VITAMIN A METABOLISM, STORAGE AND TISSUE DELIVERY MECHANISMS

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I. INTRODUCTION

Vitamin A is an essential micronutrient that must be acquired from the diet either as preformed vitamin A or as provitamin A carotenoid. By definition, all-\textit{trans}-retinol is vitamin A, although retinol metabolites, including retinyl esters and retinoic acid, are often referred to collectively as vitamin A. Throughout this chapter, vitamin A is used solely to refer to all-\textit{trans}-retinol. The term retinoid, which was first coined by Sporn in the mid-1970s, refers to a family of chemicals, both natural and synthetic, that bear a structural resemblance to all-\textit{trans}-retinol, with or without the biologic activity of vitamin A (Sporn \textit{et al.}, 1976). Thus, the retinoid family of compounds comprises both naturally-occurring vitamin A metabolites and synthetic compounds that bear a structural resemblance to vitamin A.

Within the body, the two most abundant vitamin A species are retinol and retinyl esters. Both retinol and retinyl esters are central to the metabolism, storage and delivery of vitamin A to tissues. The great majority of the vitamin A present in the body is stored as retinyl esters, primarily in the liver, but also in other tissues. Dietary vitamin A is also packaged as retinyl esters in nascent chylomicrons and is thus taken into the body. Because of this, retinyl esters are a transport form of vitamin A that can
reach high concentrations in the postprandial circulation. Retinol also is a transport form of vitamin A in the circulation, where it is found bound to retinol-binding protein (RBP4, hereafter named RBP; the intracellular RBP1-3 proteins being commonly referred to as CRBP1 [cellular retinol-binding protein-1], CRBP2, and IRBP [inter-photoreceptor retinoid-binding protein]).

In the fasting state, retinol bound to RBP is the predominant form of vitamin A present in the circulations of humans and rodents. Retinol is also the metabolic precursor for the biologically active vitamin A metabolites, 11-cis-retinaldehyde and retinoic acid. 11-cis-retinaldehyde is the visual chromophore which, as a first event in the visual cycle, undergoes photoisomerization to all-trans-retinaldehyde when activated by a photon of light (Wald, 1968) (Chapter 18). Retinoic acid, both the all-trans- and 9-cis-isomers of retinoic acid, are transcriptionally active forms of vitamin A (Chambon, 1994; Mangelsdorf et al., 1994). The binding of retinoic acid to retinoic acid receptors (RARs) or to retinoid X receptors (RXRs) can affect the transcription of vitamin A-responsive genes (Chambon, 1994; Mangelsdorf et al., 1994) (Part II in this book).

Most of the knowledge of vitamin A metabolism, storage and mobilization has been obtained from studies of humans and rodents; this chapter primarily focuses on this information. However, it should be noted that these processes vary markedly across different species. For instance, retinol is the predominant vitamin A species present in the circulations of fasting humans, mice and rats. However, this is not universally true for other mammals, or even primates, and may even be the exception. Great apes have relatively high fasting plasma retinyl ester levels compared to humans (García et al., 2006). Unlike the fasting human circulation, where retinol accounts for 95% or more of the total vitamin A, retinol accounts for only approximately 80% of the total vitamin A present in the fasting circulations of chimpanzees and orangutans, with most of the remainder being present as retinyl esters (García et al., 2006). Retinyl esters are the predominant form of vitamin A present in the fasting circulations of dogs (Schweigert, 1988; Raila et al., 2002b, 2004), domestic cats (Raila et al., 2001), and ferrets (Ribaya-Mercado et al., 1992; Raila et al., 2002a).

Similarly, retinyl ester concentrations in the lungs of humans and rats are relatively low compared to those of the liver (Schmitz et al., 1991; Ross and Li, 2007). Yet, unlike humans or rats, the lungs of mice contain very high concentrations of retinyl esters (O’Byrne et al., 2005). The physiological significance of these or other species differences are not understood. Nevertheless, the reader should be aware that there are very pronounced species differences in how vitamin A is transported in the circulation and stored in tissues among different species.

II. VITAMIN A METABOLISM RELEVANT TO ITS STORAGE

Unlike most other vitamins, vitamin A can be accumulated from the diet and stored at relatively high concentrations within the body. When adult rodents, which are maintained on a conventional vitamin A-sufficient chow diet, are placed on a vitamin A-deficient diet, it can take 6–9 weeks, or even longer, for the retinyl ester stores of these rodents to become exhausted (Lamb et al., 1974; Kato et al., 1985; Shankar and De Luca, 1988). The ability to accumulate vitamin A stores and the linked ability to
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Mobilize these stores in times of dietary vitamin A insufficiency affords the organism a great evolutionary advantage, since these stores relieve the organism of the obligate need for regular dietary vitamin A intake.

A scheme for the metabolism of vitamin A is provided in Figure 1.1. Since vitamin A is stored as retinyl ester and mobilized from tissue stores primarily as retinol, this chapter focuses primarily on the metabolic events on the left-hand side of the scheme in Figure 1.1.

Only long chain fatty acyl esters of retinol are synthesized within the body. The retinyl esters found in humans and rodents consist of retinyl palmitate, retinyl oleate, retinyl stearate and retinyl linoleate (Goodman et al., 1965; Tanumihardjo et al., 1990). Within the liver, approximately 70-80% of the total retinyl ester is present as retinyl palmitate (Goodman et al., 1965; Tanumihardjo et al., 1990). Other long chain acyl esters are also found in the body but these usually constitute less than 1–2% of all of the retinyl ester. Retinyl acetate, which is commonly found in food supplements and vitamin formulations, is not naturally present in animals. The retinyl acetate consumed in food supplements and vitamin formulations is quickly hydrolyzed by gut hydrolases to retinol, which is then rapidly taken up by the small intestine and re-esterified as long chain retinyl esters.

A. Vitamin A Esterification to Retinyl Ester

Two pathways for retinyl ester formation were proposed in the early literature. These are summarized in Figure 1.2. One, originally suggested by Huang and Goodman in the mid-1960s, proposed that retinyl esters were formed through a
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A transesterification reaction involving the transfer of the sn-1 acyl group from membrane phosphatidylcholine to retinol (Huang and Goodman, 1965; Goodman et al., 1966). This deduction preceded by about twenty-five years the discovery of a lecithin:retinol acyltransferase (LRAT) activity in tissues (Ong et al., 1987, 1988; Saari and Bredberg, 1988; Yost et al., 1988). Another, proposed by Ross (Ross,

Figure 1.2. Two biochemical pathways for retinyl ester formation: LRAT versus ARAT. (A) LRAT catalyzes a phosphatidylcholine-dependent transesterification reaction, transferring the sn-1 acyl group from membrane phosphatidylcholine to retinol (either nonprotein bound retinol or retinol bound to CRBP1, CRBP2, or CRBP3). The products formed upon LRAT action are lysophosphatidylcholine and retinyl ester (and apo-CRBP when retinol was bound to a CRBP). (B) ARAT catalyzes the acyl-CoA-dependent transfer of the acyl group from acyl-CoA to free retinol, forming CoA and retinyl ester as products.
It is now clear from investigations of induced mutant mice that the predominant pathway for retinyl ester formation in the body involves a transesterification reaction catalyzed specifically by one enzyme, LRAT (Batten et al., 2004; Liu and Gudas, 2005; O’Byrne et al., 2005). Batten et al. (2004) reported that the targeted disruption of the Lrat gene in all mouse tissues results in no developmental abnormalities; however, the mutant mice have only trace levels of retinyl esters in the liver, lung, eye, and blood. O’Byrne et al. (2005) identified adipose tissue of Lrat−/− mice as the only substantial tissue site of retinyl ester accumulation, reporting a twofold to threefold elevation of retinyl esters compared to age-, genetic background-, and gender-matched wild-type mice. Moreover, O’Byrne et al. reported that some retinyl esters were present in chylomicrons of Lrat−/− mice when these mice were given an oral physiologic dose of vitamin A. Liu and Gudas (2005) reported that Lrat−/− mice much more readily develop vitamin A deficiency than wild-type mice when maintained on a vitamin A-insufficient diet. Subsequent studies by the Gudas laboratory demonstrated that Lrat−/− mice, when fed a diet high in vitamin A, regulate vitamin A homeostasis differently than wild-type mice, by enhancing cytochrome (CYP26A1)-catalyzed catabolism and elimination of the dietary retinol (Liu et al., 2008). This is unlike wild-type mice, which simply accumulate greater concentrations of retinyl esters in tissue stores.

