Retinoid Production and Catabolism: Role of Diet in Regulating Retinol Esterification and Retinoic Acid Oxidation\textsuperscript{1,2}

A. Catharine Ross\textsuperscript{3}

Department of Nutritional Sciences, The Pennsylvania State University, University Park, PA 16802

ABSTRACT Retinoic acid (RA), a transcriptionally active metabolite of vitamin A (retinol), activates two families of nuclear retinoid receptors that have the potential to regulate the expression of a large number of genes. Although it may be presumed that the concentration of RA is closely regulated, the mechanisms underlying such regulation are not well understood. Our research has examined the expression and function of two enzymes, lecithin:retinol acyltransferase (LRAT) and a cytochrome P450, CYP26, in the liver and lung of rats and mice, over a wide range of vitamin A status or after treatment of vitamin A–deficient animals with exogenous RA. LRAT expression at both the mRNA and protein activity levels and CYP26 mRNA are regulated by dietary vitamin A in a steady-state model and are acutely regulated by RA in an acute repletion model. In the liver, the level of expression of LRAT and CYP26 is as follows: vitamin A deficient < vitamin A marginal < vitamin A adequate < vitamin A supplemented < RA treated. The regulation of LRAT shows strong tissue specificity (highly regulated in liver and lung but not in small intestine), whereas CYP26 is strongly regulated in the liver, lung, testis and intestine. RA may function as a signal of the body’s vitamin A adequacy. The regulated expression of LRAT, CYP26 and other genes by RA may provide a sensitive response mechanism that overall serves to adjust the metabolism of vitamin A to maintain retinoid homeostasis and prevent retinoid excess. J. Nutr. 133: 291S–296S, 2003.

KEY WORDS: • retinoic acid • lecithin:retinol acyltransferase • cytochrome P450 • CYP26 • autoregulation • liver • lung • rat • mouse

A central question concerning vitamin A homeostasis is, Can the body “sense” its vitamin A status? If so, what signal-response mechanisms are involved? Do the homeostatic adjustments that are made help to conserve vitamin A when dietary intake is suboptimal or eliminate retinoids when they are present in excess of physiological requirements? Interestingly, a number of genes involved in vitamin A metabolism appear to be regulated by an active metabolite of vitamin A, retinoic acid (RA)\textsuperscript{4} (1). This review first introduces some of the general features of the molecular regulation of retinoid-responsive genes and then focuses in more depth on two enzymes, lecithin:retinol acyltransferase (LRAT) and a cytochrome P450, CYP26, that we have come to believe may be critical for the regulation of retinol oxidation and the disposition of RA, respectively. Our interest in these enzymes as possible key factors in retinoid homeostasis is due in large part to the strong regulation that each exhibits in response to differences in vitamin A nutrition and exposure to exogenous RA.

Mechanisms of gene regulation by retinoids

Dietary vitamin A is an essential precursor of tissue retinol, which is metabolized by sequential oxidation reactions into two classes of biologically active retinoids. The 11-cis-retinoids function specifically in vision, whereas the acidic retinoids, principally all-trans-RA and 9-cis-RA, are potent regulators of many biological processes through their ability to activate two families of nuclear hormone receptors. All-trans-RA is capable of binding with high affinity to members of the RA receptor family (RAR\textsubscript{a}, \textbeta, and \textgamma), whereas 9-cis-RA also is capable of binding to RARs but is thought to function mainly as a high-affinity ligand for members of the retinoid X receptor family (RXR\textalpha, \textbeta, and \textgamma) (1–3). The RAR and RXR proteins interact with each other to form dimers that may be composed of an RAR/RXR or RXR/RXR pair, mostly as RAR/RXR heterodimers under physiological conditions. Retinoid receptor dimers are capable of binding to specific DNA sequences, termed retinoid responsive elements (RARE or RXRE), that usually are located within the 5’-regulatory region of retinoid-regulated genes but occasionally are found within introns. A
large number of genes contain one or more RARE and therefore are potentially responsive to RA. Because the RXR functions as a partner for several families of nuclear receptors other than the RAR, the RXR and the retinoid ligands that bind to them have the potential to influence an ever-wider variety of genes than are regulated by the RARs (Fig. 1).

