Published in final edited form as:

Biochim Biophys Acta. 2012 January ; 1821(1): 124–136. doi:10.1016/j.bbali.2011.06.023.

HEPATIC METABOLISM OF RETINOIDS AND DISEASE ASSOCIATIONS

Yohei Shirakami¹, Seung-Ah Lee¹, Robin D. Clugston, and William S. Blaner

Department of Medicine, College of Physicians and Surgeons, Columbia University, 630 W. 168th St., New York, NY 10032

Abstract

The liver is the most important tissue site in the body for uptake of postprandial retinoid, as well as for retinoid storage. Within the liver, both hepatocytes and hepatic stellate cells (HSCs) are importantly involved in retinoid metabolism. Hepatocytes play an indispensable role in uptake and processing of dietary retinoid into the liver, and in synthesis and secretion of retinol-binding protein (RBP), which is required for mobilizing hepatic retinoid stores. HSCs are the central cellular site for retinoid storage in the healthy animal, accounting for as much as 50–60% of the total retinoid present in the entire body. The liver is also an important target organ for retinoid actions. Retinoic acid is synthesized in the liver and can interact with retinoid receptors which control expression of a large number of genes involved in hepatic processes. Altered retinoid metabolism and the accompanying dysregulation of retinoid signaling in the liver contribute to hepatic disease. This is related to HSCs, which contribute significantly to the development of hepatic disease when they undergo a process of cellular activation. HSC activation results in the loss of HSC retinoid stores and changes in extracellular matrix deposition leading to the onset of liver fibrosis. An association between hepatic disease progression and decreased hepatic retinoid storage has been demonstrated. In this review article, we summarize the essential role of the liver in retinoid metabolism and consider briefly associations between hepatic retinoid metabolism and disease.

1. Overview

The liver is quantitatively the most important storage site for retinoid in the body [1–3]. It is also quantitatively the most important tissue site of postprandial retinoid uptake in the body, accounting for uptake of 66–75% of all of dietary retinoid that is absorbed by the intestine [1–3]. And, the liver is the major organ site for retinol-binding protein (RBP) synthesis and secretion, accounting for 70–80% of all RBP that is normally present in the circulation [4, 5]. Note that RBP is also referred to in the literature as RBP4. In the fasting circulation of retinoid-sufficient animals, RBP maintains constant circulating levels of retinol, assuring continuous retinoid delivery to target tissues [4–6]. Because of its large role in each of these processes, the liver is the central organ in the body involved in retinoid storage and metabolism. This will be discussed in more detail below in Section II. Hepatic Metabolism.

© 2011 Elsevier B.V. All rights reserved.

Address Correspondence to: William S. Blaner, Ph.D., Department of Medicine, College of Physicians and Surgeons, Columbia University, 630 W. 168th St., New York, NY 10032, (P) 212 305-5429, (F) 212 305-2801, wsb2@columbia.edu.

¹Contributed equally to this work.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The liver is also an important target organ for retinoid actions. The 3 retinoic acid receptors (RAR α , RAR β , and RAR γ) are expressed in liver, as are the 3 retinoid X receptors (RXR α , RXR β and RXR γ) [7]. The importance of retinoid signaling for maintaining a healthy liver is evidenced by many observations. For instance, transgenic mice, which express in a hepatocyte-specific manner a dominant-negative form of RAR α that ablates retinoic acid and RAR signaling, are predisposed to spontaneously developing hepatocellular carcinoma [8]. There also are established associations between hepatic disease development and impaired hepatic retinoid storage. As illustrated in Figure 1, progressively worsening stages of hepatic disease observed for alcoholic patients are associated with increasingly diminished hepatic retinoid stores [9]. The diminished hepatic retinoid stores observed in this alcoholic population could not be accounted for by lessened dietary retinoid intake [9]. Thus, retinoids are needed for maintaining normal hepatic health, and hepatic retinoid stores are adversely affected by liver insult and injury. These processes will be discussed in more detail in Section 3. Associations between hepatic retinoid physiology and disease.

The key linkage between hepatic retinoid storage and metabolism and hepatic disease resides in hepatic stellate cells (HSCs; referred to as Ito cells, fat-storing cells or lipocytes but the preferred nomenclature for these cells is now HSCs [10, 11].) HSCs are the major cellular site for retinoid storage within the liver, accounting for 70–90% of all retinoid present in the liver. These cells are also importantly involved in the process of hepatic disease development. When HSCs become activated upon hepatic injury, they contribute significantly to aberrant extracellular matrix synthesis and consequently hepatic fibrosis [10, 11]. Moreover, when HSCs undergo activation, their retinoid stores are lost [10, 11].

2. Hepatic metabolism

There are two hepatic cell types centrally involved in retinoid processing and storage: the parenchymal cells (also known as hepatocytes) and HSCs [12]. The hepatocytes account for approximately 67% of all cells present in the liver and approximately 90% of hepatic protein; whereas the HSCs account for only approximately 8% of total hepatic cells and 1% of hepatic protein [10, 11]. It is well-established that hepatocytes are critically involved in the uptake and processing of dietary retinol into the liver and in the secretion of RBP, and that HSCs play a central role in storing hepatic retinoid. This section will discuss advances in our understanding of hepatic retinoid metabolism, including the uptake and processing of chylomicron remnant-retinyl ester by hepatocytes, the transfer of this retinoid to HSCs for storage, molecular aspects of retinoid in HSCs, and retinol mobilization from hepatic stores by RBP. These processes are summarized in Figure 2, which gives an overview of hepatic retinoid metabolism, as well as a general summary of whole body retinoid metabolism.

2.1. Processing of dietary retinoids by the liver

2.1.1. Dietary uptake—The main dietary sources of vitamin A are from proretinoid carotenoids, obtained primarily from vegetable food sources, and from preformed retinoid, obtained primarily as retinyl ester from animal sources. Retinyl esters consumed in the diet are hydrolyzed in the lumen of the small intestine before absorption by enterocytes [13]. Proretinoid carotenoids are absorbed intact and converted to retinol within the enterocyte, or other tissues after uptake of dietary carotenoid into the general circulation. At least three enzymes can esterify retinol within enterocytes: lecithin:retinol acyltransferase (LRAT), diacylglycerol acyltransferase 1 (DGAT1), which acts as an acyl CoA:retinol acyltransferase (ARAT), and an as yet to be identified additional ARAT activity that only becomes active upon excessive dietary retinol intake [14, 15]. When normal physiological amounts of retinol are consumed in the diet LRAT is responsible for esterifying more than 90% of this retinol [16], with DGAT1 accounting for the esterification of the remainder [15]. When large supraphysiological or pharmacological doses of retinol are consumed, another as yet to

be identified ARAT can contribute to esterification [15]. For more details regarding retinoid and carotenoid metabolism within the gastrointestinal (GI) tract, the reader is referred to the recent review by D'Ambrosio et al. [14].

The preponderance of dietary retinol absorbed by enterocytes is secreted into the lymphatic system as retinyl ester in chylomicrons, which also contain other dietary lipids, consisting of primarily triglyceride and cholesteryl ester. Approximately 66–75% of chylomicron retinyl ester is removed from the circulation by the liver with the remaining 25–33% by extrahepatic tissues, including skeletal muscle, adipose tissue, heart, spleen, and kidney [3]. The majority of retinyl esters present in chylomicrons remain with these particles as they are metabolized to chylomicron remnants in the peripheral blood, through a process that involves lipolysis of the dietary triglycerides and recruitment of apolipoprotein E (apoE) to the particles. Chylomicron remnants are thought to be internalized solely by hepatocytes [17]. Other hepatic cell types do not appear to participate in hepatic uptake of these intestinally derived particles. The uptake process involves chylomicron remnant sequestration in the space of Disse, further lipolytic processing, followed by receptor-mediated uptake of the retinyl ester containing chylomicron remnant into hepatocytes. ApoE plays an essential role in the uptake of chylomicron remnants by the liver. After acquisition of apoE, either in the peripheral blood or in the space of Disse, chylomicron remnants are sequestered in close proximity to hepatocytes through binding to heparin sulfate proteoglycans (HSPGs) present on the surfaces of hepatocytes. Subsequently, the remnant undergoes endocytosis and uptake into the hepatocyte. Several distinct cell surface receptors are proposed to be involved in the receptor-mediated uptake of chylomicron remnants. Among those are the low density lipoprotein (LDL) receptor (LDL-R), the LDL receptor related protein (LRP), and the lipolysis-stimulated receptor (LSR) [17]. A detailed discussion of the actions of these receptors can be found in an excellent review from Yu et al [18].

Once chylomicron remnant retinyl esters have been taken up by hepatocytes, a rapid hydrolysis of the retinyl ester takes place. This hydrolysis may be catalyzed by a number of enzymes that are often referred to as retinyl ester hydrolases (REHs). These include carboxylesterases and/or lipases that recognize retinyl esters as substrates. However, the specific protein species or species physiologically responsible for catalyzing this process have not been conclusively identified. This was extensively reviewed recently by Harrison [19, 20]. Among the REHs proposed to hydrolyze newly internalized chylomicron remnant retinyl ester are both neutral and acidic bile salt-independent REHs. All are organelle associated, present either in microsomes derived from the plasma membrane early endosome or in lysosome preparations, with almost no activity associated with the soluble fraction [21, 22]. This subcellular localization allows the REHs to play a role in the initial hydrolysis of retinyl esters delivered to the liver in chylomicron remnants. The potential importance of a bile salt-independent, neutral REH activity in retinoid metabolism is underscored by evidence that this enzymatic reaction is stimulated by apo-CRBPI at physiological concentrations, suggesting that apo-CRBPI may be a regulator of retinyl ester hydrolysis in vivo [21]. Other studies have been directed at determining whether neutral and acidic, bile salt-independent retinyl ester hydrolases associated with plasma membrane and endosomes fractions of rat liver homogenates are involved in the hepatic retinyl ester metabolism [23]. For these investigations, chylomicrons containing ^3H -labeled retinyl esters were injected intravenously into rats to study the fate of retinyl esters during and immediately following uptake into the liver. It was shown that labeled retinyl esters were rapidly cleared from plasma and appeared in the liver. Within the liver, the radiolabel first appeared in plasma membrane/endosomal fractions that were also enriched in both neutral and acidic, bile salt-independent retinyl ester hydrolases activities. Since ^3H -labeled retinol next appeared in fractions enriched in endoplasmic reticulum (ER), this was taken to suggest that the retinyl

esters were hydrolyzed early after uptake [23]. These studies further demonstrated the colocalization of newly delivered retinyl esters and bile salt-independent REH activities, suggesting a probable role for this enzyme(s) in the hepatic uptake and processing of chylomicron retinoid. Thus, it has been postulated that the neutral REH acts on chylomicron remnant-retinyl ester at the cell surface or when this retinyl ester enters the early endosomes. As the pH drops in late endosomes, retinyl ester hydrolysis is continued by acidic REH [19]. In later *in vitro* studies, it was shown that rat liver carboxylesterases, ES-2 and ES-10 can function as neutral bile salt-independent REHs. More recently, ES-22 has been added to the list of hepatic REH that localize to the ER [24].

A number of *in vitro* studies have identified well characterized lipases, specifically hormone sensitive lipase (HSL) and lipoprotein lipase (LPL), as possessing REH activity [25]. HSL is expressed in liver, as is LPL, albeit at relatively low levels. However, the physiological significance of these enzymes in mediating retinyl ester hydrolysis in the liver remains to be established.

2.1.2. Intercellular transfer within the liver of newly absorbed retinoid—After hydrolysis within the hepatocyte, the majority of chylomicron remnant retinol is transferred to the HSCs for storage [26, 27]. In the retinoid-sufficient state, almost all of the chylomicron remnant retinyl ester taken up by hepatocytes is rapidly transferred, probably as the alcohol retinol, to HSCs for storage [26, 27]. However, in times of dietary retinoid-insufficiency, less of the recently ingested retinol is directed towards storage and more is secreted into the circulation bound to RBP. Early studies suggested that a plausible candidate for a carrier mediating intercellular transfer from hepatocytes to HSCs was RBP. Seminal early work by Blomhoff et al. established that during *in situ* perfusion of rat livers previously given chylomicrons labeled with radioactive retinyl esters, retinol was transferred from hepatocytes to HSCs, and that the addition of antibodies against RBP blocked the transfer, suggesting that RBP was the retinol carrier [28]. This observation was supported by later data from Senoo et al., which showed that antibodies to RBP blocked retinol transfer from HepG2 cells to rat HSCs, when these different cell types were co-cultured [29]. These and other early studies led to the suggestion that RBP could be bound and internalized by HSCs, supporting the notion that RBP mediates intercellular transfer of retinol from hepatocytes to HSCs.

However, more recent studies of RBP-deficient mice suggest that this idea is incorrect. Quadro et al. generated RBP-deficient mice and showed that hepatic retinoid is stored in HSCs within the livers of these mutant mice. This result establishes that the absence of RBP does not impair hepatic storage of retinol in HSCs [30]. Moreover, there was no quantitative or qualitative differences in the lipid droplets present in the HSCs of 3-month-old RBP-deficient compared to wild type mice. These *in vivo* studies provide strong evidence that RBP does not act in an essential manner in the transfer of retinol from hepatocytes to HSCs.

