Effect of vitamin A deficiency and retinoic acid repletion on intestinal and hepatic apolipoprotein A-I mRNA levels of adult rats

Reza Zolfaghari and A. Catharine Ross[1]

Department of Biochemistry, Medical College of Pennsylvania, 2900 Queen Lane, Philadelphia, PA 19129

Abstract Apolipoprotein A-I (apoA-I) gene expression is known to be regulated by nutritional and hormonal factors. Experiments were conducted to determine the effects of vitamin A deficiency and retinoic acid repletion on the in vivo expression of apoA-I in rat intestine and liver. The relative abundance of apoA-I mRNA (apoA-I/β-actin ratio) in the intestine did not differ significantly between vitamin A-deficient and -sufficient rats. However, the relative abundance of hepatic apoA-I mRNA of vitamin A-deficient rats was 2.2- to 6-times that of sufficient rats. Even marginal vitamin A status resulted in a significant increase in hepatic apoA-I mRNA expression. Treatment of vitamin A-deficient rats with a single dose of retinoic acid (20 μg, 20 h before tissues were collected) reduced the hepatic apoA-I mRNA/β-actin ratio by about 40%, while further reduction (about 60–65%) was observed after two treatments with retinoic acid. By nuclear run-on assay, the increase in hepatic apoA-I mRNA in vitamin A-deficient rats was attributable to increased transcription of the apoA-I gene. However, immunoblot analysis showed no apparent differences in apoA-I protein in either liver homogenates or plasma of vitamin A-deficient and -sufficient rats. These data indicate that apoA-I gene expression in vivo is sensitive to retinoid status and suggest that there is additional regulation of post-transcriptional events.—**Zolfaghari, R., and A. C. Ross.** Effect of vitamin A deficiency and retinoic acid repletion on intestinal and hepatic apolipoprotein A-I mRNA levels of adult rats. *J. Lipid Res.* 1994. **35:** 1985–1992.

Supplementary key words retinoids • apolipoproteins • gene expression

Retinol and its natural metabolite, retinoic acid (RA), play important roles in cell proliferation and differentiation and are required by higher animals for embryonic development and the maintenance of many adult tissues (1). The RA isomers, *all-trans* and *9-cis*-RA, exert their biological activity as the ligands of nuclear RA receptors (RARs) and retinoid X receptors (RXRs), respectively, that are members of the thyroid/steroid hormone receptor superfamily (2–10). These proteins function as ligand-dependent transcription factors that modulate the expression of target genes by binding to cognate DNA elements, also known as response elements, in the enhancer/promoter regions of retinoid-responsive genes (4, 10).

It has been suggested that RA may regulate several genes involved in lipid metabolism or transport. Apolipoprotein A-I (apoA-I), the major protein constituent of plasma high density lipoprotein, is predominantly expressed in intestine and liver and has been shown to be regulated by diet, hormones, and development (11–25). Recent analysis of the human apoA-I gene has revealed at least three DNA elements upstream from the transcription start site that bind nuclear proteins from HepG2 cells and rat and rabbit liver (26–28). Deletion of these DNA regions resulted in the loss of most of the liver-specific enhancer activity of the apoA-I gene (26). In the human apoA-I gene, this region extends from −222 to −110 DNA bp upstream of the transcription start site (26). This region contains a site (site A, −214 to −192 bp) to which a repressor gene product known as apoA-I regulatory protein-1 (ARP-1) (29) and RXR-alpha may bind as a heterodimer (30). Transfection of HepG2 cells with the cDNAs encoding these two receptors together with a DNA construct containing the promoter/regulatory regions of the apoA-I gene linked to a chloramphenicol acetyltransferase (CAT) reporter gene was shown to activate CAT expression in the presence of RA.

It has not yet been determined whether vitamin A status in vivo is an important determinant of apoA-I gene expression. In the present investigation, vitamin A status was manipulated to explore the effects of vitamin A deficiency and RA repletion on the steady-state abundance of apoA-I mRNA in the liver and intestine of intact adult rats.

Abbreviations: RA, retinoic acid; RAR, retinoic acid receptor; RXR, retinoid X receptor; apoA-I, apolipoprotein A-I; ARP-1, apoA-I regulatory protein-1; CAT, chloramphenicol acetyltransferase; PBS, phosphate-buffered saline; LPL, lipoprotein lipase; LCAT, lecithin:cholesterol acyltransferase; HNF-4, Hepatocyte nuclear factor 4.

[1]Address for correspondence and reprint requests: Department of Nutrition, Pennsylvania State University, 126-S Henderson Building, University Park, PA 16802.

Downloaded from www.jlr.org by guest, on June 17, 2012

MATERIALS AND METHODS

Animals and diets

Lewis strain rats were raised in our animal facility following the breeding and dietary protocols described elsewhere (31). All experimental protocols were in compliance with the Guide for the Care and Use of Laboratory Animals of the Medical College of Pennsylvania. Throughout pregnancy and lactation, dams were fed a vitamin A-free semisynthetic diet (Dyets, Inc., Bethlehem, PA) (31). When pups were 21 days old they were weaned onto the vitamin A-free diet and housed conventionally in plastic cages with free access to water in a room maintained at 22°C with a 12-h light-dark cycle.