Although the published reports focused on Lrat−/− mice have established that LRAT is the key enzyme responsible for retinyl ester synthesis in the body, these studies also provide clear evidence that another enzyme(s) acts in the synthesis of retinyl esters. Several enzymes that can catalyze the acyl-CoA-dependent formation of retinyl esters have been identified by in vitro studies. These include diacylglycerol acyltransferase 1 (DGAT1) (O’Byrne et al., 2005; Orland et al., 2005; Yen et al., 2005), multifunctional acyltransferase (MFAT) (Yen et al., 2005), and acyl-CoA:monoacylglycerol acyltransferase (MGAT) (Yen et al., 2005).

Of these enzymes, the only one that has been studied in vivo as an ARAT is DGAT1. DGAT1 is one of two enzymes, the other being diacylglycerol acyltransferase 2 (DGAT2), which catalyzes the final step of triglyceride synthesis, transferring an acyl group from acyl-CoA to a diglyceride (Yen et al., 2008; Ruggles et al., 2013). It should be noted, based on in vitro studies, that DGAT2 does not possess ARAT activity (O’Byrne et al., 2005; Yen et al., 2005). DGAT1 has been extensively studied because of its importance in metabolic disease development (Yen et al., 2008; Ruggles et al., 2013).

With regards to a role for DGAT1 as a physiologically relevant ARAT, two studies have been reported. Wongsirojo et al. reported that mice totally lacking expression of both Lrat and Dgat1 (Lrat−/−;Dgat1−/− mice) are unable to incorporate any retinyl esters in nascent chylomicrons in response to an oral challenge with a physiological dose of retinol (Wongsirojo et al., 2008). This is unlike...
Lrat−/− mice, which still synthesize and incorporate some retinyl ester into nascent chylomicrons in response to a physiologic dose of retinol (O’Byrne et al., 2005). This implies that DGAT1 can act in vivo as an intestinal ARAT. Wongsiriroj et al. further reported that the Lrat−/−;Dgat1−/− mice possess elevated concentrations of retinyl esters in adipose tissue, identical to those of Lrat−/− mice. Thus, DGAT1 cannot be responsible for the observed increase in retinyl ester synthesis and accumulation in adipose tissue of Lrat−/− mice. In other studies, Farese and colleagues studied the actions of DGAT1 in maintaining vitamin A homeostasis in murine skin using Dgat1−/− and matched wild-type mice (Shih et al., 2009). These investigators concluded that DGAT1 is the major ARAT activity present in murine skin. Based on these studies, it appears that DGAT1 acts as an ARAT in both intestine (Wongsiriroj et al., 2008) and skin (Shih et al., 2009). However, it remains unclear whether MFAT and/or MGAT act as ARATs in vivo or whether either of these enzymes is responsible for the elevated retinyl ester concentrations observed in adipose tissue of Lrat−/− and Lrat−/−;Dgat1−/− mice.

B. Retinyl Ester Hydrolysis to Vitamin A

Unlike retinyl ester synthesis, in which only a few enzymes have been identified as being able to catalyze retinyl ester formation in vitro and only one of these is responsible for most retinyl ester synthesis within the body, many enzymes have been identified which possess retinyl ester hydrolyase (REH) activity in vitro. Some of these are relatively abundant and well characterized lipases, including pancreatic triglyceride lipase (van Bennekum et al., 2000), hepatic lipase (Krapp et al., 1996), lipoprotein lipase (LpL) (Blaner et al., 1994; van Bennekum et al., 1999), cholesteryl ester lipase (CEL) (Lindstrom et al., 1988), and hormone sensitive lipase (HSL) (Wei et al., 1997). Others are less extensively studied; these include pancreatic lipase-related protein 2 (Reboul et al., 2006), intestinal brush border membrane REH (also identified as a calcium-independent brush border membrane phospholipase B) (Rigtrup and Ong, 1992; Rigtrup et al., 1994a, 1994b), and a number of carboxylesterases (ES-2, ES-4, ES-10 and ES-22) (Mentlein and Heymann, 1987; Schindler et al., 1998; Linke et al., 2005; Schreiber et al., 2009).

A few of these enzymes have been established to have roles within the body in facilitating vitamin A homeostasis, acting specifically as REHs. For instance, LpL acts importantly in facilitating uptake of postprandial vitamin A from chylomicrons by extrahepatic tissues (Blaner et al., 1994; van Bennekum et al., 1999). It is generally accepted that pancreatic triglyceride lipase, brush border membrane REH, and, probably, CEL act to hydrolyze newly ingested dietary retinyl ester into retinol, which is then taken up by the enterocyte for processing. These are discussed in more detail in the next chapter. There is consensus that HSL is a physiologically significant REH in adipocytes (Wei et al., 1997; Ström et al., 2009). HSL was proposed to be the enzyme in adipocytes responsible for triglyceride hydrolysis but in the last decade, with the identification and characterization of adipocyte triglyceride lipase (ATGL), it is now clear that both HSL and ATGL can act to hydrolyze adipocyte triglycerides; ATGL has a more prominent role in this process (Lampidonis et al., 2011; Lass et al., 2011; Ruggles et al., 2013).
What remains unclear, however, is which enzyme or enzymes are importantly involved *in vivo* in the hydrolysis of retinyl esters within the liver, where the majority of dietary vitamin A is taken up and where the majority of the body's vitamin A stores are found. Retinyl esters can be found in many tissues, including lung, testis, skin, and eye. However, as with the liver, there is little general consensus as to the molecular identities of physiologically relevant REHs present in these tissues.

**C. Mobilization of Vitamin A from Tissue Stores**

The ability of the body to alternatively store or mobilize vitamin A in response to its dietary availability is unique amongst the vitamins. Healthy, well-nourished individuals who have accumulated vitamin A stores throughout life can go many weeks or even months before they succumb to the adverse physiological effects of vitamin A deficiency. As depicted in Figure 1.3, the biology that underlies this selective advantage involves enzymes able to synthesize retinyl esters (LRATs and ARATs), enzymes able to hydrolyze retinyl esters to retinol (REHs), a capacity to facilitate the mobilization of tissue vitamin A stores, and a capacity to accumulate retinyl esters in lipid droplets within cells and tissues.