The possible involvement of CRBPI in the transfer of dietary retinol from hepatocytes to HSCs was proposed by Ghyselinck et al., whose studies of CRBPI-deficient mice suggested CRBPI may help mediate the transfer of retinol from hepatocytes to HSCs [31]. It has long been established that CRBPI serves as an intracellular transporter of retinol, linking and facilitating the processes of retinol uptake, metabolism, and mobilization within cells that express this protein. It had been suggested that free retinol never accumulates inside cells, as the concentration of CRBPI always exceeds that of retinol [32]. Early studies clearly showed that CRBPI is important for efficient retinyl ester synthesis and storage *in vivo*, and its absence results in wasting of retinol. In keeping with this early work, Ghyselinck et al. showed that CRBPI-deficiency resulted in significantly lower hepatic retinyl esters, with the mutant mice having an ~50% reduction of retinyl palmitate, the main ester form found in the

liver. Light microscopy also revealed that the HSCs of CRBPI-deficient mice displayed a reduction in both the number and size of lipid droplets. This suggested to these authors that a decrease in HSC retinyl ester accumulation may be due to impaired delivery of retinol to the retinyl ester synthesizing enzyme LRAT, which is highly expressed in HSCs [12]. Indeed, LRAT utilizes CRBPI-bound retinol as a substrate, providing optimal activity *in vitro* [33]. Thus, Ghyselinck et al. raised the possibility that CRBPI may help facilitate retinol transfer from hepatocytes to HSCs [31].

As noted above, the molecular processes involved in facilitating movement of newly absorbed dietary retinoid from hepatocytes to HSCs for storage remain to be identified. Contrary to what had been proposed in the older literature [28, 29], genetic studies definitively establish that RBP is not essentially needed to catalyze transfer [30]. Although CRBPI likely has a role in the transfer process [31], CRBPI cannot be solely responsible for transfer since there is no evidence that CRBPI ever leaves its intracellular environment moving into the extracellular space or between cells. We have wondered whether retinoid transfer may involve movement between hepatocytes and HSCs of small lipid-rich particles which encapsulate the retinoid. Alternatively, it is possible that some of the older literature regarding dietary retinoid uptake and storage within the liver may be incorrect. This older literature indicates that chylomicron remnant retinoid is taken up first by hepatocytes [26] and then transferred to HSCs through a process which requires that the chylomicron remnant retinyl ester first be hydrolyzed to retinol [34]. The older literature also indicates that chylomicron remnant retinoid is removed from the remnant particle before the particle reaches the early endosome phase of the endocytic process [23]. This earlier literature needs to be reevaluated. It is possible to hypothesize a scenario which agrees with much of the older data where chylomicron remnant retinyl ester is hydrolyzed by extracellular lipases associated with the plasma membrane, like hepatic lipase, endothelial lipase or lipoprotein lipase, and taken up directly by HSCs without first entering hepatocytes. Although this or other hypotheses for explaining intercellular transfer within the liver must still be considered speculative, it is clear that new experimentally testable ideas regarding the transfer process are needed if we are to move forward towards obtaining molecular understanding of retinoid uptake by and storage in the liver.

2.1.3. Recycling of retinol-RBP from the periphery back to the liver—Tracer kinetic studies have established that circulating retinol, bound to RBP, enters and leaves the liver several times prior to its elimination from the body, in a process known as retinol recycling [35]. Prior to the undertaking of these studies, conventional wisdom held that newly absorbed dietary vitamin A was transported to the liver where it was either stored or resecreted into the circulation bound to RBP (i.e., as holo-RBP) for delivery to target tissues for uptake and utilization. The notion that retinol was recycled back to the liver from the periphery via RBP had not been considered earlier. By applying mathematical modeling to *in vivo* kinetic data, Green and colleagues have been able to describe and quantitate whole body and organ level retinoid metabolism under different nutritional conditions. These studies have convincingly established that retinol transport via RBP is complicated and bidirectional, involving transport both to and from the liver. Moreover, these studies revealed that the liver is not the only source of input of retinol-RBP into plasma and predicted roles for extrahepatic tissues in whole body retinoid dynamics. Importantly, Green and colleagues have demonstrated the significance of plasma retinol pool size in retinoid utilization and predicted contributions of extrahepatic tissues to retinoid storage and plasma retinol homeostasis [36, 37].

The kinetic studies have shown that there is extensive recycling of retinol between plasma and tissues prior to its irreversible loss from the body. This is predicted by models developed for rats with low, marginal, and high retinoid stores. Experimentally, these

studies involved the administration of doses [^3H]retinol-RBP intravenously and the monitoring of [^3H]retinol levels in plasma for use in estimating retinoid disposal rate (utilization rate). For rats with marginal liver retinoid stores (liver total retinol < 350 nmol) [38], the kinetic model predicted that the plasma retinol turnover rate was 13-times the disposal rate (24 nmol/day), and that 48% of plasma retinol was recycled to the liver while 52% was transferred to nonhepatic tissues. For these rats, 44% of the whole body retinoid pool was predicted to be present in extrahepatic tissues, suggesting that significant amounts of retinoid are present or stored in extrahepatic tissues. In rats experiencing low or marginal retinoid nutritional status (liver total retinol ~3.5 nmol) [39], the plasma retinol turnover rate (70 nmol/day) was about 12-times the disposal rate, indicating that, even though plasma retinol levels and liver retinoid stores were low in these rats (0.35 $\mu\text{mol/L}$ and 3.5 nmol, respectively), retinoid recycling was still high. Although dietary retinoid intake was very low for these animals, plasma retinol concentrations were only about 20% of normal, and liver retinoid stores were essentially depleted, these rats grew normally and appeared healthy. Thus, the animals appeared to have adapted to the chronic condition of low dietary retinoid intake. Of interest, the kinetic model predicted that “carcass” contained 39-times more total retinol (retinol + retinyl ester) than the liver of these animals. Another study was undertaken in rats with very adequate retinoid stores (liver total retinol ~4550 nmol) [40]. Of note, the model generated for rats with high hepatic retinoid stores predicted that < 0.1% of plasma retinol turnover went to the eyes or adrenal, < 1% to the lungs or testes, 4% to the liver or small intestines, 30% to the remaining carcass, and 60% to the kidneys, respectively [41]. The irreversible utilization rate was predicted to be 36 nmol/day, which is only 10% of the plasma retinol turnover rate (378 nmol/day). In the case of the kidneys, the models predicted rapid exchange of retinol between the fast turning over kidney compartment and plasma, with numerous cycles before loss from the body [39]. Thus, the kinetic modeling studies carried out in rodents have led to the hypothesis that both hepatic and extrahepatic pools of retinoid contribute towards maintaining normal plasma retinol levels even when dietary retinoid intake is low. These studies also have identified plasma retinol as the main determinant of irreversible utilization of retinol [36, 42]. Moreover, they have established that retinoid recycling in plasma from all organs to and from the liver is a prominent feature of the plasma retinol kinetics.

Tracer kinetic studies have also been carried out in human subjects and these have resulted in kinetic models for predicting human retinol turnover. In humans, ~50 $\mu\text{mol/day}$ (14.3 mg/day) of retinol passes through plasma, compared with an estimated disposal rate of 4 $\mu\text{mol/day}$ (1.14 mg/day) [43]. This model predicts that a large portion of retinol that is taken up by organs and tissues from circulating retinol-RBP is recycled back into plasma and that only a minor portion is either converted to active metabolites or degraded, confirming the suspected similarities in many aspects of retinoid metabolism in humans compared with rats. Ross and Zolfaghari [44] have suggested that recycling provides an ideal means for the liver to sample constantly and adjust the concentration of retinol available in plasma for peripheral tissues. Further, there is a growing body of studies on plasma retinol kinetics in non-human primates aimed at developing reliable assessment tools for assessing human retinoid status. Stable isotope-labeled retinol is currently available for safely studying retinoid metabolism in humans. The deuterated retinol dilution (DRD) technique provides a quantitative estimate of total body retinol pool size and the paired-DRD technique permits calculation of the change (positive or negative) in total body retinol pool size that occurs in response to different intakes of retinoid [45, 46]. Surles et al. assessed retinoid status using breast milk of sows by administering an oral dose of 3,4-didehydroretinyl acetate, which circulates in the blood as 3,4-didehydroretinol (DR). In this study, the milk DR:R (R, serum retinol) ratio was investigated for its value as an indicator of maternal retinoid status over a number of lactation cycles during retinoid depletion and compared with retinoid-replete sows [47]. These authors were interested in developing a model that allows for the use of

breast milk to assess the mother's retinoid status and current dietary intake and the possible effects that these may have on the nursing piglet's retinoid status [47].

The conclusions obtained from tracer kinetic studies are now well supported by biochemical and molecular investigations. It is now understood that extrahepatic tissues have considerable capacity for retinoid storage, metabolism and mobilization. Biochemical studies reported by Soprano et al. [42] corroborated the modeling studies by showing that extrahepatic tissues, including the kidneys, have the potential to synthesize and secrete RBP (i.e., they contain RBP mRNA). Other studies established that adipose tissue is a major site for retinoid storage and for RBP synthesis [6]. More recently, the molecular mechanisms underlying the reabsorption and resecretion of retinol by the kidneys have been established [48, 49]. Thus, solid biochemical data support the findings from kinetic modeling studies which indicate that extrahepatic tissues have important role in maintaining whole body retinoid dynamics.

2.2. Retinoid storage in the liver

2.2.1. Hepatocytes—The hepatocyte plays a key role in mediating hepatic retinoid storage and metabolism. Hepatocytes account for some retinoid storage and act essentially in the mobilization of retinol from the liver. These cells also contribute significantly to retinoid activation to retinoic acid, to retinoic acid catabolism and to the excretion of retinoid catabolic products. The hepatocyte has relatively high concentrations of total retinol (retinol + retinyl ester), RBP, CRBP-I, LRAT, bile salt stimulated and bile salt independent retinyl ester hydrolases, and enzymes which catalyze formation of retinoic acid from retinol and oxidative and/or catabolic metabolism of retinoic acid [50].

The total retinol concentration of hepatocytes isolated from rats maintained on a control diet was reported to be 5.2 ± 2.4 nmol retinol/ 10^6 cells (or 2.6 nmol retinol/mg cellular protein) [51]. Approximately 95–99% of this total retinol is present as retinyl ester, primarily as the esters of palmitic, stearic, oleic, and linoleic acid [51]. It has been estimated that approximately 10–30% of the total retinol in the livers of rats maintained on a control diet is present in hepatocytes, with the remainder in HSCs. The relative abundance of hepatocyte total retinol within the liver is thought to be inversely related to hepatic stores [52]. Thus, as hepatic retinoid stores decline, retinoid concentrations in hepatocytes increases relative to HSCs. Conversely, as hepatic retinol stores increase, hepatocyte levels decrease relative to those of HSCs. Since the hepatocyte is the site of RBP synthesis in the liver [5], this observation may suggest that hepatocyte total retinol levels are defended in order to maintain circulating retinol-RBP levels.

CRBPI concentrations in hepatocytes are relatively high, and approximately 90% of the CRBPI present in the liver is localized to hepatocytes [51]. Since the concentrations of CRBPI is present in excess of those of retinol, it has been proposed that all retinol present within these tissues is bound to CRBPI [53]. It also has been postulated that holo-CRBPI delivers retinol to newly synthesized RBP for secretion from the liver into the circulation.

2.2.2. HSCs—Approximately 50–60% of the entire body's total retinoid stores are normally found in HSCs. HSCs are nonparenchymal cells located perisinusoidally in the space of Disse, in recesses between parenchymal cells [54]. The preponderance of retinoid (> 99%) present in HSCs is in the form of retinyl ester packed in cytoplasmic lipid droplets, which are a characteristic morphological feature of these cells [1, 50]. It has been proposed that the lipid droplets within HSCs are specialized organelles for retinoid storage, implicating a strong regulatory role of retinoids in HSCs in lipid droplet formation [12].

Once unesterified retinol formed through the hydrolysis of dietary retinyl ester within hepatocytes is transferred to HSCs, it is esterified back to retinyl esters for storage in lipid droplets. It is well established that HSCs are enriched in CRBP1 and possess high levels of LRAT activity [1, 12, 50]. As discussed above, CRBP1 is an effective donor of retinol for esterification by LRAT, since mice lacking CRBP1 have impaired stores of HSC retinyl esters [31, 55]. It also has been proposed that CRBP1 binding of retinol prevents non-specific ARAT activities from converting the retinol to retinyl ester in the liver [56]. Early *in vitro* evidence showed that an unidentified ARAT may be involved in the hepatic retinyl ester synthesis along with LRAT [57]. The triglyceride synthesizing enzyme and intestinal and skin ARAT, DGAT1, is reported to be expressed in HSCs [58–60]. Thus, DGAT1 seemed to be a good candidate for *in vivo* esterification of retinol in the liver. However, this possibility was not supported by findings from Batten et al. [61], O'Byrne et al. [16] or Liu and Gudas [62], who independently showed that LRAT-deficient mice have very low levels of retinyl ester in their livers [16]. These findings suggest that LRAT may be the sole hepatic enzyme able to synthesize retinyl ester, but also that either LRAT or its product retinyl ester is essentially needed for the HSC lipid droplet formation [16]. This was further evidenced by studies from Liu and Gudas, which show that disruption of the *Lrat* gene makes mice more susceptible to retinoid-deficiency [62].

