ELSEVIER

Hepatic lecithin: Retinol acyltransferase activity is induced in vivo by retinoic acid, but not by triiodothyronine, in vitamin A-deficient, hypothyroid rats

A. Catharine Ross,*† Diana T. Foulke,* Tomokazu Matsuura,* Maria Tresini,* Joseph J. Breen,‡ and James A. Gurr‡

**The Department of Biochemistry, The Medical College of Pennsylvania, Philadelphia; the †Department of Nutrition, The Pennsylvania State University, University Park, PA, USA; and the ‡Department of Biochemistry and Fels Institute for Cancer Research, Temple University School of Medicine, Philadelphia, PA, USA*

The activity of the enzyme lecithin: retinol acyltransferase (LRAT) is extremely low in the liver of vitamin A-deficient rats, but is rapidly induced after administration of retinoic acid (RA). The nuclear receptors for RA are closely related to the receptors for thyroid hormone, and molecular cross-talk between these receptors has been observed in vitro and in cultured cells. Therefore we have examined whether retinoid status and thyroid hormone status interact in the regulation of hepatic LRAT activity in vivo. Vitamin A-deficient male Lewis rats, either euthyroid or made hypothyroid by treatment with propylthiouracil, were treated once or three times with 20 μg of all-trans-RA, 10 μg/100 gm body weight of triiodothyronine (T_3), or both hormones. Hepatic LRAT activity, which was negligible in all retinoid-deficient rats, was induced by RA ($P < 0.0001$) regardless of the animal's thyroid hormone status. T_3 by itself had no ability to induce hepatic LRAT activity ($P = 0.42$), nor did co-administration of T_3 with RA increase or decrease the response to RA ($P = 0.13$). In retinoid-sufficient rats, hypothyroidism did not alter hepatic LRAT activity; however, LRAT activity was reduced by half ($P < 0.05$) after three treatments with T_3. Therefore we conclude that thyroid hormone status alone does not regulate LRAT, nor does thyroid status affect the ability of RA to induce hepatic LRAT in retinoid-deficient animals. However, in retinoid-sufficiency, repeated treatment with T_3 may reduce the hepatic esterification of retinol by LRAT. (J. Nutr. Biochem. 8:456–460, 1997) © *Elsevier Science Inc. 1997*

Keywords: vitamin A; vitamin A deficiency; thyroid hormone; propylthiouracil; retinoid X receptor; liver; rat

Introduction

The liver plays a central regulatory role in vitamin A storage, metabolism and retinoid distribution to peripheral organs. In well-nourished animals and humans, the liver contains over 85% of total body retinoids stored as fatty acid esters of retinol. Biochemical and nutritional studies have identified lecithin: retinol acyltransferase (LRAT), a microsomal enzyme that preferentially esterifies retinol bound to the cellular retinol-binding protein, CRBP,[1] as principally responsible for the formation of retinyl esters. LRAT activity is present in homogenates and microsomes prepared from liver, isolated liver cells, and several peripheral tissues.

Hepatic LRAT activity is strongly influenced by vitamin A status. The liver of vitamin A deficient animals has essentially no hepatic LRAT activity, whereas activity is again measurable 4 to 8 hr after repletion with retinol[2] or its active metabolite, retinoic acid (RA).[3] In retinoid-deficient rats, all-*trans*-RA in doses as low as 2 μg per rat produced a measurable rise in hepatic LRAT activity within a few

Address reprint requests and correspondence to Dr. A. Catharine Ross, Ph.D. at Department of Nutrition, The Pennsylvania State University, 126-S Henderson Bldg., University Park, PA 16802 USA.
Present addresses: T. Matsuura, Department of Internal Medicine (I), Jikei University School of Medicine, Tokyo, Japan; J.J. Breen, Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, NIH, Bethesda, MD 20892 USA; J.A. Gurr, Wyeth-Ayerst Research, 145 King of Prussia Road, Radnor, PA 19087 USA.
Received February 19, 1997; accepted April 18, 1997.