Experimental design

Two main experiments were conducted as follows. In experiment 1, 24 30-day-old vitamin A-depleted female rats were divided randomly into three groups with equal mean body weights. Two groups continued to be fed the vitamin A-free diet and the third group was pair-fed the same diet containing 4 mg of retinol (as retinyl palmitate) per kg of diet (31). Twenty hours before tissues were collected, one of the two vitamin A-deficient groups received 20 μg of RA, administered intraperitoneally as described previously (32). The total experimental period was 32 days.

In experiment 2, 16 vitamin A-depleted female rats were randomly divided into four groups of 4 rats each when they were 40–45 days old. Three groups continued to receive the vitamin A-free diet: one group remained vitamin A-deficient, the second vitamin A-deficient group received 20 μg of RA as above 20 h before the end of the experiment, and the third vitamin A-deficient group received two 20-μg injections of RA, one 40 h and the second 20 h before the end of the experiment. The fourth group was fed the vitamin A-sufficient diet as above and in addition received a repletion dose of 200 μg of retinol orally at the beginning of the experiment; these rats were pair-fed with the vitamin A-deficient group for 14 days.

Two additional experiments were conducted to further compare vitamin A-deficient and -sufficient male rats, using the same diets and similar protocols. A fifth experiment was designed to compare rats with chronic, marginal vitamin A status to age-matched vitamin A-sufficient controls. As described earlier (33), rats were weaned from dams fed the vitamin A-deficient diet during lactation onto diet containing either a marginal level of vitamin A [0.18 mg retinol (as retinyl palmitate)/kg of diet] or a control level (4 mg/kg diet) and tissues were collected when rats were 50 days old.

At the end of each experimental period, the rats were killed by inhalation of CO_2 and tissues were dissected immediately. After drawing blood from the vena cava, livers were removed, washed with cold (4°C) phosphate-buffered saline (PBS), and then minced into small pieces. A segment of the jejunum was dissected, trimmed of any adherant tissue, and irrigated with cold PBS. After opening the intestine, the mucosa was scraped gently with a glass slide. Liver and intestinal samples were immediately frozen in liquid nitrogen and stored at −70°C until further use. Plasma was collected after centrifugation of the blood samples at 4°C and stored at −20°C.

RNA extraction and blot analysis

Total RNA was extracted from tissue samples by guanidine salts (34) and then dissolved in autoclaved water and stored at −70°C. For Northern blot analysis, total RNA samples were first subjected to electrophoresis through a 0.67 M formaldehyde/1.25% agarose gel (35) and then transferred overnight to Nytran membranes (Schleicher & Schuell, Inc., Keene, NH). For slot-blot analysis, up to 5 μg of total RNA per slot was applied to Nytran membranes, following the directions of the manufacturer. A full-length rat apoA-I cDNA (kindly provided by Dr. Lawrence Chan, Baylor University, Houston, TX) was labeled with deoxycytidine 5'[alpha-^{32}P]triphosphate with a specific activity of 3,000 Ci/mmol (New England Nuclear, Boston MA), using the random-primer method (35). After prehybridization, membranes were hybridized as previously described in a solution containing 10^6 cpm/ml of denatured purified radiolabeled probe (35). The membranes were then washed (35) and exposed to X-ray film (XAR-5, Eastman Kodak Co., Rochester, NY) at −70°C. The relative intensities of autoradiographic signals were quantified by scanning with a laser densitometer (Hoefer Scientific Instruments, San Francisco, CA).

Actin mRNA was measured as the control; in addition lecithin:cholesterol acyltransferase (LCAT) was analyzed as an unrelated analytical control. After hybridization with labeled apoA-I cDNA, membranes were stripped of probe and exposed to X-ray film to confirm that the signal had been removed. To measure the abundance of actin mRNA and/or LCAT mRNA, rehybridization was performed as previously, using a full-length cDNA of the rat β-actin gene (kindly provided by Lawrence Kedes, USC, Los Angeles, CA) or human LCAT cDNA (kindly provided by Dr. John McLean, Genetech, S. San Francisco, CA). The relative abundance of apoA-I mRNA in each sample was corrected using the abundance of beta-actin mRNA. The RNA samples were measured at least two times in each experiment and the mean coefficient of variability due to the analytical measurement was 6.5%.

Nuclei preparation and nuclear run-on transcription assay

In a separate experiment, nuclei were isolated from the livers of male rats and stored at −70°C essentially as described by Fisher et al. (36). Transcription in isolated nuclei and the isolation of RNA were performed by

Downloaded from www.jlr.org by guest, on June 17, 2012

modifications of the procedures of Celano, Berchtold, and Casero (37) using a final volume of 400 μl containing 200 μg of DNA, 25 mM HEPES (pH 7.5), 0.1 M KCl, 5 mM $MgCl_2$, 0.05 mM EDTA, 20% glycerol, 1 mM dithiothreitol, 200 units of RNase inhibitor (RNasin, Promega, Madison, WI), 0.25 mM each of ATP, CTP, and GTP, and 200 μCi [alpha-^{32}P]UTP. After 45 min at 25°C, the RNA was extracted and passed through 25-G Quick Spin Columns (Boehringer Mannheim Biochemicals).