The number and size of lipid droplets present within HSCs are markedly influenced by dietary retinoid intake and also by intraportal injection of retinol, but not by dietary triglyceride intake [10, 54]. In times of excessive dietary retinoid intake, the number and size of the lipid droplets increases to accommodate the retinoid. In times of insufficient dietary retinoid intake, the lipid droplet retinoid stores are mobilized to meet tissue retinoid stores, and the number and size of lipid droplets correspondingly decrease.

Analysis of the lipid composition of HSC lipid droplets isolated from chow fed rats revealed that the mean percentages of lipids present in the droplets consisted of approximately 40% retinoid and 60% non-retinoid lipid. The specific composition was 39.5% retinyl ester, 31.7% triglyceride, 15.4% cholesteryl ester, 6.3% phospholipid, 4.7% cholesterol, and 2.4% free fatty acids. The retinyl esters in these HSC lipid droplets consisted of only long chain fatty acyl moieties [12, 63]. The most abundant retinyl ester was reported to be retinyl palmitate, which accounts for approximately 75% of the retinyl ester. Retinyl palmitate was followed in abundance by retinyl stearate, retinyl oleate, and retinyl linoleate [63, 64]. Collectively, these four retinyl esters account for more than 95% of the retinyl ester present in HSC lipid droplets. As mentioned above, the concentration of retinyl ester present in HSC stores depends on dietary retinoid intake, whereas triglyceride concentrations, which comprise nearly the same amount of lipid mass as the retinyl esters present in the droplets, are not responsive to dietary fat intake.

Since retinol must be mobilized from HSC retinyl ester stores into the blood in times of dietary retinoid-insufficiency, the lipid droplets must also possess or acquire lipases able to hydrolyze the stored retinyl ester. A number of lipases that are known to possess retinyl ester hydrolase activity *in vitro*, including the carboxyesterases ES-4 and ES-10, are localized to HSCs [19, 65]. However, it is not presently clear which of these enzymes, or possibly other ones, are physiologically important for catalyzing retinyl ester hydrolysis in HSCs.

In vivo, rat liver HSCs exhibit a dual phenotype, that is, a quiescent phenotype in the healthy liver and an activated phenotype in the chronically diseased liver. The quiescent stellate cell phenotype is characterized by lipid droplets rich in retinoid, a low cell proliferative rate and low levels of collagen synthesis. In contrast, the activated or myoblast-like phenotype is distinguished by the loss of the lipid droplets and the retinoid stores they contain. The

activated stellate cell form predominates in liver fibrosis, suggesting a possible linkage between stellate cell storage of retinoids and liver diseases [10, 54]. This topic will be discussed below in Section 3.

2.2.3. Extrahepatic retinoid storage—Although HSCs are particularly important for retinoid storage, as noted above, extrahepatic (peripheral) tissues also play significant roles in the storage and mobilization of retinoid. This was first suggested by the findings of Lewis et al., for both retinoid-sufficient and deficient rats, retinol is extensively recycled among the liver, plasma, interstitial fluid, and peripheral tissues [39]. According to the multicompartamental models developed in these studies, extrahepatic tissues of rats with normal plasma retinol levels but with very low total liver stores should contain as much as 44% of the total retinoid present in the whole body. Retinol and retinyl esters as well as enzymes that are able to esterify retinol and to hydrolyze retinyl esters are found in most peripheral tissues [50]. RBP is expressed and secreted from a variety of tissues, including the kidney, lung, heart, spleen, skeletal muscle, adipose tissue, eyes, and testis [5, 42]. Moreover, lipid droplet-containing stellate cells with a similar phenotype as HSCs have been identified in lung, kidney, and intestine, in normal as well as retinoid-fed rats [64]. These cells are reported to accumulate retinyl esters in lipid droplets after the ingestion of large amounts of retinoid [66]. Both the size and number of the lipid droplets are also reported to increase in response to administration of excess dietary retinoid, suggesting that not only HSCs but also extrahepatic stellate cells play an important role in retinoid storage. Such extrahepatic storage of retinyl esters may serve as an important local supply of retinoid for organs which have a large demand for retinoid [13].

2.3. Retinoid mobilization from the liver

2.3.1. RBP (and TTR)—While the transport of dietary retinol to the liver and extrahepatic tissues is mediated by chylomicron and chylomicron remnants in response to dietary intake, the mobilization of the retinoid from liver stores and its delivery to target tissues in the fasting circulation is a regulated process involving RBP. Goodman and colleagues first demonstrated that retinol in plasma is bound to a specific transport protein, retinol-binding protein (RBP) [67]. RBP has been identified to be present in the circulations of all vertebrates that have been studied. This protein has a molecular weight of approximately 21 kDa and contains a single binding site for one molecule of all-*trans*-retinol. It is abundantly synthesized in hepatocytes, but its expression has also been detected in many other tissues including adipose tissue, the retinal pigment epithelium, the kidney, the ovary, peritubular and Sertoli cells of the testis, the choroid plexus of the brain, and at lower levels in other tissues [5]. Once bound to RBP, the retinol-RBP complex enters the bloodstream for transport to target tissues to meet the tissue retinoid requirements. The plasma concentration of retinol-RBP is strictly regulated and maintained at about 2–3 μM in humans and less in mice (about 1 μM), in spite of normal variations in daily dietary retinoid intake. In times of dietary retinoid-deficiency, when hepatic total retinol stores are exhausted, the concentration of plasma retinol-RBP decreases substantially to levels that are almost undetectable in cases of extreme retinoid-deficiency [5].

The physiological role of RBP in retinoid metabolism was demonstrated by Quadro et al. through targeted disruption of the RBP gene in mice. These investigators reported that RBP-deficient mice have reduced plasma retinol levels that are approximately 12.5% those of age-, gender-, diet- and genetic background-matched wild type mice, as well as reduced retinol and retinyl esters in the retina, and increased liver stores of retinyl ester [30]. Dietary retinol uptake was reported to be normal for these mice. Mutant mice showed impaired retinal function and visual acuity during the first months of life, but adult RBP-deficient mice fed a retinoid-sufficient diet are phenotypically normal. However, when these mice are

fed a retinoid-deficient diet, impaired vision redevelops [68]. Thus, RBP is essential for mobilization of hepatic retinol into plasma, and for the delivery of retinol to the retina, especially when the availability of dietary retinoid is marginal. Subsequent studies, involving transgenic mice that express human RBP synthesized in skeletal muscle, showed that extrahepatic RBP expression rescues the visual impairment of the RBP-deficient mice but unlike RBP expressed in the liver, extrahepatic RBP cannot mobilize liver retinoid stores [69, 70]. Hence, it was concluded that only RBP synthesized in the liver is able to mobilize hepatic retinol stores.

Retinol-RBP circulates in the blood as a 1:1 molar complex with another serum protein, the thyroxine hormone carrier transthyretin (TTR). TTR is a 55 kDa protein, mainly synthesized in the liver and choroid plexus, and secreted into the plasma by the liver and into the cerebrospinal fluid by the choroid plexus. Association of retinol-RBP with TTR makes the retinol-RBP complex less susceptible to the filtration by kidney glomeruli [68, 71, 72]. Mice totally lacking TTR were found to possess plasma retinol and RBP levels that are very low, ~5% of those observed in matched wild type mice [73, 74]. Biochemical studies by van Bennekum et al., employing TTR-deficient mice demonstrated the importance of TTR in maintaining normal levels of retinol and RBP in the circulation. When a physiologic dose of human retinol-RBP was injected intravenously into TTR-deficient mice, it was more rapidly cleared from the circulation and accumulated faster in the kidneys of TTR-deficient mice compared with wild type mice. However, the rate of infiltration of the retinol-RBP complex from the plasma to tissue interstitial fluids was identical in both strains. Thus, these data show that the reduced levels of retinol-RBP in the circulations of TTR-deficient mice arise, at least in part, due to the increased filtration of the retinol-RBP complex.

Despite low circulating levels of retinol-RBP, the total retinol (retinol + retinyl ester) levels in tissues, including liver, of TTR-deficient mice were found to be similar to those in tissues of matched wild type mice. TTR-deficient mice were shown to possess RBP protein levels in their livers that were 60% higher than those of matched wild type mice [74]. These findings establish that TTR does not have a role in hepatic uptake or storage of dietary retinol, but suggest that it may have a role in facilitating hepatic secretion of RBP.

A physiological role for lipoprotein-bound retinyl ester in meeting tissue needs for retinoid was highlighted by studies of mice lacking RBP [30]. The relatively mild phenotype of these mice led Quadro et al. to consider the possible role that postprandial and lipoprotein-bound retinoid had in meeting the physiological needs of these mice for retinoids [69]. Investigations carried out using the RBP-deficient mice revealed that these mice have a relatively high concentration of circulating retinyl ester in the chylomicron/VLDL plasma fraction, and that these mice use postprandial retinyl ester to support normal embryogenesis [69]. However, this alternative pathway for retinoid delivery does not appear to be specifically regulated in response to changes in retinol-RBP levels, since investigations of TTR-deficient mice showed that neither the rate of clearance of chylomicron retinyl ester nor its delivery to tissue is elevated despite reduced delivery of retinol-RBP to tissues. Nevertheless, lipoprotein transport of retinyl ester may play a relatively more substantial role for delivering retinol to tissues, especially when RBP and/or TTR are unavailable.

2.3.2. VLDL—In the fasting circulation, over 95% of retinoid is present as retinol bound to RBP (i.e., as retinol-RBP). The remainder is found as retinyl ester in lipoproteins of hepatic origin, very low density lipoprotein and low density lipoprotein (VLDL and LDL). Thus, VLDL and LDL normally represent a minor alternative retinoid delivery pathway for retinoid delivery from the liver to extrahepatic tissues. However, this pathway may compensate partially for the loss of RBP [75]. It is well established that retinyl esters are associated with VLDL and LDL in the fasting circulation of healthy humans, as well as mice

[76]. In humans, approximately 70% of circulating retinyl esters is associated with the VLDL fraction and the remainder with the LDL fraction [77]. It is not presently understood how retinyl esters come to be present in the VLDL and LDL fractions. It is possible in humans that some of the retinyl ester in VLDL and LDL comes to be present there through the actions of cholesteryl ester transfer protein (CETP), which is known to be able to transfer retinyl ester between triglyceride rich chylomicrons and other lipoprotein fractions [1, 78]. Alternatively, hepatocytes may package and secrete some retinyl ester in nascent VLDL. The relative importance of each of these two processes in humans has not been established.

Early studies indicated that different species show very distinct patterns of distribution of retinyl ester in fasting blood. Studies with normal and cholesterol-fed rabbits demonstrated that the rabbit liver does not secrete retinyl ester in VLDL [79], whereas fasting blood of ferrets and dogs contains very substantial levels of retinyl ester in VLDL, LDL, and HDL [80]. Studies with dogs, in particular, have suggested that lipoprotein bound retinyl ester in the fasting circulations of these animals arises from hepatic secretion of retinyl ester in VLDL [81]. Thus, for some species, VLDL and other lipoproteins may be an important delivery route through which tissues acquire retinoid.

3. Associations between hepatic retinoid physiology and disease

As mentioned above, the liver is the major organ site for retinoid uptake from the diet, retinoid storage and retinol mobilization. These processes involve primarily retinol and retinyl esters. But the liver also contains enzymes which are capable of retinoic acid synthesis, retinoic acid catabolism and excretion [82]. The liver also expresses all 3 retinoic acid receptors (RARs) and all 3 retinoid X receptors (RXRs) [7]. Retinoic acid can undergo catabolism in the liver to more polar metabolites or can be conjugated to form water soluble glucuronides which are eliminated in the urine [83]. Figure 3 shows the summary of these processes. Moreover, retinoids have long been understood to play important roles in the pathophysiology of hepatic diseases, including fatty liver, liver fibrosis, cirrhosis and hepatocellular carcinoma [8, 84]. In this section of the review, we will consider the metabolism of retinoic acid and its involvement with its receptors in the liver. In addition, this section will consider recent findings regarding the involvement of retinoids in the pathogenesis of hepatic diseases.

3.1. Retinoid actions within the liver

Within the liver, like in other tissues, retinol is converted to retinoic acid via two oxidative steps that resemble the oxidation of ethanol to acetic acid. The first enzymatic step needed for retinoic acid formation is the oxidation of retinol to retinaldehyde (also called retinal) and is catalyzed by enzymes that are collectively known as retinol dehydrogenases (RDHs). The second step, one which is attributed to several distinct enzymes referred to as retinaldehyde dehydrogenases (RALDHs), involves the irreversible oxidation of retinaldehyde to retinoic acid. CRBP1 is proposed to play a role in regulating the oxidation of retinol to retinaldehyde, like the role that has been proposed for CRBP1 in retinyl ester formation and retinyl ester hydrolysis (see Section 2 above). Retinoic acid levels are tightly regulated within cells and tissues by enzymes that catalyze the degradation of RA, primarily through its oxidation and glucuronidation.