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**Figure 1.3.** Scheme depicting the alternative regulation of vitamin A store accumulation versus the mobilization of these stores. Vitamin A is maintained at constant levels within the blood through two counteracting regulatory mechanisms. During times of dietary vitamin A sufficiency, dietary retinol is taken up and then esterified into retinyl ester, primarily via LRAT but in some tissues also through an ARAT activity. The resulting retinyl ester is then stored. During times of dietary vitamin A insufficiency, retinol is mobilized from retinyl ester stores. Retinyl ester is hydrolyzed into retinol via a REH activity. Retinol then binds apo-RBP and is released into the circulation for delivery to tissues.
As discussed in more detail later in this chapter (Section IV.), vitamin A and several of its metabolites are present in both the fasting and postprandial circulations. These include retinol, retinyl esters, retinoic acid, and the β-glucuronides of both retinol and retinoic acid. However, only retinol is thought to be mobilized from tissue retinyl ester stores in response to insufficient dietary vitamin A intake. That is to say, blood retinol levels are maintained at a constant level, or defended, in response to extended consumption of a vitamin A-insufficient diet. This is accomplished through the interaction of retinol with its specific serum transport protein, retinol-binding protein (RBP) (Kanai et al., 1968; Quadro et al., 1999). As was convincingly demonstrated through the generation and study of Rbp-deficient mice, the presence of RBP allows for the mobilization of stored vitamin A, which is liberated through hydrolysis of tissue retinyl esters (Quadro et al., 1999). Rbp-deficient mice accumulate tissue retinyl ester stores normally from the diet but are unable to mobilize retinol from these stores in response to insufficient dietary vitamin A intake (Quadro et al., 1999, 2005). Hence, Rbp-deficient mice are prone to developing vitamin A deficiency when subjected to insufficient dietary vitamin A intake.

Since retinyl esters are very highly hydrophobic, they are, thus, incorporated into intracellular lipid droplets. The formation and degradation of intracellular lipid droplets is a highly regulated process involving lipid synthesizing and degrading enzymes and many different lipid droplet-associated proteins. The best studied retinyl ester-containing lipid droplets are the retinosomes found in the retinal pigmented epithelial (RPE) cells of the eye (Imanishi et al., 2004, 2008; Orban et al., 2011), the lipid droplets of the hepatic stellate cells (HSCs) (Blaner et al., 2009), and the adipocyte lipid droplets (Lass et al., 2011; Brasaemle and Wolins, 2012; Konige et al., 2013). As is discussed in more detail later, the RPE retinosomes and the HSC lipid droplets are specialized lipid droplets that accumulate relatively high concentrations of retinyl esters. In adipocyte lipid droplets, concentration of retinyl esters are low compared to concentrations of triglycerides. However, the biochemistry of adipocyte lipid droplets is better studied. The reader is referred to recent reviews for general information regarding lipid droplet biochemistry (Lass et al., 2011; Brasaemle and Wolins, 2012; Konige et al., 2013).

III. VITAMIN A STORAGE

More than two decades ago, Blomhoff and colleagues (Blomhoff et al., 1991) provided a very thoughtful analysis of the literature concerning what proportion of the total vitamin A (retinol plus all of its metabolites) present in the body is found within the liver versus other tissues. They concluded that for a vitamin A-sufficient rat, probably greater than 90% of whole body total vitamin A is found in the liver. They went on to conclude that this percentage is undoubtedly linked to vitamin A nutritional status, with a substantially lower percentage present in the livers of animals experiencing insufficient dietary vitamin A intake. This conclusion is strongly supported by compartmental modeling studies carried out in rats receiving different quantities of vitamin A in their diets (Green et al., 1987; Lewis et al., 1990; Cifelli et al., 2005).
A more recent study by Kane et al. (2008a), one systematically exploring total vitamin A levels in tissues of 2–4 month old male SV129 mice fed the AIN-93 M diet containing 4 IU vitamin A/g diet from the time of weaning, provided data consistent with the notion that greater than 90% of whole body vitamin A is present in the liver. However, they failed to report total vitamin A levels for lungs and their data perhaps somewhat overestimated the contribution of the liver. O’Byrne et al. (2005) reported tissue total vitamin A levels for 3–4 month old male mixed genetic background (C57BL/6/SV129) mice fed from the time of weaning a chow diet containing 25 IU vitamin A/g diet. When corrected for organ weight, the lungs of these mice contained approximately 11% of the total vitamin A that is present in the liver. Thus, the data provided by O’Byrne et al. suggest that, for the mouse, approximately 80–85% of the total vitamin A in the whole body is found in liver.

In summary, there is general consensus that the liver of a healthy well-nourished animal model or of a healthy human being accounts for the great majority of the vitamin A that is present within the body.

A. The Liver

Two distinct hepatic cell types play central roles in the storage and metabolism of vitamin A within the liver: the parenchymal cells, or hepatocytes, and the nonparenchymal hepatic stellate cells (HSCs) (Blaner et al., 1985; Blomhoff et al., 1985). There is no evidence that the resident macrophages of the liver, the Kupffer cells, have a role in hepatic vitamin A physiology. There is a report in the literature employing immunohistochemical approaches indicating that hepatic endothelial cells express LRAT protein (Nagatsuma et al., 2009). However, there is no evidence that these cells accumulate significant concentrations of retinyl esters (Blaner et al., 1985; Blomhoff et al., 1985). Only the well-established roles of hepatocytes and HSCs in hepatic vitamin A storage and metabolism are considered here. Table 1.1 summarizes some similarities and differences amongst hepatocytes, HSCs, and adipocytes. It should be noted, as discussed below, that presently, there are major gaps in the knowledge regarding many details of hepatic vitamin A storage and metabolism.

1. The Hepatocyte. Hepatocytes constitute approximately two-thirds of all cells present in the liver and approximately 90% of total hepatic protein (Blaner et al., 1985; Geerts, 2001; Friedman, 2008). These large and relatively abundant cells are responsible for most of the metabolic activities associated with the liver. It is well established that the hepatocyte is the cellular site of chylomicron remnant clearance in the liver, and consequently the uptake of dietary vitamin A (Blomhoff et al., 1982, 1984; Cooper, 1992; Blaner and Olson, 1994; Redgrave, 2004; Abumrad and Davidson, 2012). The hepatocyte is also the cellular site of RBP synthesis and secretion in the liver (Blaner et al., 1985; Yamada et al., 1987; Weiner et al., 1992; Friedman et al., 1993; Sauvant et al., 2001). Thus, hepatocytes are importantly involved in both the uptake of dietary vitamin A by the liver and its mobilization from the liver. But hepatocytes account for only a relatively small proportion of the total vitamin A present within the liver. For vitamin A-sufficient rats, estimates of the hepatic total vitamin A that is found in hepatocytes range from 10–20% (Blaner et al., 1985; Batres and Olson, 1987; Blomhoff et al., 1988a). The remainder, 80-90%, is found in HSCs.
An hypothesis has been proposed regarding how newly absorbed vitamin A is processed by the liver. This proposes that normally some newly absorbed dietary vitamin A is retained in and secreted from the hepatocyte bound to RBP and that some is transferred to the HSC for storage (Batres and Olson, 1987; Blomhoff et al., 1988a; Green et al., 1988). It is thought that the relative proportion that is secreted bound to RBP depends on dietary vitamin A intake, with a relatively greater percentage being secreted in times of insufficient vitamin A intake. Thus, the relative distribution of hepatic total vitamin A between hepatocytes and HSCs changes in response to vitamin A nutritional status, the ratio of vitamin A levels in hepatocytes to HSCs increases with decreased dietary vitamin A intake (Batres and Olson, 1987; Blomhoff et al., 1988a; Green et al., 1988).