© Elsevier Science Inc. 1997
655 Avenue of the Americas, New York, NY 10010
0955-2863/97/$17.00
PII S0955-2863(97)00056-9

hours.[3] This increase could be inhibited completely by pretreating rats with cycloheximide or actinomycin D.[3]

All-*trans*-RA and its isomer, 9-*cis*-RA, function as the ligands of the nuclear retinoid receptors, RAR and RXR, respectively, which are ligand-activated transcription factors that bind to DNA to regulate the transcription of a potentially large number of genes.[4] Molecular cloning and sequence analysis has demonstrated that the nuclear transcription factors RAR and RXR are members of a gene superfamily that includes the receptors for steroid hormones, thyroid hormone (T_3) and 1,25-dihydroxyvitamin D, and the peroxisome proliferator-activated receptors and several orphan receptors.[5] Whereas all-*trans*-RA binds with high affinity to members of the RAR subfamily, 9-*cis*-RA is a high-affinity ligand of the RXR, a receptor subfamily which may interact, as a heterodimeric partner, with RARs and with the receptors for thyroid hormone (TR) and vitamin D (VDR). All the receptors in this superfamily contain a highly conserved DNA-binding domain that mediates receptor binding to specific DNA sequences in the promoter region of target genes (hormone response elements), and a less conserved domain in the C-terminus that is essential for ligand binding. The RAR and the TR share a high degree of sequence similarity in both their DNA-binding and ligand-binding domains.[5] Molecular cross-talk has been demonstrated between these receptors in in vitro transactivation assays [see Ref. 6]. Additionally, members of the RXR subfamily may enhance the expression of certain retinoid-, thyroid- or vitamin D-responsive genes by forming heterodimers that induce gene expression,[7–9] or they may function indirectly as negative regulators by sequestering the RAR, VDR, or TRs.[8] However, the interactions of retinoic acid and thyroid hormone in vivo on gene expression and protein functional activity have not been studied extensively.

The purpose of this study was to examine the physiological interaction between RA and T_3 in regulating hepatic LRAT activity in intact rats. For this purpose young rats were made retinoid-deficient and/or thyroid hormone-deficient, after which groups of rats were treated with RA, T_3, or both hormones and, after 18 hr to 3 days, hepatic LRAT activity was assayed.[2]

Methods and materials

Animals and diets

The animal protocol followed the NIH Guide for the Care and Use of Laboratory Animals and was approved by the Animal Use and Care Committee of the Medical College of Pennsylvania. Male rats of the Lewis strain, obtained as 2 to 5 day-old nurslings of virus antibody-free lactating dams, were purchased from Charles River Breeding Laboratories (Wilmington, MA USA). On arrival, the dams were fed a vitamin A-free semipurified diet[10] (Dyets Inc., Bethlehem, PA USA). When they were 20 to 21 days old, 42 rats were weaned onto the same vitamin A-free diet, and 12 were weaned onto diet of the same composition but containing 4 mg of retinol (as retinyl palmitate) per kg of diet.[10] All rats had free access to food and water throughout the study. Half of the rats undergoing retinoid depletion and nine of the vitamin A-sufficient rats were made hypothyroid by including 0.05% 6-propyl-2-thiouracil (PTU) in their drinking water.[11] When rats were 55 to 61 days old, they were treated one or three times with RA and/or T_3, or with vehicle only. All-*trans*-RA (Sigma, St. Louis, MO USA) was dissolved in ethanol, mixed with Tween 80, and diluted in sterile PBS.[3] After spectrophotometric determination of RA concentration, the solution was diluted so that a 0.5 mL ip injection contained 20 μg of RA in 0.2% Tween 80 and 2% ethanol. For rats treated three times, this solution was prepared fresh each day. L-tri-iodothyronine (T_3, Sigma) was dissolved in PBS containing 10 mmol/L of potassium hydroxide[11] and administered ip at a dose of 10 μg/100 gm body weight. Some of the rats were treated once with RA, T_3, or both, and then killed 18 hr later by CO_2 asphyxiation. Other rats were treated daily for 3 days (0, 24, and approximately 48 hr) and then killed at 72 hr. All rats were killed between 8 a.m. and 10 a.m. Heparinized blood was collected for plasma preparation and later assayed for total retinol[10] by HPLC and T_4 hormone by RIA.[11] Livers were removed, blotted, and minced so that portions from each lobe were sampled, and approximately 1 gm of tissue was rapidly frozen in liquid nitrogen and then stored at −70°C before analysis. As noted below, liver and other tissues from these rats were used for several different studies.[11–13] Data on the body weights, plasma total retinol, and total T_4 concentrations of the rats used in this study have been reported previously (study 2 in Breen et al.[11]).