3.1.1. Retinoic acid synthesis—The first enzymatic step needed for retinoic acid synthesis consists of the reversible oxidation of retinol to retinaldehyde. *In vitro* studies indicate that over a dozen enzymes can oxidize retinol to retinaldehyde [85, 86]. These RDHs, include both short chain dehydrogenase/reductases (SDRs) and several soluble medium chain alcohol dehydrogenases (ADHs) that also catalyze ethanol oxidation. Retinol

oxidation catalyzed by ADHs can be competitively inhibited by ethanol. ADHs oxidize free retinol, but not retinol bound to CRBP [87, 88]. Ubiquitously expressed ADH3 as well as tissue-restricted ADH1 and ADH4 catalyze retinol metabolism, with ADH4 being the most efficient. Mice lacking ADH3 demonstrate reduced survival and a growth defect that can be rescued by dietary retinol supplementation. The effect of loss of ADH1 is significant only in mice subjected to dietary retinoid-excess, whereas ADH4 loss is only significant when the mice are subjected to dietary retinoid-deficiency [89].

A number of membrane bound SDRs are also able to catalyze retinol oxidation to retinaldehyde. These enzymes are not inhibited by ethanol since they do not use ethanol as a substrate. These microsomal RDHs catalyze the NADPH⁺-dependent oxidation of retinol to retinaldehyde. Several of the SDRs have been shown to prefer retinol bound to CRBP as opposed to free retinol as substrate [85, 87]. The SDRs that possess RDH activity include 11-*cis*-RDH (RDH5), RoRDH4, RL-HSD, RDHL (DHRS9), retSDR1, prRDH, RDH10, RDH11, RDH12, RoRDH1, RoRDH2, CRAD1, CRAD2, CRAD3, 17 β -HSD9, RDH1, RRD, with the expression of all these enzyme except RDHL, prRDH and RDH12 in the liver [86]. For most of these SDRs, there has not been rigorous genetic study as to the possible physiological importance of each in retinoic acid synthesis. Only for 11-*cis*-RDH and RDH10 have genetic studies been undertaken [89].

There remains controversy regarding the potential physiological relevance of many of the different ADHs and SDRs for catalyzing retinol oxidation, a first step needed for retinoic acid formation. This issue will require much more research before the true roles of each of these enzymes in retinoic acid synthesis can be unequivocally resolved.

Following the oxidation of retinol to retinaldehyde by ADHs or SDRs, the next step needed for retinoic acid synthesis involves the irreversible oxidation of retinaldehyde to retinoic acid. Four enzymes termed retinaldehyde dehydrogenases (RALDHs) able to catalyze this step have been established: RALDH1 (also called ALDH1A1 and ALDH1), RALDH2 (also called ALDH1A2), RALDH3 (also called ALDH1A3 and ALDH6) and RALDH4 (also called ALDH8A1 and ALDH12) [13, 90, 91]. RALDHs are able to oxidize retinaldehyde bound to CRBP [87, 92]. This observation indicates that retinaldehyde-CRBP formed through oxidation of retinol-CRBP by the microsomal RDHs can be directly oxidized to retinoic acid through the actions of RALDHs [87, 92, 93]. Therefore, CRBP may play a significant role in channeling the metabolic activation of retinol to retinoic acid. Newly synthesized retinoic acid can be bound to cellular retinoic acid-binding proteins (CRABPs) which are expressed in many tissues [87]. One of these CRABPs, cellular retinoic acid-binding protein, type II (CRABP_{II}) has been identified to function as a facilitator of retinoic acid uptake and metabolism by cells, and as a co-regulator of retinoic acid signaling in the nucleus [94, 95]. The concentrations of CRABP expressed in hepatocytes and HSCs were 5.6 and 8.7 ng per 10⁶ cells, respectively [51]. When these data were expressed on the basis of per unit mass of cellular protein, the concentrations of CRABP in HSCs, which contain 10-fold less protein than the large hepatocytes, were shown to be more enriched than that in hepatocytes [51].

3.1.2. Catabolism of retinoic acid—Catabolism of retinoic acid is an important control mechanism regulating its levels in cells, tissues, and the whole body. Retinoic acid is catabolized to more oxidized metabolites, including 4-hydroxy-retinoic acid, 4-oxo-retinoic acid, 18-hydroxy-retinoic acid, and 5,6-epoxy-retinoic acid. These metabolites are formed primarily through the actions of cytochrome P450 (CYP) family members [96]. In general, the three most prominent enzymes involved in retinoic acid catabolism are the CYP26 family of enzymes, CYP26A1, CYP26B1, and CYP26C1. CYP26A1, the first identified CYP26 enzyme, is expressed at high levels in the liver, duodenum, colon, and placenta and

in some regions of the brain [97, 98]. The upstream promoter region of the CYP26A1 gene contains a functional retinoic acid response element (RARE), and its transcripts are thus induced by retinoic acid. This allows for a direct mechanism through which CYP26A1 can sense cellular retinoic acid levels and regulate the catabolism of excessive retinoic acid. Thatcher and Isoherranen have reported that CYP26A1 is the major CYP enzyme expressed in the human liver, contributing to endogenous retinoic acid clearance in humans [99]. CYP26B1 was cloned shortly after CYP26A1 and also was demonstrated to catabolize all-*trans*-retinoic acid to polar metabolites [100]. CYP26B1 shows a different tissue expression pattern compared with CYP26A1, but these two enzymes have similar catalytic activities [100]. CYP26C1 was cloned still more recently [101]. CYP26C1 is not widely expressed but also seems to be inducible by retinoic acid. This CYP26 isoform is reported to be able to catabolize 9-*cis* retinoic acid better than either CYP26A1 or CYP26B1. In the adult human, the expression level of CYP26A1 mRNA is highest in the liver and CYP26C1 is present in the brain and liver, while CYP26B1 mRNA is not detected at all in adult human liver but is instead abundantly expressed in the placenta, ovary, testes, and intestine [99]. Interestingly, CYP26B1 but not CYP26C1 is expressed in mouse liver. Thus, the expression patterns of the three CYP26 enzymes are generally non-overlapping, suggesting individual roles for each of the CYP enzymes in the catabolism of retinoic acid [102]. Moreover, these isoforms may possess different physiological roles in different animal species.

Although the CYP26 isoforms are importantly involved in catalyzing retinoic acid catabolism in the liver, it is likely that other CYP enzymes have important roles too. One of these, the major ethanol-induced CYP enzyme CYP2E1, has been identified to have an important role in the degradation of retinoic acid in alcoholic liver [103]. It has been shown that the enhanced catabolism of retinoic acid observed in alcohol-fed rats can be inhibited by an inhibitor of CYP2E1 both *in vitro* and *in vivo*, indicating that CYP2E1 may be a principal enzyme responsible for the alcohol-enhanced catabolism of retinoids in the liver, after exposure to alcohol [103, 104]. Qian et al. reported *in vitro* studies demonstrating that CYP2C22, a member of the rat CYP2C family with homology to human CYP2C8 and CYP2C9, is retinoid-inducible hepatic enzyme and has the potential for metabolizing all-*trans*-retinoic acid [105]. In addition, other CYP enzymes, including CYP1A2, 2A4, 2A6, 1B1, 2B1, 2B6, 2C3, 2C7, 2D6, 2E2, 2G1, 3A4/5, 3A6, 3A7, and 4A11 have been proposed to be involved in the catabolism of retinoic acid *in vitro* [106, 107], but the *in vivo* implications of these observations are still unclear.

Retinoic acid and its polar metabolites can be conjugated through glucuronidation to form water-soluble glucuronide metabolites [83, 108]. This conjugation reaction is usually thought of as a catabolic pathway leading to elimination of the retinoid from the body. However, for this pathway and the CYP26 catalyzed oxidative metabolism as well, it should be noted that several oxidized polar metabolites, including 4-hydroxy-retinoic acid and 4-oxo-retinoic acid, and retinoyl- β -glucuronide still have biological activity [109, 110].

3.1.3. Retinoid receptors—Retinoid receptors are members of the steroid/thyroid/retinoid nuclear receptor family of ligand dependent transcription factors [111]. Six retinoid receptors have been identified, and these can be divided into two classes, the retinoic acid receptors (RARs) - α , - β , and - γ and the retinoid X receptors (RXRs) - α , - β , and - γ [112]. These all are expressed in the liver, and freshly isolated HSC expressed RAR α , β , γ and RXR α and β [7]. Freshly isolated HSC showed stronger signals for RAR α , γ and RXR β compared to total liver tissue [7]. Retinoic acid can interact with RARs and RXRs, which function primarily as RAR/RXR heterodimers [111, 113]. More details regarding retinoid receptors and their functions in general organ can be found in the review articles by Germain et al. [112].

3.2. Retinoid and hepatic diseases

3.2.1. Fatty liver, liver fibrosis and cirrhosis, and hepatocellular carcinoma—

It is well established that retinoic acid transcriptional activity regulates a large number of genes related with fat metabolism [114, 115]. Studies in transgenic mice demonstrate that the expression of a RAR α -dominant negative form in hepatocytes, under the control of the albumin promoter and enhancer results in the development of steatohepatitis [8]. This mutation suppresses the activities of endogenous RAR/RXR heterodimers [116] and results in the downregulation of hepatic mitochondrial β -oxidation and an upregulation of peroxisomal β -oxidation activity. This is followed temporally by the development of liver tumors [8]. Studies of mutant mice lacking β -carotene-15'15'-monooxygenase, an enzyme required to cleave carotenoids to retinoids, demonstrate that this enzyme plays an important part in modulating hepatic fat metabolism [117], showing that these mutant mice spontaneously develop a fatty liver and are more susceptible than control mice to high fat diet-induced impairments in fatty acid metabolism. Taken together, these observations suggest that retinoids play important roles in mediating normal hepatic lipid metabolism.

Acute liver injury initiates a wound healing response which restores the liver to its healthy state [10, 11]. However, chronic liver injury leads progressively to liver fibrosis, liver cirrhosis, and hepatocellular carcinoma (HCC), regardless of etiology, such as chronic hepatitis B and C infection, chronic alcohol consumption, dietary exposure to aflatoxin B1, and non-alcoholic fatty liver disease (NAFLD) [118, 119]. Following acute or chronic liver injury, HSCs undergo a process of activation in which they convert from a quiescent condition to a myofibroblastic phenotype. Activated HSCs are characterized by synthesis of extracellular matrix and transition to fibrogenic and proliferative state. [10, 12]. One of the early events that occur during HSC activation and the development of liver fibrosis is the loss of hepatic retinyl ester stores from the lipid droplets within HSCs [120, 121]. It should be noted that excessive retinoid consumption causes liver injury and fibrosis [11, 122]. Curiously, excessive retinoid consumption induces a state of HSC transition from a cell having increased size and number of lipid droplets to an activated fibroblastic form with a complete loss of lipid droplets.

It has been proposed that serum retinol may be a marker for HCC in high risk groups [123, 124]. A progressive reduction of serum retinol levels has been noted for patients diagnosed with liver cirrhosis compared to healthy subjects and those patients with both cirrhosis and HCC had significantly lower levels than patients with cirrhosis alone [123, 125]. A number of studies have demonstrated that nutritional deficits often occur in patients with chronic liver diseases [123, 126]. This likely arises from diminished retinoid intake, impaired absorption, inadequate synthesis, or hypermetabolic state with the disease [127]. Among these, retinoid metabolism seems to be affected by insufficient synthesis of hepatic proteins, especially RBP and TTR by the diseased liver [123, 126], as well as by impaired absorption of vitamin A. The malabsorption of dietary retinoid likely arises from decreased intraluminal bile salt caused by portal hypertensive enteropathy and cholestasis [127, 128].

The relationship between retinoids and cancer has been extensively investigated, and a large number of studies have reported that retinoid-deficiency gives rise to an increase in the number of spontaneous and chemically induced tumors in animals [129, 130]. It is well established that retinoids act as therapeutic and chemopreventive agents in patients with acute promyelotic leukemia [131, 132] and leukoplakia, premalignant lesions of head and neck cancer [133, 134]. Many experimental and clinical investigations have demonstrated the loss of retinoid activity or retinoid-responsiveness in HCC cells [135], decreased retinoid stores in the liver and serum retinol levels [9, 123], and altered retinoid signaling in patients with cirrhosis and HCC [136, 137]. Based on these studies, preclinical and clinical studies of retinoid for HCC have been promoted.