Because all-trans-RA and 9-cis-RA bind to their receptors with high affinity \([K_d \text{ values in the nanomolar range (4)}]\) and regulate a very broad range of biological functions, it is logical that the production and catabolism of bioactive retinoids should be closely controlled. At the present time, a number of enzymes from several gene families have been proposed to function in the oxidative activation of retinol to form RA [reviewed in Ross et al. (5) and Napoli (6)]. But it remains uncertain whether these enzymes are essential for regulating cellular retinoid concentrations in vivo. Even less is known of how their activities might change in response to consumption of vitamin A or alterations in retinoid status.

The concept of retinoid “autoregulation” at the level of gene transcription was first proposed based on the findings of an RARE or RXRE element within the promoter of genes encoding several retinoid-related proteins, such as an RARE in the promoter of the RAR, \(\beta\) gene (7), and an RXRE in the promoter of the cellular retinoic acid–binding protein (CRABP)-II gene (8). In addition to transcriptional regulation, it is likely that other autoregulatory mechanisms contribute to retinoid homeostasis, such as is demonstrated by the retinoid-dependent proteolytic degradation of retinoid receptor proteins (9) and the regulation of receptor protein stability by phosphorylation (10).

This review focuses on recent investigations concerning two enzymes that we believe are key factors in retinoid homeostasis, both of which are regulated in response to RA. The first of these is LRAT, which catalyzes the esterification of retinol in several retinol-metabolizing tissues (small intestine, liver, retina, skin, testis, lung and probably more). As is illustrated in Figure 2, LRAT is situated at the head of the pathway of RA biosynthesis. Although the role of LRAT in the conversion of retinol into retinyl ester for storage is well appreciated, it is speculated that another crucial function of LRAT may be to divert retinol away from oxidative activation, thus aiding in the down-regulation of RA biosynthesis. The second enzyme is CYP26, a newly recognized member of the cytochrome P450 family, which is expressed in numerous embryonic and adult tissues. Interestingly, all-trans-RA is both the principal inducer of the CYP26 gene and the principal substrate of the CYP26 protein (11–14). Retinoid-induced expression of the CYP26 gene is mediated through RA-induc ed transactivation of an RARE located in the CYP26 promoter (15), whereas the CYP26-mediated oxidation of all-trans-RA results in the formation of polar metabolites (hydroxy- or keto- metabolites of RA), which in general are less bioactive than all-trans-RA. Our research has tested the hypothesis that LRAT and CYP26 are both responsive to rapid changes in retinoid status (acute model), as well as being regulated by vitamin A nutritional status over long periods (steady-state chronic model).

### Regulation of LRAT and CYP26 expression by diet and exogenous retinoids

**Regulation of LRAT gene expression.** Previous research revealed that LRAT enzyme activity is very low (oftentimes nil) in the liver of vitamin A–deficient rats (16–19). Because LRAT activity in liver micromes was assayed in the presence of an adequate supply of retinol as substrate, the lack of formation of retinyl esters indicated that the animals were lacking enzyme activity per se (16). After intact vitamin A–deficient rats were fed a single oral dose of retinol, their liver LRAT activity was measurable within 6–8 h (16). In contrast, adding retinol directly to the liver micromesomes of vitamin A–deficient rats had no effect. From these data we inferred that a metabolite of retinol is formed in vivo that is responsible for the regulation of LRAT activity. Subsequent experiments showed that all-trans-RA (17), or retinoid analogs with RAR-binding but not RXR-binding activity (20), could induce hepatic LRAT activity more rapidly and at lower doses than could retinol. Our initial studies also provided evidence that the regulation of LRAT activity is tissue specific. Although LRAT activity in the liver was exquisitely sensitive to vitamin A and exogenous retinoids, there were no differences in LRAT activity in the small intestine under the same conditions (16,18).