Preparation of liver homogenates and assay of liver LRAT activity

Liver samples were thawed and homogenized in four volumes of ice-cold 0.25 M sucrose using a Potter-Elvejhem homogenizer. LRAT activity was assayed in duplicate for each sample in a final reaction volume of 0.2 mL containing approximately 1 mg of homogenate protein, 5 μM of ^{3}H-retinol bound to rat liver CRBP,[14] 0.15 M potassium phosphate buffer, pH 7.4, and 2 mM dithiothreitol, which was incubated at 37°C for 4 min. These conditions have been shown to yield ^{3}H-retinyl esters in proportion to the time of incubation and amount of protein assayed.[2] The reaction was stopped by the addition of 1 mL of ethanol containing 0.1% butylated hydroxytoluene and the neutral lipids were quantitatively partitioned into hexanes.[15] A boiled aliquot of each homogenate was run in parallel as a control and served as the blank that was later subtracted. The product ^{3}H-retinyl esters were separated from ^{3}H-retinol by chromatography on open columns of deactivated aluminum oxide, and eluted with diethyl ether/hexane (3:97 v:v) into scintillation vials. The solvent was evaporated and the ^{3}H-retinyl ester activity determined by liquid scintillation spectrometry.[15] The protein concentration of each homogenate was determined by the method of Markwell et al.[16] The LRAT specific activity for each liver was expressed as the pmol of retinyl ester formed/(min of incubation · mg of homogenate protein).

Statistical analysis

Data for LRAT activity are reported as the mean ± SD for each treatment group. The data from retinoid-deficient rats were analyzed by a 2-factor analysis of variance (ANOVA) followed up by a least significant difference test, whereas the data from retinoid-sufficient rats were analyzed by a one-factor ANOVA and Student's *t* test.

Results

Body weights, plasma retinol, and T_3 concentrations

Data on the body weights, plasma total retinol, liver total retinol, and plasma T_4 concentrations of the rats used in this study, as reported previously,[11] are summarized in *Table 1*

Table 1 Effects of vitamin A and thyroid status on the body weight, plasma and liver retinol, and plasma T4 concentrations of male Lewis rats

	Body weight (g)	Plasma retinol (μM)	Liver total retinol (nmol/g)	Plasma T_4 (μg/dL)
Euthyroid				
Retinoid-sufficient	289 ± 7.6 (9)	1.09 ± 0.17 (3)	197 ± 41 (3)	4.73 ± 0.83 (3)
Retinoid-deficient	251 ± 12 (9)[a]	<0.05 (3)[a]	<1.0 (20)[a]	5.35 ± 0.35 (3)
Hypothyroid (PTU treated)				
Retinoid-sufficient	169 ± 5.8 (21)[b]	0.75 ± 0.27 (9)[b]	196 ± 33 (9)	<0.5 (9)[b]
Retinoid-deficient	174 ± 1.2 (21)[b]	<0.05 (21)[a]	<0.1 (20)[a]	<0.5 (9)[b]

*Data are the mean ± SD for the numbers of animals indicated in parentheses. *a*, Significantly different from retinoid-sufficient rats of the same thyroid status, $P < 0.02$; *b*, significantly different from euthyroid rats of the same retinoid status, $P < 0.02$.
PTU, propylthiouracil; T_4, thyroxine

for convenience. Regardless of retinoid status, the body weights of hypothyroid rats were lower than those of euthyroid rats. Plasma total retinol concentration averaged 1.09 μM in vitamin A-sufficient, euthyroid rats and 0.75 μM in hypothyroid rats. Plasma retinol was essentially undetectable in all retinoid-deficient rats (<0.05 μM). Liver retinol concentration was not affected by hypothyroidism, but was nil in vitamin A-deficient rats. Plasma T_4, a measure of thyroid hormone production, was very low (<0.5 μg/dL) in all hypothyroid rats.