Clinical studies have shown that the administration of acyclic retinoid (ACR), a synthetic retinoid, reduced the incidence of post-therapeutic recurrence of HCC, and thereby improved the survival of patients with HCC [138, 139]. Many studies investigating the mechanisms of ACR in the chemoprevention of HCC have shown that the efficacy of ACR is mediated through suppression of Ras/MAP kinase signal transduction and reducing phosphorylated RXR α , which is resistant to proteasomal degradation and accumulates in the nucleus. This later effect interferes with normal RXR α functions and promotes tumor growth [135, 140–143]. Other research, involving cell lines and animals, has demonstrated that ACR may suppress HCC by activating RAR β and inducing p21 expression [143–146]. This suppresses transforming growth factor α (TGF α) expression [147, 148] and modulates fibroblast growth factor signaling [149]. Recently, Yang et al. showed that another synthetic retinoid fenretinide, in combination with histone deacetylase inhibitors, induces apoptosis of human HCC cells, though a process mediated by Nur77 and RAR β , and they considered that this function may be through non-genomic action of retinoids [150]. There is increasing evidence of novel non-genomic mechanisms of signal transduction through nuclear hormone receptors, including retinoid receptors [151–153]. Overexpression of RAR γ plays a role in the growth of HCC cells through non-genomic activation of the phosphatidylinositol 3-kinase (PI3K)/Akt and NF-kappaB signaling pathways [154]. RAR γ often resides in the cytoplasm of HCC cells and interacts with the p85 α regulatory subunit of PI3K, resulting in activation of Akt and NF-kappaB [154, 155].

CRBPI expression in HCC tissue has been reported to be correlated with cellular proliferation and patient survival [55]. It was reported that accumulation of CRBPI in cell nuclei seemed to be beneficial for diagnosis of HCC and the detection of CRBPI-positive intratumoral myofibroblasts was associated with a better prognosis for survival. This finding implies that modulation of CRBPI may affect retinoid metabolism in tumor cells [55].

3.2.2. Alcoholic liver diseases—Chronic and excessive alcohol intake induces abnormalities in retinoid metabolism by the mechanisms that have been proposed in the literature to involve (i) competitive inhibition of the first step of retinol oxidation needed to form retinoic acid that is catalyzed by ADHs, (ii) accelerated catabolism of retinoic acid by inducing CYP enzymes, specifically CYP2E1, and (iii) enhanced retinoid mobilization from liver to peripheral tissues [103, 156]. This leads to fatty liver, hepatitis, fibrosis, cirrhosis and even liver cancer [157, 158]. In many inflammation in the development of alcoholic liver diseases [159–161].

Alteration of the mitogen-activated protein kinase (MAPK) pathways is one effect of chronic and excessive alcohol intake on proliferative signaling [156]. This influences downstream cascades, resulting in activation of c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK), and increased expression and activity of c-Jun and c-Fos [137]. Wang et al. demonstrated that chronic alcohol intake significantly increased c-Jun and c-Fos protein levels in the liver of rats compared to control animals [137]. Alcohol also activates AP-1, a transcriptional factor formed as a c-Jun homodimer or heterodimer with c-Fos, in HepG2 hepatoma cell lines [162]. Alcohol-induced overexpression of c-Jun and cyclin D1, as well as phosphorylation of JNK were shown to be inhibited dramatically in the liver of alcohol-fed rats by retinoic acid treatment [104, 163, 164]. Moreover, retinoic acid supplementation was able to reverse phosphorylation of JNK in the livers of alcohol-fed rats [11, 83, 164]. Since JNK and cyclin D1 play key roles in carcinogenesis and tumor progression in HCC [165, 166], they may be considered as potential targets for retinoic acid actions to protect against alcohol-promoted hepatic cell proliferation and carcinogenic transformation [156].

3.2.3. Cause or effect?—A number of researchers have reported an association of altered retinoid metabolism and action with hepatic disease development, including hepatic steatosis, hepatic fibrosis, cirrhosis, HCC, and those arising from chronic alcohol consumption. This raises fundamental questions regarding whether these alterations contribute to the pathogenesis of hepatic disease, or whether they are simply secondary changes arising out of the process of disease development.

It has been noted that hepatic retinoid stores are progressively lost upon development of liver disease in human subjects [9]. A significant decrease of hepatic retinoid levels occurs in patients diagnosed with alcohol-induced fatty livers, and patients with alcoholic hepatitis and cirrhosis have much lower hepatic total retinol levels, approximately 10% and 5% respectively, of control subjects.

Two hypotheses have been generated to account for these relations and the loss of retinoid stores from HSCs in hepatic disease [14]. One hypothesis predicts that hepatic retinoid stores play protective roles against disease development [59]. This theory implies that the liver is more susceptible to liver injury and easy to develop hepatic diseases if hepatic retinoid stores are less or absent. However, LRAT knockout mice which completely lack hepatic retinoid stores are not predisposed to developing spontaneous and even chemically induced hepatic fibrosis [16, 167]. The other hypothesis is that dysregulation of hepatic retinoid metabolism induced by liver damage result in a “toxic burst” of transcriptionally active retinoid metabolites which contribute to altered gene expression patterns [104, 168].

Details of how hepatic disease relates to impaired hepatic retinoid homeostasis have not been clearly addressed. Further studies are necessary in order for these findings to be systematically investigated and directly verified.

Acknowledgments

The authors wish to acknowledge the support of grants RC2 AA019413, R01 DK68437, and R01 DK079221 from the National Institutes of Health which supported the research carried out in their laboratory and which was cited in this review.

References

1. Blaner, WS.; Olson, JA. Retinol and retinoic acid metabolism. In: Sporn, MB.; Roberts, AB.; Goodman, DS., editors. *The Retinoids: Biology, Chemistry, and Medicine*. Raven Press, Ltd; New York: 1994. p. 229-256.
2. Blomhoff R, Green MH, Green JB, Berg T, Norum KR. Vitamin A metabolism: new perspectives on absorption, transport, and storage. *Physiol Rev*. 1991; 71:951–990. [PubMed: 1924551]
3. Goodman, DS.; Blaner, WS. Biosynthesis, absorption, and hepatic metabolism of retinol. In: Sporn, MB.; Roberts, AB.; Goodman, DS., editors. *The Retinoids*. Academic Press; New York: 1984. p. 1-39.
4. Goodman, DS. Plasma retinol-binding protein. In: Sporn, MB.; Roberts, AB.; Goodman, DS., editors. *The Retinoids*. Academic Press; Orlando, FL: 1984. p. 41-88.
5. Soprano, DR.; Blaner, WS. Plasma retinol-binding protein. In: Sporn, MB.; Roberts, AB.; Goodman, DS., editors. *The Retinoids: Biology, Chemistry, and Medicine*. Raven Press, Ltd; New York: 1994. p. 257-282.
6. Tsutsumi C, Okuno M, Tannous L, Piantadosi R, Allan M, Goodman DS, Blaner WS. Retinoids and retinoid-binding protein expression in rat adipocytes. *J Biol Chem*. 1992; 267:1805–1810. [PubMed: 1370481]
7. Hellems K, Verbuyst P, Quartier E, Schuit F, Rombouts K, Chandraratna RA, Schuppan D, Geerts A. Differential modulation of rat hepatic stellate phenotype by natural and synthetic retinoids. *Hepatology*. 2004; 39:97–108. [PubMed: 14752828]

8. Yanagitani A, Yamada S, Yasui S, Shimomura T, Murai R, Murawaki Y, Hashiguchi K, Kanbe T, Saeki T, Ichiba M, Tanabe Y, Yoshida Y, Morino S, Kurimasa A, Usuda N, Yamazaki H, Kunisada T, Ito H, Shiota G. Retinoic acid receptor alpha dominant negative form causes steatohepatitis and liver tumors in transgenic mice. *Hepatology*. 2004; 40:366–375. [PubMed: 15368441]
9. Leo MA, Lieber CS. Hepatic vitamin A depletion in alcoholic liver injury. *N Engl J Med*. 1982; 307:597–601. [PubMed: 7202119]
10. Friedman SL. Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. *Physiol Rev*. 2008; 88:125–172. [PubMed: 18195085]
11. Geerts A. History, heterogeneity, developmental biology, and functions of quiescent hepatic stellate cells. *Semin Liver Dis*. 2001; 21:311–335. [PubMed: 11586463]
12. Blaner WS, O'Byrne SM, Wongsiriroj N, Kluwe J, D'Ambrosio DM, Jiang H, Schwabe RF, Hillman EM, Piantedosi R, Libien J. Hepatic stellate cell lipid droplets: a specialized lipid droplet for retinoid storage. *Biochim Biophys Acta*. 2009; 1791:467–473. [PubMed: 19071229]
13. Blomhoff R, Blomhoff HK. Overview of retinoid metabolism and function. *J Neurobiol*. 2006; 66:606–630. [PubMed: 16688755]
14. D'Ambrosio DN, Clugston RD, Blaner WS. Vitamin A metabolism: An update. *Nutrients*. 2011; 3:63–103. [PubMed: 21350678]
15. Wongsiriroj N, Piantedosi R, Palczewski K, Goldberg IJ, Johnston TP, Li E, Blaner WS. The molecular basis of retinoid absorption: a genetic dissection. *J Biol Chem*. 2008; 283:13510–13519. [PubMed: 18348983]
16. O'Byrne SM, Wongsiriroj N, Libien J, Vogel S, Goldberg IJ, Baehr W, Palczewski K, Blaner WS. Retinoid absorption and storage is impaired in mice lacking lecithin:retinol acyltransferase (LRAT). *J Biol Chem*. 2005; 280:35647–35657. [PubMed: 16115871]
17. Cooper AD. Hepatic uptake of chylomicron remnants. *J Lipid Res*. 1997; 38:2173–2192. [PubMed: 9392416]
18. Yu KC, Cooper AD. Postprandial lipoproteins and atherosclerosis. *Front Biosci*. 2001; 6:D332–354. [PubMed: 11229885]
19. Harrison EH. Lipases and carboxylesterases: possible roles in the hepatic utilization of vitamin A. *J Nutr*. 2000; 130:340S–344S. [PubMed: 10721902]
20. Harrison EH. Mechanisms of digestion and absorption of dietary vitamin A. *Annu Rev Nutr*. 2005; 25:87–103. [PubMed: 16011460]
21. Boerman MH, Napoli JL. Cholate-independent retinyl ester hydrolysis. Stimulation by Apo-cellular retinol-binding protein. *J Biol Chem*. 1991; 266:22273–22278. [PubMed: 1939249]
22. Gad MZ, Harrison EH. Neutral and acid retinyl ester hydrolases associated with rat liver microsomes: relationships to microsomal cholesteryl ester hydrolases. *J Lipid Res*. 1991; 32:685–693. [PubMed: 1856611]
23. Harrison EH, Gad MZ, Ross AC. Hepatic uptake and metabolism of chylomicron retinyl esters: probable role of plasma membrane/endosomal retinyl ester hydrolases. *J Lipid Res*. 1995; 36:1498–1506. [PubMed: 7595074]
24. Schreiber R, Taschler U, Wolinski H, Seper A, Tamegger SN, Graf M, Kohlwein SD, Haemmerle G, Zimmermann R, Zechner R, Lass A. Esterase 22 and beta-glucuronidase hydrolyze retinoids in mouse liver. *J Lipid Res*. 2009; 50:2514–2523. [PubMed: 19723663]
25. Wei S, Lai K, Patel S, Piantedosi R, Shen H, Colantuoni V, Kraemer FB, Blaner WS. Retinyl ester hydrolysis and retinol efflux from BFC-1 beta adipocytes. *J Biol Chem*. 1997; 272:14159–14165. [PubMed: 9162045]
26. Blomhoff R, Helgerud P, Rasmussen M, Berg T, Norum KR. In vivo uptake of chylomicron[3H]retinyl ester by rat liver: evidence for retinol transfer from parenchymal to nonparenchymal cells. *Proc Natl Acad Sci U S A*. 1982; 79:7326–7330. [PubMed: 6961410]
27. Blomhoff R, Holte K, Naess L, Berg T. Newly administered[3H]retinol is transferred from hepatocytes to stellate cells in liver for storage. *Exp Cell Res*. 1984; 150:186–193. [PubMed: 6420172]
28. Blomhoff R, Berg T, Norum KR. Transfer of retinol from parenchymal to stellate cells in liver is mediated by retinol-binding protein. *Proc Natl Acad Sci U S A*. 1988; 85:3455–3458. [PubMed: 3368453]