Inserts for rat apoA-I and β-actin were excised from their respective plasmids and 1 μg of each DNA was bound to Nytran membranes (Schleicher & Schuell, Inc.) by dot blotting. The membrane filters were prehybridized in 0.5 ml of 4 × SSPE (20 × SSPE = 3 M NaCl + 0.2 M Na phosphate + 20 mM EDTA, pH 7.4), 50% formamide, 1% SDS, 0.5% Blotto (1 × Blotto = 5% nonfat dry milk + 0.02% Na azide), and 0.1 mg/ml sheared and denatured herring sperm DNA at 42°C for 48 h. Filters were then hybridized at 42°C for 72 h in 0.5 ml of the same solution (except using 3× instead of 4× SSPE) with 1 × 10^6 cpm of ^{32}P-labeled nuclear RNA. After hybridization the filters were washed with 0.1 × SSC (20 × SSC = 3 M NaCl + 0.3 M Na citrate, pH 7.0), 0.2% SDS 3 times at 42°C each for 1 h. The filters were then dried, exposed to Kodak XAR films at −70°C, and autoradiograms were quantified as described above.

Immunoblot Analysis

Diluted samples of plasma and liver homogenates were subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on 10% gels under reducing conditions according to the protocols recommended by Hoefer Scientific Instruments (San Francisco, CA). The separated proteins were transferred from the gel to a nitrocellulose membrane which was blocked for 1 h in 3% bovine serum albumin in PBS, incubated with a rabbit anti-rat apoA-I polyclonal antiserum (kindly provided by Dr. Julian B. Marsh, Medical College of Pennsylvania, Philadelphia, PA) for 30 min, and followed by incubation with biotinylated anti-rabbit IgG (Organon Teknika Corporation, Durham, NC) for 30 min. After washing, the blot was developed using streptavidin coupled to alkaline phosphatase and a tetrazolium base-coupled substrate solution.

RESULTS

Steady-state expression of apoA-I mRNA

Our major objective was to determine the effects of vitamin A status in vivo on the expression of the intestinal and hepatic apoA-I genes of intact adult rats. After pilot studies (not shown) we conducted two major independent experiments that included a vitamin A-deficient group, one or two groups of vitamin A-deficient rats that were repleted with RA, and a group of pair-fed, vitamin A-sufficient controls. All of the rats fed the vitamin A-deficient diet had a plasma retinol concentration less than 0.15 μM, but none showed physical signs of vitamin A deficiency. Whereas no differences were observed in the average body weight among all the groups in experiment 1, the vitamin A-sufficient group in experiment 2 weighed slightly more than the other groups [mean of 219 ± 4.8 g (mean ± SD) for vitamin A-sufficient rats versus 191 ± 8.8 g for vitamin A-deficient rats, $P < 0.05$].

In experiment 1, vitamin A-depleted rats were divided into three equal groups; two groups continued to receive the vitamin A-free diet and the third group was pair-fed the vitamin A-sufficient diet for 32 days. One of the two vitamin A-deficient groups received 20 μg of RA 20 h before tissues were collected. **Figure 1** shows the Northern blot of total RNA from the intestine and liver samples probed with ^{32}P-labeled apoA-I cDNA, beta-actin cDNA

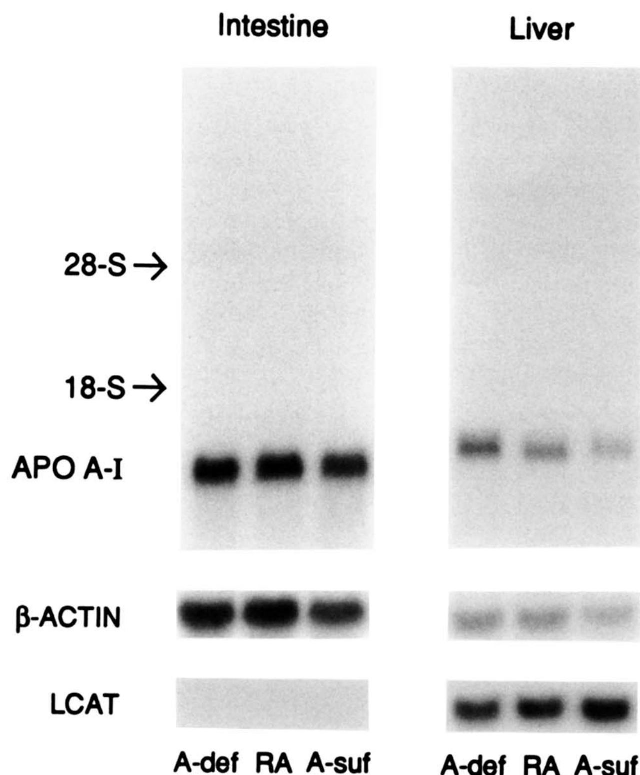

Fig. 1. Northern blot analysis of intestinal and liver total RNA from vitamin A-deficient, RA-treated, and vitamin A-sufficient adult rats. Total RNA was extracted from pooled tissues (two pools of four animals each per treatment), separated by denaturing electrophoresis in agarose gel and transferred to nylon membranes. The membranes were first hybridized to ^{32}P-labeled rat liver apoA-I cDNA probe (upper panel), stripped, and then rehybridized to ^{32}P-labeled rat β-actin cDNA (middle panel) and LCAT cDNA (lower panel). The entire length of the gel is shown for apoA-I and the positions of the migration of ribosomal 28S and 18S RNA are indicated. A-def, vitamin A-deficient rats; RA, RA-treated rats; A-suf, vitamin A-sufficient rats.