Hepatic LRAT activity

Table 2 presents the LRAT specific activities for all treatment groups. In each of the retinoid-depleted, vehicle-injected rats, regardless of thyroid status, LRAT activity was essentially undetectable. It should be noted that CRBP-retinol substrate is not rate-limiting in these in vitro assays.[2] After treatment with 20 μg of RA (18 hr after a single dose), hepatic LRAT activity was induced equally in euthyroid and hypothyroid rats ($P < 0.0001$ versus the respective vehicle-treated group). No induction was observed after a single dose of T_3 (10 μg/100 gm body weight). The combination of RA and T_3 was neither more nor less effective than RA alone in inducing hepatic LRAT activity in retinoid-depleted rats.

When hormone treatments were repeated three times, the results were qualitatively similar but, for RA, quantitatively lower than for a single dose. Seventy-two hours after the first dose (approximately 24 hr after the third dose) of RA, hepatic LRAT activity was still induced significantly ($P < 0.0001$ versus the respective vehicle-treated group), but the specific activity was about 50 to 70% of that after a single dose of RA. As was the case after a single dose of T_3, three doses of T_3 did not induce hepatic LRAT activity. In both euthyroid and hypothyroid retinoid-depleted rats, the combination of RA and T_3 produced the same hepatic LRAT response as RA alone ($P < 0.0001$ versus the respective vehicle-treated group).

Four groups of retinoid-sufficient rats were included as references. Comparison of hepatic LRAT activity in euthyroid and hypothyroid rats shows that hypothyroidism alone did not affect enzyme activity. In retinoid-sufficient hypothyroid rats treated once with T_3, the mean LRAT activity was not significantly different from the hypothyroid, vehicle-injected group. However, the hepatic LRAT activity of hypothyroid rats treated three times with T_3 was reduced by about half ($P < 0.05$).

Discussion

The main observations from this study are, first, that neither hypothyroidism nor the administration of T_3 to hypothyroid rats influenced the ability of RA to induce hepatic LRAT activity in retinoid-depleted rats. The induction of LRAT by

Table 2 Effects of retinoid and thyroid hormone deficiency, and of retinoic acid and/or thyroid hormone repletion, on the activity of hepatic lecithin: retinol acyltransferase*

	Thyroid status, LRAT specific activity, pmol RE formed (min · mg liver protein)	
	Euthyroid	*Hypothyroid*
Retinoid-deficient rats†		
Vehicle	0.05 ± 0.12	0.05 ± 0.04
18 hr		
RA[a]	7.91 ± 1.46	7.06 ± 1.70
T_3	0.05 ± 0.06	0.10 ± 0.09
RA + T_3[a]	6.60 ± 0.85	6.76 ± 1.68
72 hr		
RA[a,b]	3.90 ± 0.63	5.03 ± 0.06
T_3	0.14 ± 0.04	0.06 ± 0.06
RA + T_3[a,b]	5.50 ± 0.47	3.54 ± 1.18
Retinoid-sufficient rats‡		
Vehicle	13.53 ± 3.59	11.83 ± 1.59
18 hr T_3	—	8.11 ± 2.57
72 hr T_3	—	5.48 ± 3.65[c]

*Results are the mean ± SD, $n = 3$ rats/group.
†Results for retinoid-deficient rats were analyzed by a 2-factor ANOVA. The effect of hormone treatment was significant ($P < 0.0001$), whereas that of thyroid status was not ($P = 0.42$), and there was no interaction ($P = 0.13$). By Fisher's least significant difference test, all groups treated with RA (± T_3) differed significantly from the vehicle-treated controls as well as the groups that received T_3 alone ($P \leq 0.0001$, denoted by superscript *a*). Groups treated with T_3 alone did not differ from the vehicle-treated groups. The 72-hr treatments with RA (± T_3) three times differed significantly from the 18-hr groups that received the same treatments once ($P \leq 0.002$, superscript *b*).
‡Results for vitamin A-sufficient rats were analyzed by a 1-factor ANOVA. Hypothyroid rats treated with T_3 for 72 hr differed significantly from the vehicle-treated groups ($P < 0.05$), superscript *c*. No other differences were significant.
LRAT, lecithin: retinol acyltransferase; RE, retinyl ester; RA, all-*trans*-retinoic acid; T_3, triiodothyronine.