29. Senoo H, Smeland S, Malaba L, Bjerknes T, Stang E, Roos N, Berg T, Norum KR, Blomhoff R. Transfer of retinol-binding protein from HepG2 human hepatoma cells to cocultured rat stellate cells. *Proc Natl Acad Sci U S A*. 1993; 90:3616–3620. [PubMed: 8386378]
30. Quadro L, Blaner WS, Salchow DJ, Vogel S, Piantadosi R, Gouras P, Freeman S, Cosma MP, Colantuoni V, Gottesman ME. Impaired retinal function and vitamin A availability in mice lacking retinol-binding protein. *EMBO J*. 1999; 18:4633–4644. [PubMed: 10469643]
31. Ghyselinck NB, Bavik C, Sapin V, Mark M, Bonnier D, Hindelang C, Dierich A, Nilsson CB, Hakansson H, Sauvant P, Azais-Braesco V, Frasson M, Picaud S, Chambon P. Cellular retinol-binding protein I is essential for vitamin A homeostasis. *EMBO J*. 1999; 18:4903–4914. [PubMed: 10487743]
32. Napoli JL. Retinoid binding-proteins redirect retinoid metabolism: biosynthesis and metabolism of retinoic acid. *Semin Cell Dev Biol*. 1997; 8:403–415. [PubMed: 15001079]
33. Herr FM, Ong DE. Differential interaction of lecithin-retinol acyltransferase with cellular retinol binding proteins. *Biochemistry*. 1992; 31:6748–6755. [PubMed: 1322170]
34. Blaner WS, Dixon JL, Moriwaki H, Martino RA, Stein O, Stein Y, Goodman DS. Studies on the in vivo transfer of retinoids from parenchymal to stellate cells in rat liver. *Eur J Biochem*. 1987; 164:301–307. [PubMed: 3569264]
35. Green MH, Green JB. Quantitative and conceptual contributions of mathematical modeling to current views on vitamin A metabolism, biochemistry, and nutrition. *Adv Food Nutr Res*. 1996; 40:3–24. [PubMed: 8858804]
36. Cifelli CJ, Green JB, Green MH. Dietary retinoic acid alters vitamin A kinetics in both the whole body and in specific organs of rats with low vitamin A status. *J Nutr*. 2005; 135:746–752. [PubMed: 15795428]
37. Cifelli CJ, Green JB, Green MH. Use of model-based compartmental analysis to study vitamin A kinetics and metabolism. *Vitam Horm*. 2007; 75:161–195. [PubMed: 17368316]
38. Green MH, Green JB. Experimental and kinetic methods for studying vitamin A dynamics in vivo. *Methods Enzymol*. 1990; 190:304–317. [PubMed: 2087182]
39. Lewis KC, Green MH, Green JB, Zech LA. Retinol metabolism in rats with low vitamin A status: a compartmental model. *J Lipid Res*. 1990; 31:1535–1548. [PubMed: 2246607]
40. Green MH, Uhl L, Green JB. A multicompartamental model of vitamin A kinetics in rats with marginal liver vitamin A stores. *J Lipid Res*. 1985; 26:806–818. [PubMed: 4040952]
41. Green, MH.; Green, JB. The application of model-based compartmental analysis to the study of the kinetic behavior of vitamin A in vitamin A-sufficient rats: Validation of a theoretical model. In: Hargrove, JL.; Berdanier, CD., editors. *Mathematical modeling in nutrition and toxicology*. Mathematical Biology Press; Athens: 2005. p. 175-187.
42. Soprano DR, Soprano KJ, Goodman DS. Retinol-binding protein messenger RNA levels in the liver and in extrahepatic tissues of the rat. *J Lipid Res*. 1986; 27:166–171. [PubMed: 3754273]
43. von Reinertsdorff D, Green MH, Green JB. Development of a compartmental model describing the dynamics of vitamin A metabolism in men. *Adv Exp Med Biol*. 1998; 445:207–223. [PubMed: 9781391]
44. Ross AC, Zolfaghari R. Regulation of hepatic retinol metabolism: perspectives from studies on vitamin A status. *J Nutr*. 2004; 134:269S–275S. [PubMed: 14704332]
45. Haskell MJ, Jamil KM, Pearson JM, Wahed MA, Brown KH. The paired deuterated retinol dilution technique can be used to estimate the daily vitamin A intake required to maintain a targeted whole body vitamin A pool size in men. *J Nutr*. 2011; 141:428–432. [PubMed: 21248191]
46. Haskell MJ, Mazumder RN, Pearson JM, Jones AD, Wahed MA, Mahalanabis D, Brown KH. Use of the deuterated-retinol-dilution technique to assess total-body vitamin A stores of adult volunteers consuming different amounts of vitamin A. *Am J Clin Nutr*. 1999; 70:874–880. [PubMed: 10539748]
47. Surles RL, Hutson PR, Valentine AR, Mills JP, Tanumihardjo SA. 3, 4-Didehydroretinol kinetics differ during lactation in sows on a retinol depletion regimen and the serum:milk 3, 4-didehydroretinol:retinol ratios are correlated. *J Nutr*. 2011; 141:554–559. [PubMed: 21310863]
48. Green, MH.; Green, JB. The use of model-based compartmental analysis to study vitamin A metabolism in a non-steady state. In: Novotny, JA.; Green, MH.; Boston, RC., editors.

- Mathematical modeling in nutrition and the health sciences. Kluwer Academic/Plenum Publishers; New York: 2003. p. 159-172.
49. Raila J, Willnow TE, Schweigert FJ. Megalin-mediated reuptake of retinol in the kidneys of mice is essential for vitamin A homeostasis. *J Nutr.* 2005; 135:2512–2516. [PubMed: 16251603]
 50. Vogel, S.; Gamble, MV.; Blaner, WS. Biosynthesis, absorption, metabolism and transport of retinoids. In: Nau, H.; Blaner, WS., editors. *The Handbook of Experimental pharmacology, The Retinoids.* Springer Verlag; Heidelberg, Germany: 1999. p. 31-96.
 51. Blaner WS, Hendriks HF, Brouwer A, de Leeuw AM, Knook DL, Goodman DS. Retinoids, retinoid-binding proteins, and retinyl palmitate hydrolase distributions in different types of rat liver cells. *J Lipid Res.* 1985; 26:1241–1251. [PubMed: 4067418]
 52. Batres RO, Olson JA. A marginal vitamin A status alters the distribution of vitamin A among parenchymal and stellate cells in rat liver. *J Nutr.* 1987; 117:874–879. [PubMed: 3585540]
 53. Ong, DE.; Newcomer, ME.; Chytil, F. Cellular retinoid-binding proteins. In: Sporn, MB.; Roberts, AB.; Goodman, DS., editors. *The Retinoids: Biology, Chemistry, and Medicine.* Raven Press, Ltd; New York: 1994. p. 283-317.
 54. Senoo H, Kojima N, Sato M. Vitamin A-storing cells (stellate cells). *Vitam Horm.* 2007; 75:131–159. [PubMed: 17368315]
 55. Schmitt-Graff A, Ertelt V, Allgaier HP, Koelble K, Olschewski M, Nitschke R, Bochaton-Piallat ML, Gabbiani G, Blum HE. Cellular retinol-binding protein-1 in hepatocellular carcinoma correlates with beta-catenin, Ki-67 index, and patient survival. *Hepatology.* 2003; 38:470–480. [PubMed: 12883492]
 56. O'Byrne, SM.; Blaner, WS. Introduction to retinoids. In: Packer, L.; Obermuller-Jevic, U.; Kraemer, K.; Sies, H., editors. *Carotenoids and Retinoids: Molecular Aspects and Health Issues.* AOCS Press; Champaign, IL: 2005. p. 1-22.
 57. Ross AC. Retinol esterification by rat liver microsomes. Evidence for a fatty acyl coenzyme A: retinol acyltransferase. *J Biol Chem.* 1982; 257:2453–2459. [PubMed: 7061433]
 58. Orland MD, Anwar K, Cromley D, Chu CH, Chen L, Billheimer JT, Hussain MM, Cheng D. Acyl coenzyme A dependent retinol esterification by acyl coenzyme A: diacylglycerol acyltransferase 1. *Biochim Biophys Acta.* 2005; 1737:76–82. [PubMed: 16214399]
 59. Yamaguchi K, Yang L, McCall S, Huang J, Yu XX, Pandey SK, Bhanot S, Monia BP, Li YX, Diehl AM. Diacylglycerol acyltransferase 1 anti-sense oligonucleotides reduce hepatic fibrosis in mice with nonalcoholic steatohepatitis. *Hepatology.* 2008; 47:625–635. [PubMed: 18000880]
 60. Yen CL, Monetti M, Burri BJ, Farese RV Jr. The triacylglycerol synthesis enzyme DGAT1 also catalyzes the synthesis of diacylglycerols, waxes, and retinyl esters. *J Lipid Res.* 2005; 46:1502–1511. [PubMed: 15834126]
 61. Batten ML, Imanishi Y, Tu DC, Doan T, Zhu L, Pang J, Glushakova L, Moise AR, Baehr W, Van Gelder RN, Hauswirth WW, Rieke F, Palczewski K. Pharmacological and rAAV gene therapy rescue of visual functions in a blind mouse model of Leber congenital amaurosis. *PLoS Med.* 2005; 2:e333. [PubMed: 16250670]
 62. Liu L, Gudas LJ. Disruption of the lecithin:retinol acyltransferase gene makes mice more susceptible to vitamin A deficiency. *J Biol Chem.* 2005; 280:40226–40234. [PubMed: 16174770]
 63. Moriwaki H, Blaner WS, Piantadosi R, Goodman DS. Effects of dietary retinoid and triglyceride on the lipid composition of rat liver stellate cells and stellate cell lipid droplets. *J Lipid Res.* 1988; 29:1523–1534. [PubMed: 3241127]
 64. Yamada M, Blaner WS, Soprano DR, Dixon JL, Kjeldbye HM, Goodman DS. Biochemical characteristics of isolated rat liver stellate cells. *Hepatology.* 1987; 7:1224–1229. [PubMed: 2824313]
 65. Mello T, Nakatsuka A, Fears S, Davis W, Tsukamoto H, Bosron WF, Sanghani SP. Expression of carboxylesterase and lipase genes in rat liver cell-types. *Biochem Biophys Res Commun.* 2008; 374:460–464. [PubMed: 18639528]
 66. Nagy NE, Holven KB, Roos N, Senoo H, Kojima N, Norum KR, Blomhoff R. Storage of vitamin A in extrahepatic stellate cells in normal rats. *J Lipid Res.* 1997; 38:645–658. [PubMed: 9144080]
 67. Kanai M, Raz A, Goodman DS. Retinol-binding protein: the transport protein for vitamin A in human plasma. *J Clin Invest.* 1968; 47:2025–2044. [PubMed: 5675424]

68. Quadro L, Hamberger L, Colantuoni V, Gottesman ME, Blaner WS. Understanding the physiological role of retinol-binding protein in vitamin A metabolism using transgenic and knockout mouse models. *Mol Aspects Med.* 2003; 24:421–430. [PubMed: 14585313]
69. Quadro L, Blaner WS, Hamberger L, Novikoff PM, Vogel S, Piantedosi R, Gottesman ME, Colantuoni V. The role of extrahepatic retinol binding protein in the mobilization of retinoid stores. *J Lipid Res.* 2004; 45:1975–1982. [PubMed: 15314099]
70. Quadro L, Blaner WS, Hamberger L, Van Gelder RN, Vogel S, Piantedosi R, Gouras P, Colantuoni V, Gottesman ME. Muscle expression of human retinol-binding protein (RBP). Suppression of the visual defect of RBP knockout mice. *J Biol Chem.* 2002; 277:30191–30197. [PubMed: 12048218]
71. Gaetani S, Bellovino D, Aprea M, Devirgiliis C. Hepatic synthesis, maturation and complex formation between retinol-binding protein and transthyretin. *Clin Chem Lab Med.* 2002; 40:1211–1220. [PubMed: 12553421]
72. Monaco HL, Rizzi M, Coda A. Structure of a complex of two plasma proteins: transthyretin and retinol-binding protein. *Science.* 1995; 268:1039–1041. [PubMed: 7754382]
73. Episkopou V, Maeda S, Nishiguchi S, Shimada K, Gaitanaris GA, Gottesman ME, Robertson EJ. Disruption of the transthyretin gene results in mice with depressed levels of plasma retinol and thyroid hormone. *Proc Natl Acad Sci U S A.* 1993; 90:2375–2379. [PubMed: 8384721]
74. Wei S, Episkopou V, Piantedosi R, Maeda S, Shimada K, Gottesman ME, Blaner WS. Studies on the metabolism of retinol and retinol-binding protein in transthyretin-deficient mice produced by homologous recombination. *J Biol Chem.* 1995; 270:866–870. [PubMed: 7822324]
75. van Bennekum AM, Wei S, Gamble MV, Vogel S, Piantedosi R, Gottesman M, Episkopou V, Blaner WS. Biochemical basis for depressed serum retinol levels in transthyretin-deficient mice. *J Biol Chem.* 2001; 276:1107–1113. [PubMed: 11036082]
76. Krasinski SD, Cohn JS, Russell RM, Schaefer EJ. Postprandial plasma vitamin A metabolism in humans: a reassessment of the use of plasma retinyl esters as markers for intestinally derived chylomicrons and their remnants. *Metabolism.* 1990; 39:357–365. [PubMed: 2325560]
77. Schindler R, Klopp A. Transport of esterified retinol in fasting human blood. *Int J Vitam Nutr Res.* 1986; 56:21–27. [PubMed: 3710714]
78. Tall A. Plasma lipid transfer proteins. *Annu Rev Biochem.* 1995; 64:235–257. [PubMed: 7574481]
79. Thompson KH, Hughes LB, Zilversmit DB. Lack of secretion of retinyl ester by livers of normal and cholesterol-fed rabbits. *J Nutr.* 1983; 113:1995–2001. [PubMed: 6619978]
80. Lederman JD, Overton KM, Hofmann NE, Moore BJ, Thornton J, Erdman JW. Ferrets (*Mustela putorius furo*) inefficiently convert beta-carotene to vitamin A. *J Nutr.* 1998; 128:271–279. [PubMed: 9446855]
81. Wilson DE, Hejazi J, Elstad NL, Chan IF, Gleeson JM, Iverius PH. Novel aspects of vitamin A metabolism in the dog: distribution of lipoprotein retinyl esters in vitamin A-deprived and cholesterol-fed animals. *Biochim Biophys Acta.* 1987; 922:247–258. [PubMed: 3689810]
82. Ashla AA, Hoshikawa Y, Tsuchiya H, Hashiguchi K, Enjoji M, Nakamuta M, Taketomi A, Maehara Y, Shomori K, Kurimasa A, Hisatome I, Ito H, Shiota G. Genetic analysis of expression profile involved in retinoid metabolism in non-alcoholic fatty liver disease. *Hepatol Res.* 2010; 40:594–604. [PubMed: 20618457]
83. Ross AC, Zolfaghari R, Weisz J. Vitamin A: recent advances in the biotransformation, transport, and metabolism of retinoids. *Curr Opin Gastroenterol.* 2001; 17:184–192. [PubMed: 11224677]
84. Okuno M, Kojima S, Akita K, Matsushima-Nishiwaki R, Adachi S, Sano T, Takano Y, Takai K, Obora A, Yasuda I, Shiratori Y, Okano Y, Shimada J, Suzuki Y, Muto Y, Moriwaki Y. Retinoids in liver fibrosis and cancer. *Front Biosci.* 2002; 7:d204–218. [PubMed: 11779708]
85. Gottesman ME, Quadro L, Blaner WS. Studies of vitamin A metabolism in mouse model systems. *Bioessays.* 2001; 23:409–419. [PubMed: 11340622]
86. Pares X, Farres J, Kedishvili N, Duester G. Medium- and short-chain dehydrogenase/reductase gene and protein families: Medium-chain and short-chain dehydrogenases/reductases in retinoid metabolism. *Cell Mol Life Sci.* 2008; 65:3936–3949. [PubMed: 19011747]
87. Napoli JL. Retinoic acid biosynthesis and metabolism. *FASEB J.* 1996; 10:993–1001. [PubMed: 8801182]