Since the hepatocyte is the cellular site of dietary vitamin A uptake by the liver and the HSC is the cellular site of vitamin A storage within the liver, this raises a question as to how newly absorbed dietary vitamin A is transferred from the hepatocyte to the HSC. Studies involving injection of chylomicrons labeled with [3H]retinyl ester into the circulation of vitamin A-sufficient rats established that the radiolabel is initially taken up by hepatocytes (Blomhoff et al., 1982, 1984), but after two hours the radiolabel is primarily associated with HSCs (Blomhoff et al., 1984). This is taken to indicate that dietary vitamin A is first taken up by hepatocytes and then rapidly transferred to HSCs. Another study, making use of chylomicrons labeled with a radiolabeled nonhydrolyzable ether analog of retinyl palmitate, established that chylomicron retinyl ester is not transferred directly from hepatocytes to HSCs without first undergoing hydrolysis to retinol (Blaner et al., 1987b). This finding may suggest that retinol is being transferred from hepatocytes to HSCs. The REH(s) in hepatocytes responsible for catalyzing the hydrolysis of newly absorbed retinyl ester has not been definitively established, although Harrison and colleagues have reported that ES-10 is present within hepatocytes and proposed that this enzyme is responsible for the hydrolysis of chylomicron remnant retinyl ester (Sun et al., 1997; Harrison, 1998; Linke et al., 2005). However, this hypothesis remains to be established through in vivo studies.

But how is vitamin A transferred from the hepatocyte to the HSC for storage? A considerable amount of in vivo and in vitro work carried out in the 1980s and 1990s had suggested that this required the actions of RBP (Blomhoff et al., 1988b, 1991). Although when it was first proposed, this hypothesis was generally thought to be valid, subsequent investigations involving mice that totally lacked expression of Rbp observed no differences in the cellular distribution of hepatic total vitamin A between matched wild type, Rbp deficient, or Rbp-deficient mice expressing human RBP in skeletal muscle and, therefore, concluded that RBP is not required for intrahepatic transport and storage of vitamin A (Quadro et al., 2004). Quadro et al. observed no differences in the cellular distribution of hepatic total vitamin A between matched wild type, Rbp deficient, or Rbp-deficient mice expressing human RBP in skeletal muscle and, therefore, concluded that RBP is not required for intrahepatic transport and storage of vitamin A (Quadro et al., 2004).

It also had been suggested early on that CRBP1, which is highly expressed in both hepatocytes and HSCs (Blaner et al., 1985; Blomhoff et al., 1985), may play a role in this process (Eriksson et al., 1984). But this, too, seems unlikely, since totally Crbp1-deficient mice accumulate vitamin A in HSCs (Ghyselinck et al., 1999). Thus, at present, it remains to be established through what process(es) dietary vitamin A is transferred from hepatocytes to HSCs for storage.
2. The Hepatic Stellate Cell (HSC). HSCs, along with Kupffer and hepatic endothelial cells, constitute the nonparenchymal cells of the liver (Wake, 1971; Geerts, 2001; Friedman, 2008; Blaner et al., 2009). They account for approximately 5–8% of the total cells present in the liver and about 1% of total hepatic protein. The hallmark characteristic of the HSCs is the presence of numerous large lipid droplets within the cytoplasm (Wake, 1971; Blomhoff et al., 1988b; Geerts, 2001; Friedman, 2008). This can be readily seen in Figure 1.4, which shows a light micrograph obtained from primary mouse liver HSCs that had been cultured overnight.

The lipid composition of purified rat HSC lipid droplets has been reported by different laboratories and these are in good agreement (Hendriks et al., 1987; Yamada et al., 1987; Moriwaki et al., 1988). Moriwaki et al. (1988) reported that lipid droplets purified from HSCs isolated from rats fed a purified diet containing 8 IU vitamin A/g diet had an average lipid composition that consisted of 39.5% retinyl ester, 31.7% triglyceride, 15.4% cholesteryl ester, 4.7% cholesterol, 6.3% phospholipids, and 2.4% free fatty acids. These authors further reported that the HSC lipid droplet lipid composition was markedly responsive to changes in dietary vitamin A intake but not to changes in dietary fat intake. Based on their content of retinyl ester and their responses to dietary vitamin A intake, it would appear that HSC lipid droplets are specialized for vitamin A storage and not for storage of other neutral lipids (triglyceride or cholesteryl ester).

![Figure 1.4](image)

**Figure 1.4.** Light micrograph of an overnight culture of primary HSCs obtained from three-month-old wild type C57BL/6J mice fed a chow diet throughout life. The numerous cytoplasmic lipid droplets are the sites of retinyl ester storage within these cells and a distinguishing characteristic of HSCs. If culture of these cells were continued for approximately one week, the HSCs would activate and the vitamin A-containing lipid droplets would be totally lost.
A number of well studied lipid droplet-associated proteins present in adipocytes and other cell types are reported to be associated with HSC lipid droplets. Straub et al. (2008) reported immunolocalization studies showing that perilipin 2 (adipocyte differentiation-related protein, ADRP) and perilipin 3 (TIP47) are present in HSC lipid droplets for normal mouse, bovine and human liver. These investigators convincingly demonstrated that perilipins 2 and 3 colocalize with the retinoid autofluorescence of HSC lipid droplets as well as with vimentin and desmin, which are established markers used to identify HSCs. Moreover, Straub et al. reported that perilipin 1 colocalizes in HSC lipid droplets for bovine liver but not mouse or human liver. Thus, these members of the so-called PAT protein family, which are known to play crucial roles in the stabilization of lipid droplets in other cell types, likely act in a similar manner in HSCs. This idea is further supported by cell culture studies from two independent laboratories that demonstrate that either adenovirus-mediated expression of perilipin 2 or its knockdown using siRNA in cultured HSCs affects lipid droplet formation and vitamin A levels in the cytoplasm of treated cells (Fukushima et al., 2005; Lee et al., 2010).

As might be expected based on their high concentrations of retinyl esters, HSCs express relatively high levels of LRAT. Matsuura and colleagues reported immunoelectron microscopic studies showing that LRAT is localized to both the rough endoplasmic reticulum and the “multivesicular bodies” present within HSCs (Nagatsuma et al., 2009). This subcellular localization is believable and may be very important for allowing lipid droplet formation, considering that the multivesicular bodies have been proposed to be a precursor structure needed for the formation of membrane-bound lipid droplets present in HSCs (Yamamoto and Ogawa, 1984; Geerts et al., 1990).

It is not clear which REHs act to hydrolyze HSC retinyl esters. ES-10, LpL, and HSL mRNAs are all expressed by HSCs (Mello et al., 2008), but it remains to be established whether these or other REHs have a role in vivo in catalyzing retinyl ester hydrolysis. Although some literature published more than two decades ago claimed that the HSC was a cellular site for RBP synthesis and secretion from the liver (Blomhoff et al., 1988b, 1991; Andersen et al., 1992), this is now known to be incorrect. Little if any RBP mRNA or protein can be detected in highly purified populations of primary HSCs (Blaner et al., 1985; Yamada et al., 1987; Weiner et al., 1992; Friedman et al., 1993; Sauvant et al., 2001). The inability of HSCs to synthesize and secrete RBP raises an unresolved question regarding how stored vitamin A is mobilized from these cells in times of dietary insufficiency. A possible explanation is that retinol generated upon hydrolysis of HSC retinyl esters is transferred by a transporter across the HSC plasma membrane to apo-RBP present in the extracellular space, but no such transporter has yet been identified experimentally. This remains an important question for future research aimed at explaining how retinol is mobilized from HSC vitamin A stores.