Downloaded from www.jlr.org by guest, on June 17, 2012

ASBMB JOURNAL OF LIPID RESEARCH

TABLE 1. Results of four independent experiments on the effect of vitamin A status on the relative abundance of hepatic apoA-I mRNA of adult rats

Experiment No.	Sex	ApoA-I/β-Actin mRNA Ratio			
		Vitamin A-Sufficient	RA-Repleted (2×)	RA-Repleted (1×)	Vitamin A-Deficient
1	F	1.00 ± 0.29		1.40 ± 0.29	2.16 ± 0.40
2	F	1.00 ± 0.08	2.46 ± 0.15	4.30 ± 0.31	6.00 ± 0.46
3	M	1.00 ± 0.13			2.33 ± 0.12
4	M	1.00 ± 0.07			2.50 ± 0.13

Results are from slot-blots hybridized with ^{32}P-labeled probes. These were eight rats per group in experiment #1 and four rats per group in the other three experiments. Tissues were pooled to form two independent pools per treatment. Data for each experiment show the mean ± SD of these pools.

as the control, and LCAT cDNA as an independent control probe. As expected (15), apoA-I mRNA was more abundant in the intestines of all groups than in the corresponding livers. There was no significant difference due to vitamin A status in the steady-state level of intestinal apoA-I mRNA. However, apoA-I mRNA abundance was clearly greater in the livers of vitamin A-deficient rats as compared to the vitamin A-sufficient controls. This difference was confirmed with quantitative slot-blot analysis of total RNA (**Table 1**). When corrected for β-actin, the relative abundance of liver apoA-I mRNA was more than double in the vitamin A-deficient group as compared to the vitamin A-sufficient group. Without correction for actin, the apoA-I mRNA level was about 3.5 times higher in vitamin A-deficient rats than in controls (data not shown). A small but consistent increase in β-actin mRNA abundance was observed in the livers of vitamin A-deficient rats, and therefore a second, unrelated liver-specific probe, LCAT, was also used. For this probe, no differences due to the animals' vitamin A status were apparent (Fig. 1). Injection of a single 20-μg dose of RA in vitamin A-deficient rats lowered the hepatic apoA-I mRNA level by about 40% relative to β-actin (Fig. 1 and Table 1).

Experiment 2 was conducted to confirm and extend these observations. This experiment included treatments similar to those in experiment 1 and, in addition, a group of vitamin A-deficient rats that were repleted with two 20-μg doses of RA 40 and 20 h before the end of the experiment. **Figure 2** shows the slot-blot results of total RNA from rat liver using the rat apoA-I, β-actin, and LCAT cDNA probes. Again, in this experiment the steady-state level of apoA-I mRNA was much higher in the livers of vitamin A-deficient rats than in controls. Densitometric scanning of slot-blot autoradiographs demonstrated a 6-fold increase in hepatic apoA-I mRNA abundance in the vitamin A-deficient group compared to the vitamin A-sufficient controls when the apoA-I mRNA level was compared to actin. Similar to the results of experiment 1, intraperitoneal injection of 20 μg of RA to vitamin A-deficient rats 20 h before the end of experimental period lowered the hepatic apoA-I mRNA level by about 30–50%. Further reduction (about 60–65%) was observed in the apoA-I mRNA abundance when 20 μg of RA was injected twice (Table 1).

To further confirm these findings, two additional studies (experiments 3 and 4) were conducted with vitamin A-deficient and -sufficient male rats. The results of these ex-

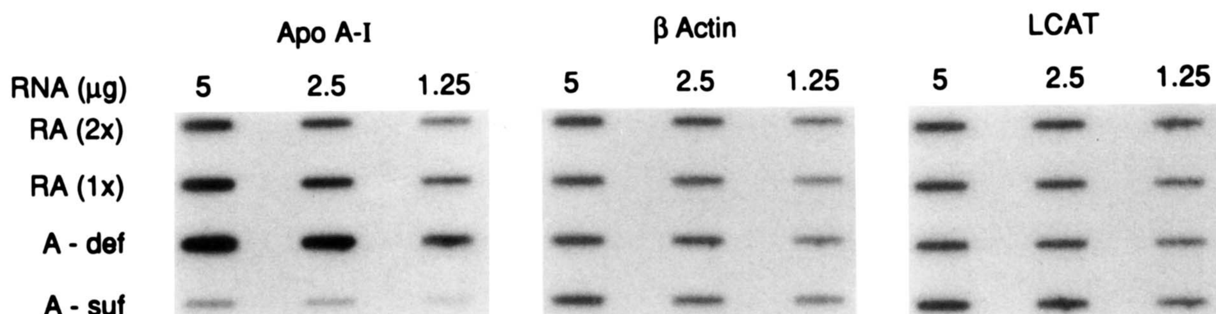

Fig. 2. Slot-blot analysis of total RNA from liver of vitamin A-deficient, RA-treated, and vitamin A-sufficient adult rats. Total RNA was extracted from pooled tissues (two pools of two animals each per treatment) and slot-blotted onto nylon membranes at three amounts from 1.25 to 5 μg. The membranes were first hybridized to ^{32}P-labeled rat liver apoA-I cDNA probe (left panels), stripped, and then rehybridized to ^{32}P-labeled rat β-actin cDNA (middle panels) and LCAT cDNA (right panels). A-def, vitamin A-deficient rats; RA, RA-treated rats; A-suf, vitamin A-sufficient rats. (1×) and (2×) indicate one and two injections of 20 μg of RA per rat, respectively.