The cloning of LRAT cDNA (21,22) revealed that the LRAT gene is unrelated to any known genes and that the LRAT protein is highly homologous (80–94%) among human, bovine, mouse and rat species (22,23). With new molecular tools in hand, it became possible to evaluate whether the regulation of LRAT activity that had been observed previously is due to molecular regulation at the level of LRAT gene expression. Similar to LRAT protein, LRAT mRNA was essentially absent from the liver of vitamin A–deficient rats and mice (22). However, within a few hours after treating vitamin A–deficient rats or mice with RA (acute model), LRAT mRNA was expressed at levels equal to or greater than that of the control, vitamin A–adequate, animals. LRAT enzyme activity and mRNA were also measured in rats fed a vitamin A–restricted diet throughout their life (chronic or steady-state model) (22,24). The diet used was designed to sustain a state of marginal vitamin A deficiency, without an...
impaired of growth, as animals progressed from youth to middle age to old age (25). In rats of all ages, marginal vitamin A deficiency resulted in liver LRAT mRNA and activity levels that were significantly reduced compared with those of vitamin A–adequate rats of the same age (22,24). Conversely, when rats were fed a vitamin A–supplemented diet, which significantly elevated their hepatic vitamin A stores, LRAT mRNA and activity were significantly increased (22,24). Overall, these studies using both acute and chronic models to investigate the effects of a wide range of vitamin A status on retinoid metabolism revealed that LRAT mRNA and LRAT activity in the liver are regulated progressively in the order: vitamin A deficient (nil) < vitamin A marginal (low but detectable) < vitamin A adequate (control) < vitamin A supplemented or RA treated (significantly increased but generally less than twofold above control).

As noted earlier, the regulation of LRAT gene expression by vitamin A and RA is tissue specific, with regulated expression in the liver and constitutive expression in the small intestine. To better understand the regulation of LRAT in various tissues, we recently conducted a study of LRAT expression in the adult rat lung. The lung had been shown to contain esterified retinol, at concentrations well below those in the liver, but the mechanism and potential regulation of retinol esterification were unknown (26,27). Using Northern blot analysis and quantitative real-time polymerase chain reaction, LRAT mRNA was demonstrated to be present in the vitamin A–adequate adult lung, as was LRAT activity, which was measured by the esterification of cellular retinol-binding protein (CRBP)-bound retinol by lung homogenates or microsomes (28). As had been shown previously for liver, the levels of RNA expression and retinol esterifying activity in the lung were well correlated (r = 0.92, P < 0.0001) (28). Also similar to the liver, LRAT mRNA and enzyme activities in the lung were regulated by vitamin A status and treatment with RA. LRAT levels were significantly reduced in the lung of vitamin A–deficient rats (Fig. 3). However, 16 h after treatment of vitamin A–deficient rats with a low dose of RA (100 μg), lung LRAT mRNA did not differ from that in vitamin A–adequate rats. The administration of a pharmacological dose of RA (5 mg) to either vitamin A–deficient or control rats resulted in a significant elevation of both LRAT mRNA and retinol esterifying activity [6.2- to 8.9-fold above control, respectively (28) and Fig. 3B]. Thus although the relative expression of LRAT mRNA and activity is lower (~20%) in the lung compared with the liver, LRAT expression is regulated by vitamin A in both organs. Previously, it was suggested that the down-regulation of LRAT expression in the liver, such as occurs during the onset of vitamin A deficiency (16), could provide a mechanism for conserving retinol for as long as possible for use in other organs, including oxidative activation or secretion into plasma (22). Similarly, the down-regulation of LRAT in the lung could be a mechanism to preserve the local supply of retinol of the lung for the local production of RA. Although LRAT expression was induced by exogenous RA in both vitamin A–deficient liver and lung, the response of the lung to RA was proportionately greater. Moreover RA increased the level of LRAT expression in the lung of normal vitamin A–adequate rats (Fig. 3) (28), but in comparison caused only a small increase in liver LRAT expression (22). These relative tissue-specific differences in the regulation of LRAT between organs could be due to quantitative differences in RA concentration within tissues and/or to qualitative differences in the mechanism of regulation of LRAT by RA, independent of RA concentration. Despite the sensitive regulation of LRAT expression by RA and RAR receptor agonists, evidence for the direct transcriptional regulation of the LRAT gene has yet to be presented. The regulation of LRAT mRNA by posttranscriptional mechanisms would also be consistent with the regulation of LRAT mRNA expression and the high correlation between LRAT mRNA and enzyme activity. Further research is required to understand the mechanisms by which tissue RA levels are controlled, the potentially numerous mechanisms by which LRAT expression is regulated and the effects of vitamin A status and retinoid treatment on these mechanisms within various tissues.