As discussed above, HSCs normally account for 80–90% of the total vitamin A present in the liver, and the liver accounts for 80–90% of the total vitamin A present in the body. Thus, this small and relatively scarce liver cell type is responsible for the storage of the majority of the vitamin A that is present in the body of a healthy well-nourished animal. This raises a provocative question as to why this is. What evolutionary pressure or selective advantage gave rise to this very
disproportionate distribution of vitamin A in the body? One possibility is that this allows for very specific regulation within the body for the accumulation and mobilization of vitamin A stores. An alternative hypothesis is that HSC vitamin A stores are needed to buffer against the development of hepatic disease. The HSC is best studied for its role in the process of hepatic disease development, progressing from fibrosis to cirrhosis and hepatocellular carcinoma. The development of hepatic disease involves the activation of HSCs and this is accompanied by the rapid loss of cellular vitamin A stores (Geerts, 2001; Friedman, 2008; Blaner et al., 2009). It has been suggested that the decline in HSC vitamin A stores upon HSC activation represents a futile attempt to block HSC activation and their transdifferentiation to myofibroblasts (Geerts, 2001; Friedman, 2008). If this hypothesis was correct, it would explain why the HSCs account for the great majority of vitamin A stored within the body.

B. Adipose Tissue

Adipose tissue accumulates both retinol and retinyl esters. Tsutsumi et al. (1992) reported that the inguinal, dorsal, mesenteric, epididymal, perinephric, and brown adipose depots of chow-fed rats each contain approximately 6–7 μg total vitamin A (retinol + retinyl ester) per gram tissue. Based on these levels and estimates of the total adipose tissue present in a 300–450 g rat, these authors estimated that adipose tissues contains as much as 15–20% of the total vitamin A found in the liver. However, based on the adipose tissue total vitamin A levels reported by Kane et al. (2008a) for mice fed a purified diet and by O’Byrne et al. (2005) for mouse fed a chow diet, it would appear that the mouse accumulates much less vitamin A in adipose tissue than the rat, approximately 2–4% of the liver levels. However, studies of Lrat−/− mice fed a totally vitamin A-deficient diet have established that adipose tissue vitamin A is indeed mobilized to defend circulating vitamin A levels (Liu and Gudas, 2005; O’Byrne et al., 2005; Liu et al., 2008). Thus, adipose tissue vitamin A stores are true stores.

Tsutsumi et al. (1992) further reported that, of the cell types found in rat adipose tissue, only adipocytes contain detectable amounts of vitamin A (as retinol and retinyl ester), at levels ranging from 0.60 to 0.85 μg vitamin A/10⁶ adipocytes. By comparison, rat hepatocytes are reported to contain between 0.10 and 0.34 μg total vitamin A/10⁶ cells and HSCs 10.9 μg total vitamin A/10⁶ cells (Blaner et al., 1985). The adipocyte is also the cellular site of RBP synthesis and secretion from adipose tissue. mRNA levels of RBP in adipose tissue of chow-fed rats are approximately 15–20% those of the liver (Tsutsumi et al., 1992) (A summary is given in Table 1.1.)

Investigations of BFC-1β preadipocyte differentiation to adipocytes established that RBP mRNA expression is indeed differentiation dependent, showing a temporal pattern of expression that resembles those of the adipocyte-specific genes encoding LpL and fatty acid-binding protein 4 (FABP4 or aP2) (Zovich et al., 1992). Studies of RBP expression carried out using human adipose tissue explants have shown that adipose RBP mRNA levels are higher in women than in men and that RBP protein secretion from this ex vivo model is stimulated by leptin but not by insulin (Kos et al., 2011).
Over the last few years, there has been considerable research interest in adipose-derived RBP. A large number of published reports have suggested a link between RBP synthesized by adipocytes and obesity, diabetes and insulin signaling. The first of these studies looked at the impact of RBP levels in various mouse and human models (Yang et al., 2005; Graham et al., 2006; Polonsky, 2006). RBP levels were found to be increased in human obesity and this increase was proposed to impair insulin signaling, possibly contributing to the development of type 2 diabetes, although the published studies are sometimes not in agreement (Yang et al., 2005; Graham et al., 2006; Janke et al., 2006; Polonsky, 2006; Munkhtulga et al., 2007; Gómez-Ambrosi et al., 2008; Kotnik et al., 2011). The fact that this relationship has also been reported for apo-RBP suggests that RBP per se and not its ligand retinol is responsible for linking adipose-derived RBP with impaired insulin responsiveness (Mills et al., 2008; Erikstrup et al., 2009; Norseen et al., 2012).

As noted previously, HSL is the sole physiologically relevant REH present in adipocytes (Wei et al., 1997; Ström et al., 2009). Adipose tissue of Lrat–/– mice shows an increase in total vitamin A levels as compared to matched wild-type mice (Liu and Gudas, 2005; O’Byrne et al., 2005). This is taken to indicate that LRAT is not responsible for catalyzing retinyl ester formation in adipose tissue. Similarly, since adipose tissue retinyl ester levels are elevated in Lrat–/–:Dgat1–/– double knockout mice, DGAT1 also cannot be responsible for retinyl ester formation in adipose tissue (Wongsiriroj et al., 2008). The enzyme(s) responsible for retinyl ester synthesis in adipose tissue remains to be established. Adipose tissue also expresses both Crbp1 and Crbp3 (Rbp7) mRNAs (Tsutsumi et al., 1992; Vogel et al., 2001). Interestingly, Crbp1 mRNA is associated with the adipose stromal-vascular cells; whereas Crbp3 mRNA is associated with adipocytes. The significance of this later finding is not understood.

### Table 1.1. Estimated distribution of vitamin A-related parameters in rat hepatocytes, hepatic stellate cells (HSCs), and adipocytes

<table>
<thead>
<tr>
<th></th>
<th>Hepatocytes</th>
<th>HSCs</th>
<th>Adipocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimated total vitamin A in body (%)</td>
<td>8–18</td>
<td>64–81</td>
<td>10–20</td>
</tr>
<tr>
<td>Vitamin A levels (µg vitamin A/10⁶ cells)</td>
<td>0.10–0.34e</td>
<td>10.9c</td>
<td>0.60–0.85d</td>
</tr>
<tr>
<td>Subcellular localization of vitamin A</td>
<td>Not known</td>
<td>In lipid droplets</td>
<td>In lipid droplets</td>
</tr>
<tr>
<td>RBP synthesis and secretion</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Chylomicron vitamin A uptake</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

---

aMeasurements of vitamin A-related parameters for chow fed rats.
bVitamin A levels as total vitamin A (retinol + retinyl esters).
cTaken from Blaner et al., 1985.
dTaken from Tsutsumi et al., 1992.
C. The Eye

Going back to the studies of Wald in the 1930s, the metabolism and action of vitamin A are perhaps most extensively studied in the eye (Palczewski, 2012). It has long been known that the retina and retinal pigmented epithelium (RPE) contain high concentrations of both the 11-cis- and all-trans-isomers of retinaldehyde, retinol and retinyl ester (Wald, 1968; Palczewski, 2012). The photoreceptors of the retina are the site of phototransduction and are very highly enriched in retinaldehyde. RPE cells are the site of vitamin A storage in the eye and these cells are highly enriched in retinyl esters. These cells express high levels of LRAT (Saari and Bredberg, 1988; Saari et al., 1993; Mata and Tsin, 1998; Huang et al., 2009) and possess REH enzymatic activity (Blaner et al., 1987a; Mata et al., 1992). In addition, they express RBP mRNA and protein (Martone et al., 1988; Ong et al., 1994a). It has been proposed that RBP synthetized in the RPE provides a means for intraocular translocation of retinol (Martone et al., 1988; Ong et al., 1994a). However, this proposed role for RBP within the eye cannot be essential, since Rbp−/− mice are not completely blind (Quadro et al., 1999).

