis, V. (1988). Apolipoprotein gene expression in rabbit: abundance, size, and distribution of apolipoprotein mRNA species in different tissues. *J. Lipid Res.* **29,** 755–764

3 Elshourbagy, N.A., Boguski, M.S., Liao, W.S.L., Jefferson, L.S., Gordon, J.I., and Taylor, J.M. (1985). Expression of rat apolipoprotein A-IV and A-I genes: mRNA induction during development and in response to glucocorticoids and insulin. *Proc. Natl. Acad. Sci. (USA)* **82,** 8242–8246

4 Apostolopoulos, J.J., Howlett, G.J., and Fidge, N. (1987). Effects of dietary cholesterol and hypothyroidism on rat apolipoprotein mRNA metabolism. *J. Lipid Res.* **28,** 642–648

5 Davidson, N.O., Carlos, R.C., Drewek, M.J., and Parmer, T.G. (1988). Apolipoprotein gene expression in the rat is regulated in a tissue-specific manner by thyroid hormone. *J. Lipid Res.* **29,** 1511–1522

6 Apostolopoulos, J.J., La Scala, M.J., and Howlett, G.J. (1988). The effect of triiodothyronine on rat apolipoprotein A-I and A-IV gene transcription. *Biochem. Biophys. Res. Commun.* **154,** 997–1002

7 Go, M.F., Schonfeld, G., Pfleger, B., Cole, T.G., Sussman, N.L., and Alpers, D.H. (1988). Regulation of intestinal and hepatic apoprotein synthesis after chronic fat and cholesterol feeding. *J. Clin. Invest.* **81,** 1615–1620

8 Staels, B., Auwerx, J., Chan, L., Van Tol, A., Rosseneu, M., and Verhoeven, G. (1989). Influence of development, estrogens, and food intake on apolipoprotein A-I, A-II, and E mRNA in rat liver and intestine. *J. Lipid Res.* **30,** 1137–1146

9 Sorci-Thomas, M., Prack, M.M., Dashti, N., Johnson, F., Rudel, L.L., and Williams, D.L. (1989). Differential effects of dietary fat on the tissue-specific expression of the apolipoprotein A-I gene: relationship to plasma concentration of high density lipoproteins. *J. Lipid Res.* **30,** 1397–1404

10 Apostolopoulos, J.J., La Scala, M.J., Shen, P., and Howlett, G.J. (1993). The effect of triiodothyronine on the association of the rat

apolipoprotein A-I, C-III and A-IV genes with the nuclear matrix. *Biochem. Biophys. Res. Commun.* **161,** 576–582

11 Tang, J., Krul, E.S., and Schonfeld, G. (1991). In vivo regulation of apolipoprotein A-I gene expression by estradiol and testosterone occurs at the translational level in inbred strains of mice. *Biochem. Biophys. Res. Commun.* **181,** 1407–1411

12 Strobl, W., Chan, L., and Patsch, W. (1992). Differential regulation of hepatic apolipoprotein A-I and A-II gene expression by thyroid hormone in rat liver. *Atherosclerosis* **97,** 161–170

13 Varma, V.K., Smith, T.K., Sorci-Thomas, M., and Ettinger, Jr., W.H. (1993). Dexamethasone increases apolipoprotein A-I concentrations in medium and apolipoprotein A-I mRNA abundance from HepG2 cells. *Metabolism* **41,** 1075–1080

14 Zolfaghari, R. and Ross, A.C. (1994). Effect of vitamin A deficiency and retinoic acid repletion on intestinal and hepatic apolipoprotein A-I mRNA levels of adult rats. *J. Lipid Res.* **35,** 1985–1992

15 Berthou, L., Staels, B., Saldicco, I., Berthelot, K., Casey, J., Fruchart, J.-C., and Denèfle, P. (1994). Opposite in vitro and in vivo regulation of hepatic apolipoprotein A-I gene expression by retinoic acid: absence of effects on apolipoprotein A-II gene expression. *Arterioscler. Thromb.* **14,** 1657–1664

16 Nagasaki, A., Kikuchi, T., Kurata, K., Masushige, S., Hasegawa, T., and Kato, S. (1994). Vitamin A regulates the expression of apolipoprotein AI and CIII genes in the rat. *Biochem. Biophys. Res. Commun.* **205,** 1510–1517

17 Zolfaghari, R. and Ross, A.C. (1995). Chronic vitamin A intake affects the expression of mRNA for apolipoprotein A-I, but not for nuclear retinoid receptors, in liver of young and aging Lewis rats. *Arch. Biochem. Biophys.* **323,** 258–264