RA was seen in all RA-treated animals 18 hr after either a single dose, or 3 days after the initiation of a three-dose treatment schedule. T_3 alone had no LRAT-inducing activity in retinoid-deficient rats. Second, there was no interaction between retinoid status or RA treatment and thyroid hormone status or T_3 treatment on the in vivo induction of hepatic LRAT activity. Thus, from these results it may be concluded that RA administration restores hepatic LRAT activity in a manner that is independent of thyroid function.

The mechanisms by which LRAT activity is regulated, and the mechanism underlying the induction of its activity by RA, have not yet been elucidated. From nutritional and physiological studies, it is clear that hepatic LRAT activity falls as animals are depleted of retinoids and that activity is restored rapidly after the administration (by ip injection, gavage or in the diet) of retinoids.[2,3,17] No increase in activity is observed after adding retinoids to vitamin A-deficient microsomes or homogenates in vitro,[2,17] and the in vivo induction is blocked by pretreating animals with an inhibitor of RNA synthesis, actinomycin D, or an inhibitor of protein synthesis, cycloheximide.[3] For these reasons, it seems likely that RA plays a role, either indirectly or perhaps directly, in maintaining the basal transcription of either the LRAT gene itself (which has not yet been isolated and characterized) or another gene whose protein regulates LRAT activity. If RA regulates LRAT activity transcriptionally, then it is most likely that the nuclear receptors of the RAR and/or RXR families are involved as mediators. The inhibitory effect of actinomycin D and cycloheximide suggests the requirement for another gene to be transcribed and/or translated, which seems to favor an indirect mode of action.

Receptors in the RAR and RXR subfamilies may interact as homodimers (RAR-RAR and RXR-RXR) that bind to and transactivate RAREs and RXREs, respectively, or as RAR-RXR heterodimers. However, the RXRs, which bind 9-*cis*-RA selectively, also are capable of forming heterodimers not only with the RAR but also with several other receptor partners including the hormone receptors T_3R and VDR, as well as more distantly related transcription factors (e.g., COUP-TF,[4,18,19]). Despite the potential complexity of RAR-RXR and T_3R-RXR interactions, the evidence from our in vivo study indicates that RA regulates hepatic LRAT activity in a manner that is independent of T_3 status, as T_3 by itself had no ability to induce hepatic LRAT activity, nor did co-administration of T_3 with RA increase or decrease the response to RA. Presuming that nuclear retinoid receptors are involved in the induction of hepatic LRAT activity by RA, a parsimonious explanation of these data would be that the regulation of LRAT expression is mediated by members of the RAR family, without involvement of the RXR. However, in nearly all well-characterized examples of gene regulation by RAR, RXR plays a role as a partner, either as a ligand-independent "silent" partner or as a ligand-binding heterodimeric partner. In rat liver, the mRNAs for members of both the RAR[20,21] and RXR families[21] are expressed,[20,21] with relatively strong expression of RAR-α and RXR-α, and detectable expression of RAR-β. RAR-β mRNA was shown to be low in vitamin A-deficient rat liver and to increase rapidly after treatment with RA, whereas neither hypothyroidism nor T_3 administration significantly affected RAR mRNA levels.[20] In recent studies, retinoid analogues that selectively bind to the RXR did not induce hepatic LRAT activity, whereas RAR-selective retinoids were effective.[22] Therefore, if RXR also plays a role, it seems most likely that it may function as a silent partner of ligand-activated RAR.