Palczewski and colleagues have published a number of elegant studies identifying and characterizing retinyl ester storage particles, or retinosomes, within the RPE. Using the intrinsic fluorescence of all-trans-retinyl esters and noninvasive two-photon microscopy, these investigators identified a distinct cellular organelle, the retinosome, within the mouse RPE with an approximate length of 6.9 ± 1.1 μm and diameter of 0.8 ± 0.2 μm (Imanishi et al., 2004). Confirming the physiologic importance of retinosomes, they are reported to be absent in eyes of Lrat−/− mice, which are deficient in retinyl ester synthesis. Subsequent studies by these investigators defined the lipid composition of retinosomes isolated from bovine RPE cells (Orban et al., 2011). This composition was similar to that reported for lipid droplets isolated from rat HSCs (Moriwaki et al., 1988; Orban et al., 2011). Retinyl esters accounted for approximately 43% of the lipid mass of the retinosomes (Orban et al., 2011). Also present in the isolated bovine retinosomes were triglycerides, cholesteryl esters, cholesterol, phospholipids and free fatty acids. Other studies established a role for perilipin 2 in maintaining proper storage and trafficking of vitamin A within the eye (Imanishi et al., 2008). Based on the available literature, it would appear that the retinosomes and HSC lipid droplets share many biochemical similarities.

The reader is referred to Chapters 18 and 24 for greater consideration of vitamin A and vision.

D. Vitamin A Stores in Other Tissues

Retinyl esters and, consequently, vitamin A stores are found at relatively low levels in many tissues. Kane et al. (2008a) reported that readily measurable concentrations of retinyl esters were present in the liver, kidney, white adipose tissue, brown adipose tissue, muscle, spleen, testis, skin, and brain of 2–4 month old male mice fed a purified vitamin A-sufficient diet throughout life. The lung of a chow-fed male mouse also possesses relatively high concentrations of retinyl esters (O’Byrne et al., 2005). Although these extrahepatic stores may account for as much as 15–20% of the total vitamin A present in the body, there is very little information available regarding the
cellular sites of retinyl ester storage in these tissues. Ong and colleagues have reported that testicular Sertoli cells possess LRAT activity and, consequently, the Sertoli cells may be the cellular site of retinyl ester storage within the testis (Shingleton et al., 1989). Nagy et al. (1997) reported that lipid droplet-containing stellate cells are present in liver, lung, kidney, and intestine of normal and excess vitamin A-fed rats. Similarly, Apte et al. (1998) identified periacinar stellate shaped cells in rat pancreas and provided evidence consistent with the notion that these lipid droplets contain vitamin A.

E. Tissue Provitamin A Carotenoids

Since many tissues, including liver, lungs, testes, and eyes, express β-carotene-15, 15’-monooxygenase 1 (BCMO1), the enzyme responsible for β-carotene cleavage to vitamin A, in principle, intact provitamin A carotenoids delivered to these tissues may be converted in situ to vitamin A (Kiefer et al., 2001; Paik et al., 2001; Redmond et al., 2001; Wyss et al., 2001; Yan et al., 2001; Lindqvist and Andersson, 2002; Chichili et al., 2005). It is also well established that carotenoids are taken up from the diet along with other dietary lipids in nascent chylomicrons (Huang and Goodman, 1965; Goodman et al., 1966). Erdman and colleagues have reported high performance liquid chromatography (HPLC)-based measurements of carotenoid levels in human tissues obtained post mortem within 24 hours of death (Schmitz et al., 1991). Liver was reported to contain concentrations of the common dietary carotenoids, β-carotene, α-carotene, β-cryptoxanthin, and lycopene, that ranged from undetectable to greater than 20 nmol/g tissue. Kidney and lung were also found to accumulate each of these dietary carotenoids, albeit at concentrations which were an order of magnitude less than liver.

The mouse, owing to its ability to be genetically manipulated, is presently being used to study β-carotene uptake from the diet, as well as β-carotene metabolism and physiologic actions. But rodents are very poor absorbers of β-carotene. Consequently, studies involving the feeding of β-carotene to mice must employ very high concentrations of β-carotene in the diet, concentrations that are many times greater than those which would be found in any human diet. Hessel et al. reported β-carotene concentrations ranging from 1 to 2 nmol/g tissue for lung, kidney, testis and uterus of wild-type mice fed 1 mg β-carotene/g diet for 16 weeks (Hessel et al., 2007). This is equivalent to a 70 kg man consuming 8.4 g β-carotene per day, as opposed to the more normal human consumption of milligram quantities of β-carotene per day. Fierce et al., who reported feeding wild-type mice diets containing 0.1 mg/kg diet for up to seven weeks, observed β-carotene accumulation only in liver, lung, and intestine and at much lower levels than were reported by Hassel et al. (Fierce et al., 2008).

At present, it remains to be established whether tissue provitamin A carotenoid pools can truly be considered to be “stores” that are used in a regulated manner for vitamin A synthesis. Specifically, are these “stores” drawn upon to defend blood vitamin A levels in times of insufficient dietary vitamin A intake? This has not been unequivocally established. Thus, although tissues can accumulate significant concentrations of β-carotene and other carotenoids, it remains unclear whether or how this is integrated into the whole body economy of vitamin A.
IV. VITAMIN A TRANSPORT IN THE CIRCULATION

A number of different vitamin A metabolites are found in the circulation; these differ quantitatively between the fasting and postprandial states. These include: retinyl esters in chylomicrons and chylomicron remnants; retinyl esters in very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL); retinol bound to RBP; retinoic acid bound to albumin; and the water-soluble beta-glucuronides of retinol and retinoic acid. As can be surmised from the text below, the delivery of vitamin A to tissues is complex, involving many different vitamin A forms and carriers. Quantitatively the two most important pathways are those involving retinol bound to RBP and the postprandial delivery pathway involving chylomicron retinyl esters.

However, the importance of retinoic acid delivery to tissues from the blood should not be discounted, since the accumulation of retinoic acid from the blood is tissue dependent (Kurlandsky et al., 1995). This notion is further substantiated by the effective use of orally administered retinoic acid in experimental studies and its pharmacological administration in humans. In this regard, the delivery of vitamin A to tissues, as either retinol or retinoic acid, is not different from the delivery of vitamin D or thyroid hormone, involving the presence of relatively large concentrations of the transcriptionally-inactive precursor (retinol, 25-hydroxy-vitamin D, or T₄) and relatively low concentrations of the transcriptionally active metabolite (retinoic acid, 1,25-dihydroxy-vitamin D, or T₃). This implies that it is not incorrect to refer to retinoic acid as a hormone although, according to its mode of action, it is probably better defined as an autacoid molecule (further discussed in Chapter 3).