**Possible effect of LRAT activity on plasma retinol concentrations.** The liver performs several, potentially competing, functions with respect to retinol metabolism and transport. Retinol-binding protein (RBP), which transports nearly all plasma retinol, is synthesized within liver parenchymal cells as apo-RBP and, after combination with retinol in the endoplasmic reticulum, is secreted from liver into plasma as holo-RBP. In a series of nutritional studies, Goodman and colleagues (30) showed that the abundance of RBP mRNA in the liver and the efficiency of translation of RBP mRNA into RBP protein did not differ between vitamin A–deficient and control rats. However, apo-RBP accumulated in vitamin A–deficient rat liver and parenchymal cells (see 31–33 for reviews). Because the provision of retinol to intact rats or perfused rat liver

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**FIGURE 3** Regulation by vitamin A and retinoic acid (RA) of the molecular expression of lecithin:retinol acyltransferase (LRAT) mRNA and activity in rat lung. A, Northern blot analysis of poly(A)⁺ RNA from lung tissue pooled from vitamin A–sufficient (VAS) pair-fed rats, vitamin A–deficient rats and vitamin A–deficient RA-treated adult rats and from liver of vitamin A–sufficient rats. Differences between groups with different letters were significant, P < 0.005 (least squares means test, a < b < c). B, LRAT enzyme activity assay, which used [³H]retinol bound to cellular retinol-binding protein as the substrate (28). Differences between groups with different letters were significant, P < 0.0001 (least squares means test). From Zolfaghari and Ross (28), with permission of the American Society for Nutritional Sciences.
resulted in a very rapid release of holo-RBP from liver to plasma or perfusate, respectively, it was deduced that the secretion of RBP is regulated by the availability of retinol itself (32), a concept further supported by biochemical studies showing that the folding and stabilization of newly synthesized RBP is regulated in part by retinol (34). It is therefore reasonable to surmise that mechanisms that control the availability of retinol may have an important bearing on the secretion of holo-RBP from the liver and therefore on the delivery of retinol to target tissues throughout the body. Having observed that the expression of liver LRAT is up-regulated by exogenous RA and supplemental vitamin A, we wondered whether the supply of retinol available for secretion might be reduced and whether this would be evidenced by a reduction in plasma retinol concentration. Indeed, the concentration of plasma retinol in vitamin A–adequate RA-treated rats (shown to have increased lung and liver LRAT mRNA and activity) was reduced by >50% 16 h after RA administration (28). Ritter and Smith (35) also observed a rapid and very dramatic reduction in plasma retinol after RA treatment and moreover showed that the reduction was greater for all-trans-RA compared with several other retinoid compounds. Further research is needed to determine whether the reduction of holo-RBP in plasma is due specifically to reduced secretion and whether the regulation of liver LRAT actually diverts retinol away from secretion. Regardless of the cause and the mechanism of this effect, it appears that the concentration of holo-RBP to which target tissues are exposed may also be significantly reduced in response to a high dose of RA. This effect fits well with the idea, expressed again later, that RA may function as a signal of the body’s vitamin A adequacy. If so, it would be appropriate for the liver to increase an increase in RA as indicating that peripheral tissues are now retinoid sufficient, and for the liver’s response to be to reduce the flow of apo-RBP to peripheral tissues.