Downloaded from www.jlr.org by guest, on June 17, 2012
ASBMB JOURNAL OF LIPID RESEARCH

periments are also shown in Table 1. The apoA-I mRNA levels in the livers of the vitamin A-deficient male rats were 2.3- to 2.5-times higher than those of vitamin A-sufficient rats. Thus, apoA-I mRNA levels were significantly increased in the livers of rats of both genders in each of four independent experiments.

Hepatic apoA-I mRNA in marginal vitamin A deficiency

As mentioned above, the plasma retinol concentration of vitamin A-deficient rats in the preceding studies was less than 0.15 μM. To determine whether such extensive depletion of retinol is necessary to observe an elevation in hepatic apoA-I mRNA expression, an additional experiment was conducted to compare rats with marginal vitamin A status to that of litter mates fed the vitamin A-sufficient control diet. Plasma retinol concentrations and hepatic levels of apoA-I mRNA expression (relative to β-actin) were determined individually for five rats per treatment. As is shown in **Table 2,** the average plasma retinol concentrations of the marginal and control groups were 0.88 and 1.58 μM, respectively. The average apoA-I mRNA/β-actin mRNA ratio was approximately 50% higher in the marginal group as compared to the control group, and this difference was statistically significant ($P < 0.0025$). Therefore, even marginal vitamin A status, in which plasma retinol levels were moderately reduced, was associated with elevated levels of hepatic apoA-I mRNA.

Transcription of apoA-I mRNA in isolated nuclei

In order to examine whether the increase in the steady-state level of hepatic apoA-I mRNA in vitamin A-deficient rats was due to an increase in the rate of transcription, we performed nuclear run-on assays on pooled nuclei from the livers of vitamin A-deficient and -sufficient rats. The results of the experiment are shown as an autoradiograph in **Fig. 3.** As calculated from densitometry, the rate of transcription of the apoA-I gene was 2.5-fold higher in vitamin A-deficient rats compared to vitamin A-sufficient controls, whereas actin signals were equal in the two groups. For the same animals, the relative abundance of

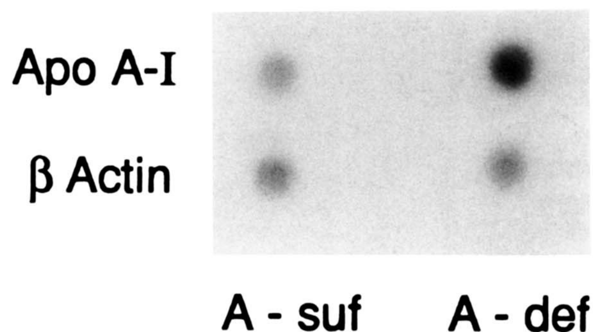

Fig. 3. Nuclear run-on assay of apoA-I and actin genes from livers of vitamin A-deficient and -sufficient rats. Nascent ^{32}P-labeled RNA was extracted from isolated liver nuclei of vitamin A-deficient (A-def) and -sufficient (A-suf) rats and hybridized to the membranes blotted with rat liver apoA-I cDNA or rat β-actin cDNA as the control. After washing, the membranes were exposed to X-ray film at −70°C as described in Experimental Procedures.

apoA-I mRNA (compared to β-actin mRNA) was 2.3-fold higher in livers of vitamin A-deficient versus -sufficient rats (experiment 3, Table 1).

TABLE 2. Effect of marginal vitamin A status on hepatic apoA-I mRNA level

Diet Group	Body Weight	Plasma Retinol	ApoA-I/β-Actin mRNA Ratio
	g	*μM*	
Marginal (5)	207 ± 11	0.88 ± 0.06	1.48 ± 0.13
Control (5)	219 ± 8	1.58 ± 0.22	1.00 ± 0.09
	NS	$P < 0.005$	$P < 0.0025$

Rats were 50 days old at the time of the measurements. Statistical differences were determined by an independent *t*-test. Values are given as mean ± SD; NS, not significant.

ApoA-I protein in liver and plasma

To determine whether the elevated levels of apoA-I mRNA in the liver of vitamin A-deficient rats resulted in an increased concentration of circulating apoA-I protein, plasma samples from the animals in experiment 2 were subjected to electrophoresis and immunoblotting using an anti-rat apoA-I antiserum. As is shown in **Fig. 4,** the intensities of the apoA-I protein bands were essentially equal among all groups. There were also no differences in apoA-I protein in liver homogenates subjected to the same procedure (data not shown).

DISCUSSION

Results from in vitro binding studies have shown that there is a complex interaction between the DNA elements located 5' upstream of the apoA-I gene transcription initi-

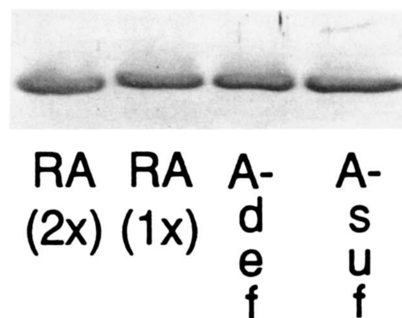

Fig. 4. Immunoblot analysis of plasma proteins from vitamin A-deficient, RA-treated, and vitamin A-sufficient adult rats. Pooled plasma (two pools from two rats each per treatment) were analyzed by immunoblotting using antiserum to rat apoA-I protein. Abbreviations are the same as for Fig. 2.