Two other observations are worthy of comment. First, a single dose of 20 μg of RA induced a higher level of hepatic LRAT activity than did the same dose given three times. All-*trans*-RA has a short half life in vivo[23] and, at least after high doses, is capable of inducing its own catabolism.[24] Thus, the lower level of LRAT activity after three doses of RA may be attributable to an increased rate of RA degradation. Although a single dose of RA induced hepatic LRAT activity rapidly (Ref. 3 and Table 2), activity was shown to decline to a low level by 72 hr in the absence of subsequent RA treatments.[3] Thus, RA apparently must be present continuously to maintain a high level of hepatic LRAT activity. In vitamin A sufficiency, the production of RA from retinol is believed to be continuous and well regulated.[25,26] It is possible that the administration of periodic, bolus doses of RA results in a conditioned increase in RA catabolism that blunts the regulatory effect of subsequent doses of RA.

Second, it was unexpected that the administration of three doses of T_3 to hypothyroid rats with normal hepatic LRAT activity would depress enzyme activity. Had there been evidence for RA-T_3 interactions, a plausible speculation would be that T_3-activated TR interacted with RXR, reducing their binding to the RAR and, therefore, reducing hepatic LRAT expression. However, as discussed above, there is as yet no strong evidence of RXR participation in the regulation of LRAT activity. Alternatively, it is possible that the effect of repeated T_3 administration is secondary to the effects of T_3 on energy balance or other aspects of metabolism. Regardless of the mechanism(s) involved, the result suggests that chronic T_3 administration could have a significant effect on the capacity of the liver to store vitamin A.

Finally, it is of interest to comment on the differences we have observed within the same animals whose tissues were also used to examine the expression of thyroid stimulating hormone (TSH) β subunit and growth hormone (GH) mRNAs in the pituitary gland,[11] the expression of ornithine aminotransferase (OAT) mRNA in liver and kidney,[12] and the expression of apolipoprotein A-I (apo A-I) mRNA in liver and small intestine.[13] For TSH-β gene expression, regulation by RA required either normal thyroid function or T_3 administration, indicating a regulatory interaction between these hormones.[11] For GH mRNA expression in the same tissue, retinoid status did not affect GH mRNA expression, although RA had been shown previously to increase GH mRNA expression in cultured cells [see Ref. 11 for references]. For OAT mRNA in liver (but not kidney), retinoid deficiency was associated with reduced expression and RA with increased expression, similar to the results for hepatic LRAT activity.[12] However, in contrast to the lack of an effect of T_3 on hepatic LRAT activity, administration of T_3 blocked the ability of RA to induce OAT mRNA in the liver of retinoid-deficient rats.[12] For apo A-I mRNA expression in liver, RA and T_3 were both regulatory, but showed little, if any, interaction.[13] A com-

parison of these results emphasizes the exquisite precision of hormonal control in vivo, as well as the importance of investigating potentially interacting hormones, or nutrients and hormones, within the intact organism.

Acknowledgments

We are grateful for research support from National Institutes of Health Grant DK-46869 (A.C.R.), National Science Foundation Grant DCB 880-1965 and a grant from Boots Pharmaceuticals, Inc. (to J.A.G.), and training support from the Howard Heinz Endowment and the Uehara Foundation.

References

1 Ong, D.E., Newcomer, M.E., and Chytil, F. (1994). Cellular retinoid-binding proteins. In: The Retinoids: Biology, Chemistry and Medicine, 2nd ed., M.B. Sporn, A.B. Roberts, and D.S. Goodman, eds.) pp. 283–317, Raven Press, New York, NY USA

2 Randolph, R.K. and Ross, A.C. (1991). Vitamin A status regulates hepatic lecithin:retinol acyltransferase activity in rats. *J. Biol. Chem.* **266,** 16453–16457

3 Matsuura, T. and Ross, A.C. (1993). Regulation of hepatic lecithin: retinol acyltransferase activity by retinoic acid. *Arch. Biochem. Biophys.* **301,** 221–227

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

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

6 Ribeiro, R.C.J., Apriletti, J.W., Yen, P.M., Chin, W.W., and Baxter, J.D. (1994). Heterodimerization and deoxyribonucleic acid-binding properties of a retinoid X receptor-related factor. *Endocrinol.* **135,** 2076–2085