88. Duester G. Families of retinoid dehydrogenases regulating vitamin A function: production of visual pigment and retinoic acid. *Eur J Biochem.* 2000; 267:4315–4324. [PubMed: 10880953]
89. Duester G, Mic FA, Molotkov A. Cytosolic retinoid dehydrogenases govern ubiquitous metabolism of retinol to retinaldehyde followed by tissue-specific metabolism to retinoic acid. *Chem Biol Interact.* 2003; 143–144:201–210.
90. Duester G. Retinoic acid synthesis and signaling during early organogenesis. *Cell.* 2008; 134:921–931. [PubMed: 18805086]
91. Lin M, Zhang M, Abraham M, Smith SM, Napoli JL. Mouse retinal dehydrogenase 4 (RALDH4), molecular cloning, cellular expression, and activity in 9-cis-retinoic acid biosynthesis in intact cells. *J Biol Chem.* 2003; 278:9856–9861. [PubMed: 12519776]
92. Yamamoto M, Drager UC, Ong DE, McCaffery P. Retinoid-binding proteins in the cerebellum and choroid plexus and their relationship to regionalized retinoic acid synthesis and degradation. *Eur J Biochem.* 1998; 257:344–350. [PubMed: 9826179]
93. Chai X, Zhai Y, Napoli JL. Cloning of a rat cDNA encoding retinol dehydrogenase isozyme type III. *Gene.* 1996; 169:219–222. [PubMed: 8647450]
94. Budhu AS, Noy N. Direct channeling of retinoic acid between cellular retinoic acid-binding protein II and retinoic acid receptor sensitizes mammary carcinoma cells to retinoic acid-induced growth arrest. *Mol Cell Biol.* 2002; 22:2632–2641. [PubMed: 11909957]
95. Lampron C, Rochette-Egly C, Gorry P, Dolle P, Mark M, Lufkin T, LeMeur M, Chambon P. Mice deficient in cellular retinoic acid binding protein II (CRABPII) or in both CRABPI and CRABPII are essentially normal. *Development.* 1995; 121:539–548. [PubMed: 7768191]
96. Petkovich PM. Retinoic acid metabolism. *J Am Acad Dermatol.* 2001; 45:S136–142. [PubMed: 11606945]
97. White JA, Beckett-Jones B, Guo YD, Dilworth FJ, Bonasoro J, Jones G, Petkovich M. cDNA cloning of human retinoic acid-metabolizing enzyme (hP450RAI) identifies a novel family of cytochromes P450. *J Biol Chem.* 1997; 272:18538–18541. [PubMed: 9228017]
98. Lampen A, Meyer S, Nau H. Effects of receptor-selective retinoids on CYP26 gene expression and metabolism of all-trans-retinoic acid in intestinal cells. *Drug Metab Dispos.* 2001; 29:742–747. [PubMed: 11302942]
99. Thatcher JE, Isoherranen N. The role of CYP26 enzymes in retinoic acid clearance. *Expert Opin Drug Metab Toxicol.* 2009; 5:875–886. [PubMed: 19519282]
100. White JA, Ramshaw H, Taimi M, Stangle W, Zhang A, Everingham S, Creighton S, Tam SP, Jones G, Petkovich M. Identification of the human cytochrome P450, P450RAI-2, which is predominantly expressed in the adult cerebellum and is responsible for all-trans-retinoic acid metabolism. *Proc Natl Acad Sci U S A.* 2000; 97:6403–6408. [PubMed: 10823918]
101. Taimi M, Helvig C, Wisniewski J, Ramshaw H, White J, Amad M, Korczak B, Petkovich M. A novel human cytochrome P450, CYP26C1, involved in metabolism of 9-cis and all-trans isomers of retinoic acid. *J Biol Chem.* 2004; 279:77–85. [PubMed: 14532297]
102. Reijntjes S, Gale E, Maden M. Generating gradients of retinoic acid in the chick embryo: Cyp26C1 expression and a comparative analysis of the Cyp26 enzymes. *Dev Dyn.* 2004; 230:509–517. [PubMed: 15188435]
103. Liu C, Russell RM, Seitz HK, Wang XD. Ethanol enhances retinoic acid metabolism into polar metabolites in rat liver via induction of cytochrome P4502E1. *Gastroenterology.* 2001; 120:179–189. [PubMed: 11208727]
104. Liu C, Chung J, Seitz HK, Russell RM, Wang XD. Chlormethiazole treatment prevents reduced hepatic vitamin A levels in ethanol-fed rats. *Alcohol Clin Exp Res.* 2002; 26:1703–1709. [PubMed: 12436060]
105. Qian L, Zolfaghari R, Ross AC. Liver-specific cytochrome P450 CYP2C22 is a direct target of retinoic acid and a retinoic acid-metabolizing enzyme in rat liver. *J Lipid Res.* 2010; 51:1781–1792. [PubMed: 20147703]
106. Marill J, Capron CC, Idres N, Chabot GG. Human cytochrome P450s involved in the metabolism of 9-cis- and 13-cis-retinoic acids. *Biochem Pharmacol.* 2002; 63:933–943. [PubMed: 11911845]
107. Theodosiou M, Laudet V, Schubert M. From carrot to clinic: an overview of the retinoic acid signaling pathway. *Cell Mol Life Sci.* 2010; 67:1423–1445. [PubMed: 20140749]

108. Samokyszyn VM, Gall WE, Zawada G, Freyaldenhoven MA, Chen G, Mackenzie PI, Tephly TR, Radominska-Pandya A. 4-hydroxyretinoic acid, a novel substrate for human liver microsomal UDP-glucuronosyltransferase(s) and recombinant UGT2B7. *J Biol Chem.* 2000; 275:6908–6914. [PubMed: 10702251]
109. Reijntjes S, Blentic A, Gale E, Maden M. The control of morphogen signalling: regulation of the synthesis and catabolism of retinoic acid in the developing embryo. *Dev Biol.* 2005; 285:224–237. [PubMed: 16054125]
110. Barua AB, Sidell N. Retinoyl beta-glucuronide: a biologically active interesting retinoid. *J Nutr.* 2004; 134:286S–289S. [PubMed: 14704335]
111. Piedrafita, FJ.; Pfahl, M. Nuclear Retinoid Receptors and Mechanisms of Action. In: Nau, H.; Blaner, WS., editors. *The Handbook of Experimental pharmacology, The Retinoids.* Springer Verlag; Heidelberg: 1999. p. 153-184.
112. Germain P, Chambon P, Eichele G, Evans RM, Lazar MA, Leid M, De Lera AR, Lotan R, Mangelsdorf DJ, Gronemeyer H. International Union of Pharmacology. LXIII. Retinoid X receptors. *Pharmacol Rev.* 2006; 58:760–772. [PubMed: 17132853]
113. Soprano DR, Qin P, Soprano KJ. Retinoic acid receptors and cancers. *Annu Rev Nutr.* 2004; 24:201–221. [PubMed: 15189119]
114. Balmer JE, Blomhoff R. Gene expression regulation by retinoic acid. *J Lipid Res.* 2002; 43:1773–1808. [PubMed: 12401878]
115. McGrane MM. Vitamin A regulation of gene expression: molecular mechanism of a prototype gene. *J Nutr Biochem.* 2007; 18:497–508. [PubMed: 17320364]
116. Saitou M, Narumiya S, Kakizuka A. Alteration of a single amino acid residue in retinoic acid receptor causes dominant-negative phenotype. *J Biol Chem.* 1994; 269:19101–19107. [PubMed: 7913468]
117. Hessel S, Eichinger A, Isken A, Amengual J, Hunzelmann S, Hoeller U, Elste V, Hunziker W, Goralczyk R, Oberhauser V, von Lintig J, Wyss A. CMO1 deficiency abolishes vitamin A production from beta-carotene and alters lipid metabolism in mice. *J Biol Chem.* 2007; 282:33553–33561. [PubMed: 17855355]
118. Bosch FX, Ribes J, Diaz M, Cleries R. Primary liver cancer: worldwide incidence and trends. *Gastroenterology.* 2004; 127:S5–S16. [PubMed: 15508102]
119. Bruix J, Boix L, Sala M, Llovet JM. Focus on hepatocellular carcinoma. *Cancer Cell.* 2004; 5:215–219. [PubMed: 15050913]
120. Bataller R, Brenner DA. Liver fibrosis. *J Clin Invest.* 2005; 115:209–218. [PubMed: 15690074]
121. Friedman SL. Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. *J Biol Chem.* 2000; 275:2247–2250. [PubMed: 10644669]
122. Erickson JM, Mawson AR. Possible role of endogenous retinoid (Vitamin A) toxicity in the pathophysiology of primary biliary cirrhosis. *J Theor Biol.* 2000; 206:47–54. [PubMed: 10968936]
123. Clemente C, Elba S, Buongiorno G, Berloco P, Guerra V, Di Leo A. Serum retinol and risk of hepatocellular carcinoma in patients with child-Pugh class A cirrhosis. *Cancer Lett.* 2002; 178:123–129. [PubMed: 11867196]
124. Yuan JM, Gao YT, Ong CN, Ross RK, Yu MC. Prediagnostic level of serum retinol in relation to reduced risk of hepatocellular carcinoma. *J Natl Cancer Inst.* 2006; 98:482–490. [PubMed: 16595784]
125. Newsome PN, Beldon I, Moussa Y, Delahooke TE, Pouloupoulos G, Hayes PC, Plevris JN. Low serum retinol levels are associated with hepatocellular carcinoma in patients with chronic liver disease. *Aliment Pharmacol Ther.* 2000; 14:1295–1301. [PubMed: 11012474]
126. Roongpisuthipong C, Sobhonslidsuk A, Nantiruj K, Songchitsomboon S. Nutritional assessment in various stages of liver cirrhosis. *Nutrition.* 2001; 17:761–765. [PubMed: 11527674]
127. Tsiaousi ET, Hatzitolios AI, Trygonis SK, Savopoulos CG. Malnutrition in end stage liver disease: recommendations and nutritional support. *J Gastroenterol Hepatol.* 2008; 23:527–533. [PubMed: 18397483]
128. Moscatiello S, Manini R, Marchesini G. Diabetes and liver disease: an ominous association. *Nutr Metab Cardiovasc Dis.* 2007; 17:63–70. [PubMed: 17164082]