Downloaded from www.jlr.org by guest, on June 17, 2012
ASBMB
JOURNAL OF LIPID RESEARCH

ation site and nuclear proteins extracted from liver cells or tissue (26–28). In this region there is at least one site (site A) which appears to be controlled by RA (30). Both ARP-1, a repressor protein (29), and RXR-alpha bind in an overlapping manner to this site as a heterodimer (30). Cotransfection of ARP-1 and RXR-alpha cDNAs together with the CAT reporter gene linked to the 5' flanking region of apoA-I gene inhibited the expression of the CAT reporter gene in the absence of RA; however, this repression was relieved in the presence of RA (30). Whereas the in vitro systems represent a simplified model of the gene, the constructs used did not contain DNA elements which, although not known to be retinoid-responsive, nonetheless may play a significant role in transcriptional regulation. Alternatively, an imbalance in the in vitro expression of receptors may influence gene expression. Recently, based on studies of the cellular retinol-binding protein-II (CRBP-II) gene promoter, Nakshatri and Chambon (38) suggested that RXRs and RARs overexpressed by transfection of their cDNAs in cells expressing ARP-1 and hepatocyte nuclear factor 4 (HNF-4), a member of the thyroid/steroid hormone receptor superfamily, may bind promiscuously to ARP-1 and HNF-4 response elements, leading to induction of the gene promoter.

Based on these in vitro observations, we initially anticipated that vitamin A deficiency would be associated with a reduction in apoA-I gene expression which would be relieved by administration of RA. However, the results of four independent in vivo experiments were consistent in showing that the steady-state level of hepatic apoA-I mRNA was higher in vitamin A-deficient rats as compared to vitamin A-sufficient controls (Table 1). This increase was observed for both male and female rats. The apoA-I mRNA/β-actin mRNA ratio was 2.2- to 2.5-times higher in the livers of vitamin A-deficient rats in three of the four studies, whereas in the fourth experiment this ratio was about 6 times higher. Although the reason for this greater value is not known, it is clear that differences of >2-fold are consistent in each study. Even marginal vitamin A deficiency was associated with a 50% increase in hepatic apoA-I mRNA levels. Rats fed the marginal vitamin A diet grew normally (33) (Table 2) and maintained plasma retinol concentrations that, while lower than the controls, were still well above those of vitamin A-deficient rats. Retinoic acid, a natural oxidative metabolite of retinol, is apparently involved in the control of hepatic apoA-I mRNA because when vitamin A-deficient rats were treated with RA, the steady-state level of hepatic apoA-I mRNA was reduced toward normal levels (Table 1). Nuclear run-on assays showed that the increase in apoA-I mRNA in retinoid-deficient rats was attributable to increased apoA-I gene transcription (Fig. 3).

Hormones have been reported to affect apoA-I gene expression and production of apoA-I protein. Thyroid hormone, unlike RA, has been shown to increase, and hypothyroidism to decrease, apoA-I mRNA in rat liver through both transcriptional and post-transcriptional processes (14, 16, 17, 21, 23). Likewise, insulin and dexamethasone have been reported to increase the apoA-I mRNA abundance in rat liver (12) and human HepG2 cells (25). Sex hormones, on the other hand, affect apoA-I expression mostly through translational control (22).

Differences in apoA-I expression due to retinoid deficiency and RA repletion in vivo were selective for the liver; there was little change in intestinal apoA-I mRNA. This observation may reflect the previously reported tissue-specific differences in regulation of the apoA-I gene by other hormones and factors. For example, whereas the hepatic apoA-I mRNA level was shown to change significantly during development in the rat, no significant changes were observed in the abundance of intestinal apoA-I mRNA (12, 13, 19). Both hyperthyroidism and hypothyroidism affected apoA-I gene expression in liver but had little or no effect on the intestine (14, 16, 17). Feeding dietary fat to non-human primates has been reported to increase apoA-I mRNA in liver without a concomitant change in the intestine (20). On the other hand, in rats fed an atherogenic diet containing cholesterol and propylthiouracil, apoA-I mRNA abundance decreased in both the liver and intestine (18). Apparently the factors that control the expression of the apoA-I gene in the intestine are distinct from those in the liver (39, 40).

Although the steady-state level of apoA-I mRNA was greater in the livers of vitamin A-deficient rats than in controls, the apoA-I protein concentration in plasma, determined by immunoblotting, was not different. This type of apparent discrepancy has been noted previously for apoA-I (14, 19, 24) and several other apolipoproteins and lipolytic enzymes (41–44). For apoA-I in several experimental conditions, transcription (as judged by the steady-state levels of apoA-I mRNA) and apoA-I protein concentration have not changed in parallel. For example, during development the hepatic apoA-I mRNA level increased sharply in the postnatal period and then decreased prior to weaning but the plasma apoA-I mass increased after birth and remained elevated as rats matured (24). In hypothyroid rats the mRNA level of the liver was shown to decrease dramatically without any changes in the plasma apoA-I protein (14). The complex regulatory events intervening between transcription and tissue or plasma levels of many proteins remain to be determined, but may include intracellular protein degradation, glycosylation, as well as post-secretory changes in catabolism.

In conclusion, the in vivo expression of the apoA-I gene in liver was increased during vitamin A deficiency and reduced after treatment with RA. It will be important in future studies to examine the in vivo expression of the transcription factors known to play a role in apoA-I gene regulation and their physiological adaptations to changes in vitamin A status.