A. Retinol-Binding Protein (RBP)

In the fasting circulation of humans and rodents, retinol bound to RBP is the predominant vitamin A species, with normal concentrations ranging from 2 to 4 μM in humans and around 1 μM in mice (Soprano and Blaner, 1994; Chuwers et al., 1997; O’Byrne et al., 2005). This accounts for approximately 95% of the vitamin A present in the fasting human and rodent circulations. However, as noted above, this is not the case for all mammals or even all primates. Importantly, aside from abnormal vision, Rbp⁻/⁻ mice are generally phenotypically normal (Quadro et al., 1999, 2005). Similarly, for the few humans who have been identified, the RBP-null phenotype is only associated with impaired vision or total blindness and other ocular defects, but not more severe systemic lesions (Biesalski et al., 1999; Seeliger et al., 1999; Cukras et al., 2012). Thus, although retinol-RBP is a major pathway for the transport of vitamin A in the circulation, it is not an essential pathway nor one where its absence is inconsistent with human or mouse life.

In the circulation, the retinol-RBP complex binds another plasma protein, transthyretin (TTR), and this stabilizes the complex, reducing renal filtration of the vitamin A (Soprano and Blaner, 1994; van Bennekum et al., 2001). As depicted in Figure 1.5, one molecule of RBP is found in the circulation bound to one molecule of tetrameric TTR. The molar concentration of tetrameric TTR in the blood exceeds that of RBP (Soprano and Blaner, 1994), thus holo-RBP is normally bound to TTR and some TTR is present unbound to RBP. The Kᵅ for retinol-RBP binding to tetrameric TTR is reported to be 0.07 μM (Noy et al., 1992). In 0.15 M NaCl, binding of RBP to TTR shows an absolute
Vitamin A metabolism, storage and tissue delivery mechanisms

requirement for retinol. The kinetics leading to the complete dissociation of the retinol–RBP–TTR complex first involves the dissociation of retinol, with a rate constant of \( 0.06/min \). Following the loss of retinol, the two proteins dissociate with a rate of dissociation of \( 0.055/h \). This slow rate of dissociation for the second dissociation step has been taken to indicate that the apo-RBP-TTR complex is an important factor in regulating serum retinol levels (Noy et al., 1992).

How retinol is taken up by cells from the circulating retinol–RBP–TTR complex has been the subject of much research interest for many years. Studies of intestinal cells imply that retinol enters by diffusion, and this is likely true for other cell types (During et al., 2002, 2005; During and Harrison, 2004, 2007). However, in 2007, a cell surface receptor for RBP termed STRA6 (product of the Stimulated by retinoic acid 6 gene) was identified (Blaner, 2007; Kawaguchi et al., 2007). STRA6 is expressed in a number of tissues/cells that have a high demand for retinoid, especially the RPE cells of the eye, but is not expressed in many others that have exceedingly important roles in vitamin A storage and metabolism, including liver cells (Bouillet et al., 1997; Blaner, 2007; Kawaguchi et al., 2007; Pasutto et al., 2007). This receptor interacts with RBP and increases cellular uptake of retinol (Kawaguchi et al., 2007). In addition, STRA6 is reported to facilitate retinol efflux from cells (Isken et al., 2008; Kim et al., 2008; Kawaguchi et al., 2012). It is proposed that both LRAT and CRBP1 couple with STRA6 within the cell (Kawaguchi et al., 2007, 2012; Amengual et al., 2012). VonLintig and colleagues have convincingly established that the functional coupling of LRAT with STRA6 increases cellular retinol uptake into tissues and have proposed that LRAT is a critical component of this process (Amengual et al., 2012). In vitro experiments have established that STRA6 facilitates retinol exchange between intracellular CRBP1 and extracellular RBP (Kawaguchi et al., 2012).

Recently, mice totally lacking STRA6 were generated and studied (Ruiz et al., 2012; Berry et al., 2013). These mice are viable and generally normal. The
primary phenotype of the \textit{Stra6}-deficient mice is a visual one, similar to the mice lacking \textit{Rbp} (Quadro \textit{et al}., 1999; Kim \textit{et al}., 2008). The results of studies of \textit{Stra6}-deficient mice suggest that there are other pathways facilitating retinol uptake into the RPE, but that the one involving STRA6 is the primary pathway in the RPE (Ruiz \textit{et al}., 2012). Outside of the RPE though, STRA6-mediated retinol uptake is not necessary for supplying target tissues with retinol (Berry \textit{et al}., 2013). In general, the absence of STRA6 seems to make very little difference in the concentrations of total vitamin A (retinol + retinyl esters) that are present in mice fed a vitamin A sufficient diet throughout life (Berry \textit{et al}., 2013). Earlier published reports proposing that STRA6 may be involved in mediating this effect of RBP on insulin signaling (Berry \textit{et al}., 2011; Berry and Noy, 2012) are further strengthened by studies of \textit{Stra6}-deficient mice (Berry \textit{et al}., 2013). Studies of these mutant mice suggest that STRA6 may function \textit{in vivo} to couple sensing of circulating RBP levels with energy metabolism and insulin-mediated responses.

B. Chylomicrons and their Remnants

In the postprandial circulation following consumption of a vitamin A-rich meal, retinyl ester concentrations can reach 5–10$\mu$M (Chuwers \textit{et al}., 1997), although this will depend directly on the quantity of vitamin A consumed in the meal. Retinyl esters in chylomicrons enter the circulation and are taken up by tissues as the chylomicron undergoes lipolysis and remodeling. Approximately 66–75\% of chylomicron retinyl ester is cleared by the liver and the remainder is cleared by peripheral tissues (Goodman \textit{et al}., 1965). Thus, 25–33\% of dietary vitamin A is delivered to peripheral tissues without first entering the liver or its stores. It has been proposed that the uptake of chylomicron vitamin A by tissues allows \textit{Rbp}$^{-/-}$ mice (Quadro \textit{et al}., 1999, 2005) and \textit{RBP}-null humans (Biesalski \textit{et al}., 1999; Seeliger \textit{et al}., 1999; Cukras \textit{et al}., 2012) to thrive, albeit without vision. This would not be possible without the regular intake of dietary vitamin A, a condition that has been common only in recent times. Nevertheless, the delivery of postprandial vitamin A to tissues is a very important pathway through which tissues acquire vitamin A.

Prior to its clearance by peripheral tissues, chylomicron retinyl ester must undergo hydrolysis. LpL is responsible for hydrolyzing chylomicron retinyl ester, facilitating the uptake of retinol by peripheral tissues (Blaner \textit{et al}., 1994). Postprandial un-esterified retinol taken up by cells is thought to bind immediately to CRBP1 that is present in most tissues (Noy and Blaner, 1991; Ong \textit{et al}., 1994b). It has been suggested that CRBP1 optimizes retinol uptake and facilitates its subsequent metabolism (Noy and Blaner, 1991; Ong \textit{et al}., 1994b). In the liver, retinyl ester hydrolysis occurs as the chylomicron remnant particle is internalized by hepatocytes during the early stages of endosome formation (Blaner and Olson, 1994; Vogel \textit{et al}., 1999). It has been proposed by Harrison that ES-10 is responsible for the hydrolysis of chylomicron remnant retinyl esters to retinol within the hepatocyte (Sun \textit{et al}., 1997; Harrison, 1998; Linke \textit{et al}., 2005). As discussed above, some of this retinol is secreted from the hepatocyte bound to RBP and the remainder is transferred to HSCs for re-esterification and storage.
C. Very Low Density Lipoprotein and Other Liver-Derived Lipoproteins

The human and rodent livers secrete some retinyl ester bound to nascent very low density lipoprotein (VLDL) and, upon metabolism of the VLDL, some of this retinyl ester can be found in low density lipoprotein (LDL) or transferred to high density lipoprotein (HDL) in species that express cholesteryl ester transfer protein. Concentrations of retinyl esters in the fasting human circulation can vary considerably but are generally in the range of 100–200 nM for individuals who do not take vitamin supplements (Relas et al., 2000; Ballew et al., 2001). Although Smith and Goodman had proposed in the 1970s that high circulating concentrations of retinyl esters are markers of vitamin A toxicity, especially hepatic toxicity (Smith and Goodman, 1976), this hypothesis is not supported by an analysis of serum retinyl ester concentrations for 6547 adults who participated in the National Health and Nutrition Examination Survey (NHANES III) (Ballew et al., 2001). Analysis of NHANES III data revealed that the prevalence of fasting serum retinyl ester concentrations that were greater than 10% of the serum total vitamin A concentration was not associated with abnormal liver function (Smith and Goodman, 1976). Thus, the presence of retinyl esters in fasting human blood is a reflection of normal vitamin A transport in the circulation.