18 Mangelsdorf, D.J., Umesono, K., and Evans, R.M. (1994). The retinoid receptors. In: *The Retinoids: Biology, Chemistry and Medicine*, 2nd ed. (Sporn, M.B., Roberts, A.B., and Goodman, D.S., eds.) pp. 319–349. Raven Press, New York, NY USA

19 Chambon, P. (1996). A decade of molecular biology of retinoic acid receptors. *FASEB J.* **10,** 940–954

20 Glass, C.K., Lipkin, S.M., Devary, O.V., and Rosenfeld, M.G. (1989). Positive and negative regulation of gene transcription by a retinoic acid-thyroid hormone receptor heterodimer. *Cell* **59,** 697–708

21 Graupner, G., Wills, K.N., Tzukerman, M., Zhang, X.K., and Pfahl, M. (1989). Dual regulatory role for thyroid-hormone receptors allows control of retinoic-acid receptor activity. *Nature* **340,** 653–656

22 Garcia-Villalba, P., Au-Filegner, M., Samuels, H.H., and Aranda, A. (1993). Interaction of thyroid hormone and retinoic acid receptors on the regulation of the rat growth hormone gene promoter. *Biochem. Biophys. Res. Commun.* **191,** 580–586

23 Mano, H., Mori, R., Ozawa, T., et al. (1994). Positive and negative regulation of retinoid X receptor gene expression by thyroid hormone in the rat. *J. Biol. Chem.* **269,** 1591–1594

24 Widom, R.L., Ladias, J.A.A., Kouidou, S., and Karathanasis, S.K. (1991). Synergistic interactions between transcription factors control expression of apolipoprotein AI gene in liver cells. *Mol. Cell. Biol.* **11,** 677–687

25 Papazafiri, P., Ogami, K., Ramji, P., Nicosia, A., Monaci, P., Cladaras, C., and Zannis, V. (1991). Promoter elements and factors involved in hepatic transcription of the human apo A-I gene positive and negative regulators bind to overlapping sites. *J. Biol. Chem.* **266,** 5790–5797

26 Reisher, S.R., Fisher, E.A., and Feinstein, S.I. (1993). Rabbit and rat liver nuclei both contain proteins which bind to the regions controlling apolipoprotein A-I gene expression. *Eur. J. Biochem.* **216,** 247–253

27 Soyal, S.M., Seelos, C., Lin-Lee, Y.-C., Sanders, S., Gotto, A.M., Jr., Hachey, D.L., and Patsch, W. (1995). Thyroid hormone influences the maturation of apolipoprotein A-I messenger RNA in rat liver. *J. Biol. Chem.* **270,** 3996–4004

28 Breen, J.J., Matsuura, T., Ross, A.C., and Gurr, J.A. (1995). Regulation of thyroid-stimulating hormone beta-subunit and growth hormone messenger ribonucleic acid levels in the rat: effect of vitamin A status. *Endocrinology* **136,** 543–549

29 Shull, J.D., Pennington, K.L., Gurr, J.A., and Ross, A.C. (1995). Cell-type specific interactions between retinoic acid and thyroid hormone in the regulation of expression of the gene encoding ornithine aminotransferase. *Endocrinology* **136,** 2120–2126

30 Ross, A.C., Foulke, D.T., Matsuura, T., Tresini, M., Breen, J.J., and Gurr, J.A. (1997). Hepatic lecithin:retinol acyltransferase activity is induced in vivo by retinoic acid, but not by triiodothyronine, in vitamin A-deficient, hypothyroid rats. *J. Nutr. Biochem.* **8,** 456–460

31 Zolfaghari, R., Chen, X., and Fisher, E.A. (1993). Simple method for extracting RNA from cultured cells and tissue with guanidine salts. *Clin. Chem.* **39,** 1408–1411

32 Crowe, T.C., Loidl, N.M., Payne, K.L., Topliss, D.J., Stockigt, J.R., and Barlow, J.W. (1996). Differential modulation of thyroid hormone responsiveness by retinoids in a human cell line. *Endocrinology* **137,** 3187–3192

33 Strobl, W., Gorder, N.L., Lin-Lee, Y.-C., Gotto, A.M., Jr., and Patsch, W. (1990). Role of thyroid hormones in apolipoprotein A-I gene expression in rat liver. *J. Clin. Invest.* **85,** 659–667

34 Tzameli, I. and Zannis, V.I. (1996). Binding specificity and modulation of the apo A-I promoter activity by homo- and heterodimers of nuclear receptors. *J. Biol. Chem.* **271,** 8402–8415