Downloaded from www.jlr.org by guest, on June 17, 2012

ASBMB JOURNAL OF LIPID RESEARCH

This work was supported by NIH grants DK46869, HL22633, AG09839, and funds from the Howard Heinz Endowment. We thank Dr. Edward A. Fisher for his helpful discussions and Dr. Tomokasu Matsuura and Diana Foulke for their technical advice and assistance.

Manuscript received 9 February 1994 and in revised form 28 April 1994.

REFERENCES

1. DeLuca, L. M. 1991. Retinoids and their receptors in differentiation, embryogenesis, and neoplasia. *FASEB J.* **5:** 2924–2933.
2. Evans, R. M. 1988. The steroid and thyroid hormone receptor superfamily. *Science.* **240:** 889–895.
3. Green, S., and P. Chambon. 1988. Nuclear receptors enhance our understanding of transcription regulation. *Trends Genet.* **4:** 309–314.
4. Glass, C. K., J. DiRenzo, R. Kurokawa, and Z. Horn. 1991. Regulation of gene expression by retinoic acid receptors. *DNA Cell Biol.* **10:** 623–638.
5. Heyman, R. A., D. J. Mangelsdorf, J. A. Dyck, R. B. Stein, G. Eichele, R. M. Evans, and C. Thaller. 1992. 9-*Cis* retinoic acid is a high affinity ligand for the Retinoid X Receptor. *Cell.* **68:** 397–406.
6. Levin, A. A., L. J. Sturzenbecker, S. Kazmer, T. Bosakowski, C. Huselton, G. Allenby, J. Speck, Cl. Kratzeisen, M. Rosenberger, A. Lovey, and J. F. Grippo. 1992. 9-*Cis* retinoic acid stereoisomer binds and activates the nuclear RXRα. *Nature.* **355:** 359–361.
7. Mangelsdorf, D. J., U. Borgmeyer, R. A. Heyman, J. Y. Zhou, E. S. Ong, A. E. Oro, A. Kakizuka, and R. M. Evans. 1992. Characterization of three RXR genes that mediate the action of 9-*cis* retinoic acid. *Genes Dev.* **6:** 329–344.
8. Zhang, X., J. Lehmann, B. Hoffmann, M. I. Dawson, J. Cameron, G. Graupner, T. Hermann, P. Tran, and M. Pfahl. 1992. Homodimer formation of retinoid X receptor induced by 9-*cis* retinoic acid. *Nature.* **358:** 587–591.
9. Durand, B., M. Saunders, P. Leroy, M. Leid, and P. Chambon. 1992. All-*trans* and 9-*cis* retinoic acid induction of CRABP II transcription is mediated by RAR-RXR heterodimers bound to DR1 and DR2 repeated motifs. *Cell.* **71:** 73–85.
10. Leid, M., P. Kastner, and P. Chambon. 1992. Multiplicity generates diversity in the retinoic acid signalling pathways. *Trends Biochem. Sci.* **17:** 427–433.
11. Ertel Miller, J. C., R. K. Barth, P. H. Shaw, R. W. Elliott, and W. D. Hastie. 1983. Identification of a cDNA clone for mouse apolipoprotein A-I (apoA-I) and its use in characterization of apoA-I mRNA expression in liver and small intestine. *Proc. Natl. Acad. Sci. USA.* **80:** 1511–1515.
12. Elshourbagy, N. A., M. S. Boguski, W. S. L. Liao, L. S. Jefferson, J. I. Gordon, and J. M. Taylor. 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.
13. 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.
14. 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.
15. Lenich, C., P. Brecher, S. Makrides, A. Chobanian, and V. Zannis. 1988. Apolipoprotein gene expression in rabbit: abundance, size, and distribution of apolipoprotein mRNA species in different tissues. *J. Lipid Res.* **29:** 755–764.
16. Davidson, N. O., R. C. Carlos, M. J. Drewek, and T. G. Parmer. 1988. Apolipoprotein gene expression in the rat is regulated in a tissue-specific manner by thyroid hormone. *J. Lipid Res.* **29:** 1511–1522.
17. Apostolopoulos, J. J., M. J. La Scala, and G. J. Howlett. 1988. The effect of triiodothyronine on rat apolipoprotein A-I and A-IV gene transcription. *Biochem. Biophys. Res. Commun.* **154:** 997–1002.
18. Go, M. F., G. Schonfeld, B. Pfleger, T. G. Cole, N. L. Sussman, and D. H. Alpers. 1988. Regulation of intestinal and hepatic apoprotein synthesis after chronic fat and cholesterol feeding. *J. Clin. Invest.* **81:** 1615–1620.
19. Staels, B., J. Auwerx, L. Chan, A. van Tol, M. Rosseneu, and G. Verhoeven. 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.
20. Sorci-Thomas, M., M. M. Prack, N. Dashti, F. Johnson, L. L. Rudel, and D. L. Williams. 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.
21. Apostolopoulos, J. J., M. J. La Scala, P. Shen, and G. J. Howlett. 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.
22. Tang, J., E. S. Krul, and G. Schonfeld. 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.
23. Strobl, W., L. Chan, and W. Patsch. 1992. Differential regulation of hepatic apolipoprotein A-I and A-II gene expression by thyroid hormone in rat liver. *Atherosclerosis.* **97:** 161–170.
24. Golder-Novoselsky, E., T. M. Forte, A. V. Nochols, and E. M. Rubin. 1992. Apolipoprotein A-I expression and high density lipoprotein distribution in transgenic mice during development. *J. Biol. Chem.* **267:** 20787–20790.
25. Varma, V. K., T. K. Smith, M. Sorci-Thomas, and W. H. Ettinger, Jr. 1993. Dexamethasone increases apolipoprotein A-I concentrations in medium and apolipoprotein A-I mRNA abundance from HepG2 cells. *Metabolism.* **41:** 1075–1080.
26. Widom, R. L., J. A. A. Ladias, S. Kouidou, and S. K. Karathanasis. 1991. Synergistic interactions between transcription factors control expression of apolipoprotein A-I gene in liver cells. *Mol. Cell. Biol.* **11:** 677–687.
27. Papazafiri, P., K. Ogami, P. Ramji, A. Nicosia, P. Monaci, C. Cladaras, and V. Zannis. 1991. Promoter elements and factors involved in hepatic transcription of the human apoA-I gene positive and negative regulators bind to overlapping sites. *J. Biol. Chem.* **266:** 5790–5797.
28. Reisher, S. R., E. A. Fisher, and S. I. Feinstein. 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.
29. Ladias, J. A., and S. K. Karathanasis. 1991. Regulation of the apolipoprotein A-I gene by ARP-1, a novel member of the steroid receptor superfamily. *Science.* **251:** 561–565.
30. Widom, R. L., M. Rhee, and S. K. Karathanasis. 1992.