7 Kliewer, S.A., Umesono, K., Mangelsdorf, D.J., and Evans, R.M. (1992). Retinoid X receptor interacts with nuclear receptors in retinoic acid, thyroid hormone and vitamin D_3 signalling. *Nature* **355,** 446–449

8 Zhang, X.-k., Hoffman, B., Tran, P.B.-V., Graupner, G., and Pfahl, M. (1992). Retinoid X receptor is an auxiliary protein for thyroid hormone and retinoic acid receptors. *Nature* **355,** 441–446

9 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

10 Pasatiempo, A.M.G., Bowman, T.A., Taylor, C.E., and Ross, A.C. (1989). Vitamin A depletion and repletion: effects on antibody response to the capsular polysaccharide of *Streptococcus pneumoniae*, type III (SSS-III). *Am. J. Clin. Nutr.* **49,** 501–510

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

12 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,** 1220–1226

13 Zolfaghari, R., and Ross, A.C. (1997). Vitamin A deficiency increases hepatic apolipoprotein A-1 mRNA expression in both euthyroid and hypothyroid rats. *J. Nutr. Biochem.* **8,** 451–455

14 Yost, R.W., Harrison, E.H., and Ross, A.C. (1988). Esterification by rat liver microsomes of retinol bound to cellular retinol-binding protein. *J. Biol. Chem.* **263,** 18693–18701

15 Ross, A.C. (1982). Retinol esterification by mammary gland microsomes from the lactating rat. *J. Lipid Res.* **23,** 133–144

16 Markwell, M.A.K., Haas, S.M., Bieber, L.L., and Tolbert, N.E. (1978). A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal. Biochem.* **87,** 206–210

17 Matsuura, T., Zhao, Z., and Ross, A.C. (1996). *N*-(4-hydroxphenyl)-retinamide increases lecthin:retinol acyltransferase activity in rat liver. *J. Nutr.* **126,** 2474–2480

18 Cooney, A.J., Leng, X., Tsai, S.Y., O'Malley, B.W., and Tsai, M-J. (1993). Multiple mechanisms of chicken ovalbumin upstream promoter transcription factor-dependent repression of transactivation by the vitamin D, thyroid hormone, and retinoic acid receptors. *J. Biol. Chem.* **268,** 4152–4160

19 Ben-Shushan, E., Sharir, H., Pikarsky, E., and Bergman, Y. (1995). A dynamic balance between ARP-1/COUP-TFII, EAR-3/COUP-TFI, and retinoic acid receptor: retinoid X receptor heterodimers regulates Oct-3/4 expression in embryonal carcinoma cells. *Molec. Cell. Biol.* **15,** 1034–1048

20 Kato, S., Mano, H., Kumazawa, T., Yoshizawa, Y., Kojima, R., and Masushige, S. (1992). Effect of retinoid status on α, β and γ retinoic acid receptor mRNA levels in various rat tissues. *Biochem. J.* **286,** 755–760

21 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

22 Shimada, T., Ross, A.C., Brouilette, W., Muccio, D.D., and Matsuura, T. (1996). Natural and synthetic retinoids induce hepatic lecithin: Retinol acyltransferase (LRAT) activity in vitamin A-deficient rats. *FASEB J.* **10,** A525

23 McPhillips, D.M., Kalin, J.R., and Hill, D.L. (1987). The pharmacokinetics of all-*trans*-retinoic acid and *N*-(2-hydroxyethyl)retinamide in mice as determined with a sensitive and convenient procedure. *Drug Metab. Dispos.* **15,** 207–211

24 el Mansouri, S., Tod, M., Leclerq, M., Petitjean, O., Perret, G., and Porthault, M. (1995). Time- and dose-dependent kinetics of all-*trans*-retinoic acid in rats after oral or intravenous administration(s). *Drug Metab. Dispos.* **23,** 227–231

25 Napoli, J.L. (1996). Retinoic acid biosynthesis and metabolism. *FASEB J.* **10,** 993–1001

26 Wolf, G., (1996). The regulation of retinoic acid formation. *Nutr. Rev.* **54,** 182–184