Further research is needed to clarify the relationships between circulating retinoid levels and tissue retinoid metabolism under both normal and pathological conditions. Tissue retinoid concentrations are often reduced in tumor tissue compared with surrounding normal tissue (36–38), and numerous differences between normal and malignant cells and tissues have been reported, including a loss or aberrant expression of retinoid receptors or binding proteins (39,40) and retinoid-metabolizing enzymes (41). A loss of capacity for retinol esterification and LRAT expression (41) has been reported for several cancer cell lines or tumor tissues. Whether aberrations in retinoid metabolism are part of the cause of carcinogenesis or an effect of tumor development remains an important, unresolved issue.

**Regulation of CYP26.** That the metabolism of all-trans-retinoids and their cis-counterparts is at least in part segregated may be deduced from the selectivity of most retinoid-binding proteins, receptors and several enzymes for either the all-trans form of a specific retinoid or one or more of its cis-isomers. The cytochrome P450 now designated as CYP26(A1) was first cloned in 1997 as P450RAI (RA inducible) and shown by several laboratories to be expressed in embryonic and adult tissues of lower and higher vertebrate species (11–14). The hallmarks of CYP26 are its selective inducibility by all-trans-RA and its metabolism of all-trans-RA. The direct and rapid induction of the CYP26 gene by all-trans-RA is due to presence of a highly conserved 32-bp segment containing a canonical, direct repeat-5 type of RARE in the 5’ upstream promoter region (15). Expression of CYP26 cDNA resulted in enzymatic activity towards all-trans-RA and the production of several polar metabolites, including 4-hydroxy-RA and 4-oxo-RA (11,14). These results imply a direct transcriptional mechanism through which the CYP26 gene may sense the concentration of RA in its environment by the binding of RA to the RAR/RXR, which binds to its RARE, and then may respond rapidly through gene transcription and the production of new CYP26 enzyme to adjust and control the concentration of the cell of all-trans-RA.

To determine whether CYP26 expression is regulated by differences in dietary vitamin A and by exogenous RA, Yamamoto et al. (42) conducted a series of studies in rats and mice, similar to those conducted to define the regulation of LRAT by vitamin A. Similar to LRAT, the expression of CYP26 in the liver of vitamin A–deficient mice and rats was nil. Also similar to LRAT, CYP26 gene expression was barely detectable in rats with marginal vitamin A deficiency. Conversely, when rats were fed a vitamin A–supplemented diet that significantly elevated their liver vitamin A stores, CYP26 expression increased dose-dependently with liver vitamin A. In rats treated with exogenous RA, CYP26 mRNA levels greatly exceeded the control level (42,43). In a recent dose-response study using a 1000-fold range of exogenous RA, the hepatic CYP26 mRNA response was essentially linear with RA dose (43). Overall, the level of CYP26 mRNA in liver was regulated in a progressive manner: vitamin A deficient < vitamin A marginal < control < vitamin A supplemented ≤ RA treated.

Although the response of CYP26A1 to RA is most dramatic in the liver, its regulation by diet and RA is still apparent in extrahepatic tissues (43). In vitamin A–deficient rats, little if any CYP26 mRNA was detected in the lung, small intestines and testis, but treatment with RA resulted in a strong induction, equaling 9-, 6- and 2.5-fold, respectively, above the control level for each of these tissues, within 16 h (43) (Fig. 4). Although the high level of RA-inducible CYP26 expression in the liver suggests that this organ is likely to be responsible for a major portion of CYP26-mediated RA metabolism, the down-regulation of CYP26 expression by vitamin A deficiency and up-regulation by RA in each of the extrahepatic tissues we examined (43) suggest that the local