129. Altucci L, Gronemeyer H. The promise of retinoids to fight against cancer. *Nat Rev Cancer*. 2001; 1:181–193. [PubMed: 11902573]
130. Niles RM. Biomarker and animal models for assessment of retinoid efficacy in cancer chemoprevention. *Acta Pharmacol Sin*. 2007; 28:1383–1391. [PubMed: 17723172]
131. Takeshita A, Shibata Y, Shinjo K, Yanagi M, Tobita T, Ohnishi K, Miyawaki S, Shudo K, Ohno R. Successful treatment of relapse of acute promyelocytic leukemia with a new synthetic retinoid, Am80. *Ann Intern Med*. 1996; 124:893–896. [PubMed: 8610919]
132. Warrell RP Jr, Frankel SR, Miller WH Jr, Scheinberg DA, Itri LM, Hittelman WN, Vyas R, Andreeff M, Tafuri A, Jakubowski A, et al. Differentiation therapy of acute promyelocytic leukemia with tretinoin (all-trans-retinoic acid). *N Engl J Med*. 1991; 324:1385–1393. [PubMed: 1850498]
133. Meyskens FL Jr, Manetta A. Prevention of cervical intraepithelial neoplasia and cervical cancer. *Am J Clin Nutr*. 1995; 62:1417S–1419S. [PubMed: 7495240]
134. Niles RM. Signaling pathways in retinoid chemoprevention and treatment of cancer. *Mutat Res*. 2004; 555:81–96. [PubMed: 15476854]
135. Kojima S, Okuno M, Matsushima-Nishiwaki R, Friedman SL, Moriwaki H. Acyclic retinoid in the chemoprevention of hepatocellular carcinoma (review). *Int J Oncol*. 2004; 24:797–805. [PubMed: 15010815]
136. Sano K, Takayama T, Murakami K, Saiki I, Makuuchi M. Overexpression of retinoic acid receptor alpha in hepatocellular carcinoma. *Clin Cancer Res*. 2003; 9:3679–3683. [PubMed: 14506158]
137. Wang XD. Retinoids and alcohol-related carcinogenesis. *J Nutr*. 2003; 133:287S–290S. [PubMed: 12514311]
138. Muto Y, Moriwaki H, Ninomiya M, Adachi S, Saito A, Takasaki KT, Tanaka T, Tsurumi K, Okuno M, Tomita E, Nakamura T, Kojima T. Prevention of second primary tumors by an acyclic retinoid, polypropenoic acid, in patients with hepatocellular carcinoma. Hepatoma Prevention Study Group. *N Engl J Med*. 1996; 334:1561–1567. [PubMed: 8628336]
139. Takai K, Okuno M, Yasuda I, Matsushima-Nishiwaki R, Uematsu T, Tsurumi H, Shiratori Y, Muto Y, Moriwaki H. Prevention of second primary tumors by an acyclic retinoid in patients with hepatocellular carcinoma. Updated analysis of the long-term follow-up data. *Intervirology*. 2005; 48:39–45. [PubMed: 15785088]
140. Hebuterne X, Wang XD, Smith DE, Tang G, Russell RM. In vivo biosynthesis of retinoic acid from beta-carotene involves an excentric cleavage pathway in ferret intestine. *J Lipid Res*. 1996; 37:482–492. [PubMed: 8728312]
141. Matsushima-Nishiwaki R, Okuno M, Adachi S, Sano T, Akita K, Moriwaki H, Friedman SL, Kojima S. Phosphorylation of retinoid X receptor alpha at serine 260 impairs its metabolism and function in human hepatocellular carcinoma. *Cancer Res*. 2001; 61:7675–7682. [PubMed: 11606411]
142. Matsushima-Nishiwaki R, Okuno M, Takano Y, Kojima S, Friedman SL, Moriwaki H. Molecular mechanism for growth suppression of human hepatocellular carcinoma cells by acyclic retinoid. *Carcinogenesis*. 2003; 24:1353–1359. [PubMed: 12807734]
143. Shimizu M, Sakai H, Shirakami Y, Iwasa J, Yasuda Y, Kubota M, Takai K, Tsurumi H, Tanaka T, Moriwaki H. Acyclic retinoid inhibits diethylnitrosamine-induced liver tumorigenesis in obese and diabetic C57BLKS/J- +(db)/+Lepr(db) mice. *Cancer Prev Res (Phila)*. 2011; 4:128–136. [PubMed: 21071580]
144. Suzui M, Masuda M, Lim JT, Albanese C, Pestell RG, Weinstein IB. Growth inhibition of human hepatoma cells by acyclic retinoid is associated with induction of p21(CIP1) and inhibition of expression of cyclin D1. *Cancer Res*. 2002; 62:3997–4006. [PubMed: 12124333]
145. Suzui M, Shimizu M, Masuda M, Lim JT, Yoshimi N, Weinstein IB. Acyclic retinoid activates retinoic acid receptor beta and induces transcriptional activation of p21(CIP1) in HepG2 human hepatoma cells. *Mol Cancer Ther*. 2004; 3:309–316. [PubMed: 15026551]
146. Adachi S, Okuno M, Matsushima-Nishiwaki R, Takano Y, Kojima S, Friedman SL, Moriwaki H, Okano Y. Phosphorylation of retinoid X receptor suppresses its ubiquitination in human hepatocellular carcinoma. *Hepatology*. 2002; 35:332–340. [PubMed: 11826406]

147. Kagawa M, Sano T, Ishibashi N, Hashimoto M, Okuno M, Moriwaki H, Suzuki R, Kohno H, Tanaka T. An acyclic retinoid, NIK-333, inhibits N-diethylnitrosamine-induced rat hepatocarcinogenesis through suppression of TGF- α expression and cell proliferation. *Carcinogenesis*. 2004; 25:979–985. [PubMed: 14742314]
148. Nakamura N, Shidoji Y, Moriwaki H, Muto Y. Apoptosis in human hepatoma cell line induced by 4,5-didehydro geranylgeranoic acid (acyclic retinoid) via down-regulation of transforming growth factor- α . *Biochem Biophys Res Commun*. 1996; 219:100–104. [PubMed: 8619789]
149. Shao RX, Otsuka M, Kato N, Taniguchi H, Hoshida Y, Moriyama M, Kawabe T, Omata M. Acyclic retinoid inhibits human hepatoma cell growth by suppressing fibroblast growth factor-mediated signaling pathways. *Gastroenterology*. 2005; 128:86–95. [PubMed: 15633126]
150. Yang H, Zhan Q, Wan YJ. Enrichment of Nur77 mediated by retinoic acid receptor beta leads to apoptosis of human hepatocellular carcinoma cells induced by fenretinide and histone deacetylase inhibitors. *Hepatology*. 2011; 53:865–874. [PubMed: 21319187]
151. Losel R, Wehling M. Nongenomic actions of steroid hormones. *Nat Rev Mol Cell Biol*. 2003; 4:46–56. [PubMed: 12511868]
152. Masia S, Alvarez S, de Lera AR, Baretino D. Rapid, nongenomic actions of retinoic acid on phosphatidylinositol-3-kinase signaling pathway mediated by the retinoic acid receptor. *Mol Endocrinol*. 2007; 21:2391–2402. [PubMed: 17595318]
153. Zeng J-Z, Zhang X-k. Nongenomic actions of retinoids: role of Nur77 and RXR in the regulation of apoptosis and inflammation. *Anti-inflammatory & anti-allergy agents in medicinal chemistry*. 2007; 6:315–331.
154. Yan TD, Wu H, Zhang HP, Lu N, Ye P, Yu FH, Zhou H, Li WG, Cao X, Lin YY, He JY, Gao WW, Zhao Y, Xie L, Chen JB, Zhang XK, Zeng JZ. Oncogenic potential of retinoic acid receptor- γ in hepatocellular carcinoma. *Cancer Res*. 2010; 70:2285–2295. [PubMed: 20197465]
155. Han YH, Zhou H, Kim JH, Yan TD, Lee KH, Wu H, Lin F, Lu N, Liu J, Zeng JZ, Zhang XK. A unique cytoplasmic localization of retinoic acid receptor- γ and its regulations. *J Biol Chem*. 2009; 284:18503–18514. [PubMed: 19416983]
156. Wang XD. Alcohol, vitamin A, and cancer. *Alcohol*. 2005; 35:251–258. [PubMed: 16054987]
157. Morgan TR, Mandayam S, Jamal MM. Alcohol and hepatocellular carcinoma. *Gastroenterology*. 2004; 127:S87–96. [PubMed: 15508108]
158. Stewart S, Jones D, Day CP. Alcoholic liver disease: new insights into mechanisms and preventative strategies. *Trends Mol Med*. 2001; 7:408–413. [PubMed: 11530336]
159. Gyamfi MA, Tanaka Y, He L, Klaassen CD, Wan YJ. Hepatic effects of a methionine-choline-deficient diet in hepatocyte RXR α -null mice. *Toxicol Appl Pharmacol*. 2009; 234:166–178. [PubMed: 18952117]
160. Kim MS, Sweeney TR, Shigenaga JK, Chui LG, Moser A, Grunfeld C, Feingold KR. Tumor necrosis factor and interleukin 1 decrease RXR α , PPAR α , PPAR γ , LXR α , and the coactivators SRC-1, PGC-1 α , and PGC-1 β in liver cells. *Metabolism*. 2007; 56:267–279. [PubMed: 17224343]
161. Wang K, Wan YJ. Nuclear receptors and inflammatory diseases. *Exp Biol Med* (Maywood). 2008; 233:496–506. [PubMed: 18375823]
162. Roman J, Colell A, Blasco C, Caballeria J, Pares A, Rodes J, Fernandez-Checa JC. Differential role of ethanol and acetaldehyde in the induction of oxidative stress in HEP G2 cells: effect on transcription factors AP-1 and NF- κ B. *Hepatology*. 1999; 30:1473–1480. [PubMed: 10573527]
163. Chung J, Chavez PR, Russell RM, Wang XD. Retinoic acid inhibits hepatic Jun N-terminal kinase-dependent signaling pathway in ethanol-fed rats. *Oncogene*. 2002; 21:1539–1547. [PubMed: 11896582]
164. Chung J, Liu C, Smith DE, Seitz HK, Russell RM, Wang XD. Restoration of retinoic acid concentration suppresses ethanol-enhanced c-Jun expression and hepatocyte proliferation in rat liver. *Carcinogenesis*. 2001; 22:1213–1219. [PubMed: 11470752]

165. Uto H, Ido A, Moriuchi A, Onaga Y, Nagata K, Onaga M, Tahara Y, Hori T, Hirono S, Hayashi K, Tsubouchi H. Transduction of antisense cyclin D1 using two-step gene transfer inhibits the growth of rat hepatoma cells. *Cancer Res.* 2001; 61:4779–4783. [PubMed: 11406552]
166. Maeda S. NF-kappaB, JNK, and TLR Signaling Pathways in Hepatocarcinogenesis. *Gastroenterol Res Pract.* 2010; 2010:367694. [PubMed: 21151655]
167. Kluwe J, Wongsiriroj N, Troeger JS, Gwak GY, Dapito DH, Pradere JP, Jiang H, Siddiqi M, Piantedosi R, O'Byrne SM, Blaner WS, Schwabe RF. Absence of hepatic stellate cell retinoid lipid droplets does not enhance hepatic fibrosis but decreases hepatic carcinogenesis. *Gut.* 2011
168. Dan Z, Popov Y, Patsenker E, Preimel D, Liu C, Wang XD, Seitz HK, Schuppan D, Stickel F. Hepatotoxicity of alcohol-induced polar retinol metabolites involves apoptosis via loss of mitochondrial membrane potential. *FASEB J.* 2005; 19:845–847. [PubMed: 15731294]

Highlights

- Hepatocytes are responsible for the uptake and processing of postprandial retinoid into the liver.
- Hepatocytes secrete retinol-binding protein (RBP) from the liver and account for most RBP found in the circulation.
- Extrahepatic tissues store retinoid and can recycle this retinoid back to the liver.
- Hepatic stellate cells store more than 50% of all retinoid present in the body.
- Hepatic retinoid stores are lost during the development of hepatic diseases.

Figure 1.

Alcoholic patients display hepatic total retinol (retinol + retinyl ester) levels that are progressively lower with increasing severity of liver disease (Taken from [9]). Total retinol levels were assessed for human liver biopsies or samples taken at autopsy for normal liver and for livers from alcoholic patients diagnosed with persistent hepatitis, fatty liver, alcoholic hepatitis or cirrhosis. For this study, careful assessments of dietary retinoid intake indicated that differences in hepatic total retinol concentrations could not be explained by diminished retinoid intake by these alcoholic patients from their diets.

Figure 2.

Overview of retinoid metabolism in the two major hepatic cell types involved in retinoid metabolism, the hepatocytes and the hepatic stellate cells. Details of these processes are described in the text. CRBPI, cellular retinol-binding protein, type I; LRAT, lecithin:retinol acyl transferase; RBP, retinol-binding protein; REH, retinyl ester hydrolase; nREH, neutral, bile salt-independent retinyl ester hydrolase; aREH, acidic, bile salt-independent retinyl ester hydrolase; ROL, all-trans-retinol; TTR, transthyretin.

Figure 3.

Retinol is oxidized to retinaldehyde by ADHs or SDRs in a reversible reaction. Subsequent irreversible oxidation of retinaldehyde to retinoic acid is catalyzed by RALDHs. Retinoic acid can interact with members of two families of retinoid receptors (RARs and RXRs), which function primarily as RAR/RXR heterodimers. The retinoid receptors control expression of numerous genes involved in a many different biological processes. Retinoic acid is catabolized (i.e., eliminated, especially in times of excessive availability) to oxidized metabolites primarily through enzymes of the cytochrome P450 (CYP) 26 family.