Downloaded from www.jlr.org by guest, on June 17, 2012

Repression by ARP-1 sensitizes apolipoprotein A-I gene responsiveness to RXR-alpha and retinoic acid. *Mol. Cell. Biol.* **12:** 3380–3389.
31. Pasatiempo, A. M. G., M. Kinoshita, C. E. Taylor, and A. C. Ross. 1990. Antibody production in vitamin A-depleted rats is impaired after immunization with bacterial polysaccharide or protein antigens. *FASEB J.* **4:** 2518–2527.
32. Matsuura, T., and A. C. Ross. 1993. Regulation of hepatic lecithin:retinol acyltransferase activity by retinoic acid. *Arch. Biochem. Biophys.* **301:** 221–227.
33. Gardner, E. M., and A. C. Ross. 1993. Dietary vitamin A restriction produces marginal vitamin A status in young rats. *J. Nutr.* **123:** 1435–1443.
34. Han, J. H., C. Stratowa, and W. J. Rutter. 1987. Isolation of a full-length putative rat lysophospholipase cDNA using improved methods for mRNA isolation and cDNA cloning. *Biochemistry.* **26:** 1617–1625.
35. Zolfaghari, R., J. M. Glick, and E. A. Fisher. 1993. The effects of varying the expression of a neutral cholesteryl ester hydrolase on the turnover of cholesteryl ester in rat hepatoma cells. *J. Biol. Chem.* **268:** 13532–13538.
36. Fisher, E. A., A. Anbari, D. M. Klurfield, and D. Kritchevsky. 1988. Independent effects of diet and nutritional status on apolipoprotein B gene expression in rabbit. *Arteriosclerosis.* **8:** 797–803.
37. Celano, P., C. Berchtold, and R. A. Casero, Jr. 1989. A simplification of the nuclear run-off transcription assay. *Biotechnique.* **7:** 942–944.
38. Nakshatri, H., and P. Chambon. 1994. The directly repeated RG(G/T)TCA motifs of the rat and mouse cellular retinol-binding protein II genes are promiscuous binding sites for RAR, RXR, HNF-4, and ARP-1 homo- and heterodimers. *J. Biol. Chem.* **269:** 890–902.
39. Sastry, K. N., U. Seedorf, and S. K. Karathanasis. 1988. Different *cis*-acting DNA elements control expression of the human apolipoprotein A-I gene in different cell types. *Mol. Cell. Biol.* **8:** 605–614.
40. Walsh, A., N. Azrolan, K. Wang, A. Marcigliano, A. O'Connell, and J. L. Breslow. 1993. Intestinal expression of the human apoA-I gene in transgenic mice is controlled by a DNA region 3' to the gene in the promoter of the adjacent convergently transcribed apoC-III gene. *J. Lipid Res.* **34:** 617–623.
41. Li, W-H., M. Tanimura, C-C. Luo, S. Datta, and L. Chan. 1988. The apolipoprotein multigene family: biosynthesis, structure, structure–function relationships, and evolution. *J. Lipid Res.* **29:** 245–271.
42. Sparks, J. D., and C. E. Sparks. 1993. Hormonal regulation of lipoprotein assembly and secretion. *Curr. Opin. Lipidol.* **4:** 177–186.
43. Zolfaghari, R., E. H. Harrison, J. H. Han, W. J. Rutter, and E. A. Fisher. 1992. Tissue and species differences in bile salt-dependent neutral cholesteryl ester hydrolase activity and gene expression. *Arterioscler. Thromb.* **12:** 295–301.
44. Ailhaud, G., E. Amri, P. Grimaldi, L. Teboul, and C. Vannier. 1993. Fatty acids regulate the expression of lipoprotein lipase gene and the lipoprotein lipase activity in adipose cells. *Circulation.* **88:** I-463.

Downloaded from www.jlr.org by guest, on June 17, 2012

ASBMB JOURNAL OF LIPID RESEARCH JLR