![FIGURE 4 Regulation of CYP26 expression in extrahepatic tissues. Total RNA from each tissue was subjected to real-time polymerase chain reaction analysis to quantify CYP26 mRNA relative to the 18S RNA content of each sample. Vitamin A–sufficient (VAS) tissue was defined as 1.0 (fold induction) for each tissue. Each tissue from vitamin A–deficient (VAD) rats showed a similar down-regulation of CYP26 expression and a similar up-regulation after treatment of VAD rats with 5 mg of all-trans-retinoic acid (VAD + RA). From Wang et al. (43) with permission of Academic Press.](https://academic.oup.com/jn/article-abstract/133/1/291S/4687535)
control of RA oxidation by CYP26 within peripheral tissues may also be an important mechanism of retinoid homeostasis. The combined data suggest the presence of a "dual system" for regulating RA oxidation involving local control in peripheral tissues as well as strong, potentially high capacity, central metabolism in the liver.

CYP26 has been identified in a wide array of extraplateic tissues and cell types. Its activity is implicated in the emergence of RA resistance that has been observed in clinical trials (44 and references therein). In breast cancer cells and a mouse model of mammary tumor growth, inhibitors of CYP26-mediated RA metabolism reduced the concentration of RA that was needed to inhibit cell proliferation and reduced tumor growth in vivo (44). Thus CYP26 may be an important target for cancer therapy involving retinoid administration.

Isotope kinetic studies showed that most of the RA in the liver is derived by uptake from plasma (45). The liver seems ideally suited by virtue of its anatomic location to act as a sensor of the RA entering it via a portal vein from the intestines and splanchic circulation and via the hepatic vein from the systemic circulation. CYP26 may provide a biochemical mechanism for both sensing RA via its RARE and responding to RA through its induced enzymatic activity. Moreover the liver (and possibly other organs in which RA is oxidized) possesses enzymes capable of phase II metabolism such as UDP-glucuronidyl transfers capable of metabolizing 4-hydroxy-RA (46). Several oxidized water-soluble metabolites of RA, including retinoyl β-glucuronide, have been isolated from liver and bile (47). It is therefore plausible that CYP26 and phase II enzymes act in concert first to convert excess RA into polar metabolites and then to form water-soluble adducts for elimination from the body.

Although the likely catabolic function of CYP26 toward RA has been emphasized here, it is worthwhile to note that CYP26 may also oxidize other retinoids such as retinol into hydroxy- and keto-derivatives corresponding to those for RA (48). In addition, although the oxidation of RA is generally thought to reduce its activity, it appears that the oxidized forms of RA may possess significant bioactivity in some systems (49). Further research is needed to define the biochemical chemistry of CYP26 and to clarify the nature and activity of its metabolic products in different tissues.

**A model for retinoid homeostasis**

Plasma retinol circulates between liver and tissues several times before undergoing irreversible degradation (50,51). It is plausible that the retinol molecules that are taken up by the liver, whether derived from intestinal absorption or recycling, enter a "free retinol pool" which may in fact be associated with CRBP (52,53). RA enters the liver via a portal vein from the intestines and splanchic circulation and via the hepatic vein from the systemic circulation. CYP26 may provide a biochemical mechanism for both sensing RA via its RARE and responding to RA through its induced enzymatic activity. Moreover the liver (and possibly other organs in which RA is oxidized) possesses enzymes capable of phase II metabolism such as UDP-glucuronidyl transfers capable of metabolizing 4-hydroxy-RA (46). Several oxidized water-soluble metabolites of RA, including retinoyl β-glucuronide, have been isolated from liver and bile (47). It is therefore plausible that CYP26 and phase II enzymes act in concert first to convert excess RA into polar metabolites and then to form water-soluble adducts for elimination from the body.

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**LITERATURE CITED**

and can be induced through RA receptors in human breast and colon carcinoma cells. Cell Growth Differ. 9: 629–637.