Lipoprotein (VLDL, LDL, and HDL)-mediated delivery of retinyl esters to tissues accounts for most vitamin A delivery through the fasting circulations of dogs, accounting for 70% or more of the total vitamin A present in the fasting circulation (Schweigert, 1988; Raila et al., 2002b, 2004). These retinyl esters comprise primarily retinyl palmitate and retinyl stearate. Interestingly, after exercise, the plasma retinyl esters concentrations of sled dogs were found to nearly double, suggesting that exercise mobilizes retinyl esters, possibly from liver and/or adipose tissue (Raila et al., 2004). Retinyl esters are also the predominate vitamin A metabolite, accounting for approximately 70% of the total vitamin A present in the fasting circulation of domestic cats (Raila et al., 2001). This is also true for ferrets where lipoprotein-bound retinyl esters account for 80% or more of the total vitamin A present in the fasting circulation (Ribaya-Mercado et al., 1992; Raila et al., 2002a). The fasting circulations of chimpanzees and orangutans contain considerable retinyl esters, albeit at levels which contribute only approximately 20% of total vitamin A present in the circulation (García et al., 2006).

The role of lipoproteins in the delivery of vitamin A to tissues is not generally recognized and is completely underappreciated. Vitamin A present in VLDL, LDL, and HDL presumably is taken up along with the lipoprotein particles by their cell surface receptors but this has not been investigated.

D. Circulating Retinoic Acid

Retinoic acid is present in both the fasting and postprandial circulations, where it is bound found to albumin. Nau and colleagues have reported that immediately following consumption of a vitamin A-rich meal consisting of 100 g of turkey liver, human blood levels of all-trans-retinoic acid (atRA) can reach 80–90 nM (Arnhold et al., 1996). However, this level is quickly restored by the body to fasting levels that range from 1 to 3 nM (Kurlandsky et al., 1995; Arnhold et al., 1996;
Kane et al., 2008b; Arnold et al., 2012). The studies of Nau and colleagues (Arnhold et al., 1996) suggest that the intestine contributes significantly to the retinoic acid that is present in the postprandial circulation. The tissues responsible for contributing retinoic acid to the fasting circulation remain to be established. At present, it is unclear whether only one or a few tissues contribute to the circulating retinoic acid pool or whether retinoic acid is simply “leaking” into the fasting circulation from most or all tissues.

Plasma kinetic studies have established that circulating atRA contributes very significantly to tissue atRA pools and that the extent of this differs amongst tissues (Kurlandsky et al., 1995). Table 1.2 summarizes the extent to which different tissues rely on the circulating atRA as a source for the tissue pool. The half-life of plasma atRA in a fasted chow-fed rat is very short, with the plasma pool completely turning over every two minutes (Kurlandsky et al., 1995). All-trans-retinoic acid uptake into tissues is not presently thought to involve a cell surface receptor. It is well established experimentally that atRA can “flip-flop” across a phospholipid bilayer, and it is generally assumed that it is taken up into cells from the circulation through this process (Noy, 1992a, 1992b). This same “flip-flop” mechanism was earlier proposed to account for most un-esterified fatty acid uptake by cells across the plasma membrane from the circulation (Kamp et al., 1993). However, it is now widely accepted that several plasma membrane proteins are responsible for most un-esterified fatty acid uptake by tissues (Schwenk et al., 2010). The possibility that atRA or its other isomers also may be taken up into cells by one or more cell surface receptors has not been systematically explored and remains to be established.

### E. Other Vitamin A Metabolites

Blood concentrations of retinyl- and retinoyl-β-glucuronides were reported by Olson and colleagues to be in the range 5–15 nM (Barua and Olson, 1986; Barua et al., 1989). Although these authors have proposed that retinyl- and retinoyl-β-glucuronides, which are known to be readily hydrolyzed by a number of β-glucuronidases (Blaner

<table>
<thead>
<tr>
<th>Tissue</th>
<th>n</th>
<th>Contribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>8</td>
<td>88.4 ± 21.9b</td>
</tr>
<tr>
<td>Liver</td>
<td>8</td>
<td>78.2 ± 28.2</td>
</tr>
<tr>
<td>Kidney</td>
<td>8</td>
<td>33.4 ± 17.1</td>
</tr>
<tr>
<td>Epididymal fat</td>
<td>8</td>
<td>30.2 ± 29.9</td>
</tr>
<tr>
<td>Perinephric fat</td>
<td>8</td>
<td>24.5 ± 14.5</td>
</tr>
<tr>
<td>Seminal vesicle</td>
<td>8</td>
<td>23.1 ± 13.5</td>
</tr>
<tr>
<td>Spleen</td>
<td>5</td>
<td>19.0 ± 10.1</td>
</tr>
<tr>
<td>Epididymis</td>
<td>8</td>
<td>9.6 ± 6.1</td>
</tr>
<tr>
<td>Pancreas</td>
<td>6</td>
<td>2.3 ± 2.5</td>
</tr>
<tr>
<td>Testis</td>
<td>8</td>
<td>0.7 ± 0.9</td>
</tr>
</tbody>
</table>

*Table taken from Kurlandsky et al., 1995.

bAll values are given as mean ± S.D.
and Olson, 1994; Vogel et al., 1999), may serve as sources of retinoids for tissues, it is generally believed that these fully water-soluble metabolites are filtered in the kidney and eliminated quickly from the body.

**F. Provitamin A Carotenoids**

In addition to preformed vitamin A delivery through the circulation, provitamin A carotenoids such as β-carotene are absorbed intact by the intestine and can be found in the blood bound to chylomicrons and their remnants, VLDL, LDL, and HDL (Redlich et al., 1996, 1999). Fasting blood levels of the canonical provitamin A carotenoid β-carotene in humans, a species which absorbs carotenoids well, can be as great as 5–8 μM (Redlich et al., 1999). Rodents will only absorb dietary carotenoids when fed supraphysiological levels in the diet and, consequently, when maintained on a chow diet, carotenoids in the blood are usually very low. There is no evidence whether circulating carotenoids are specifically taken up by cells and tissues or whether they are simply taken up along with the other neutral lipids that may be present in the lipoprotein particles.

**V. INTEGRATION WITHIN THE INTACT ORGANISM OF VITAMIN A METABOLISM, STORAGE AND TRANSPORT**

In the context of the whole living organism, how is vitamin A storage and mobilization through the circulation regulated or controlled? This control must exist but presently there is no definitive answer to the question.

More than three decades ago Underwood and colleagues (Keilson et al., 1979) raised this same question. At that time, understanding of vitamin A metabolism and actions was much more limited and focused primarily on retinol and RBP and on now disproven theories regarding how vitamin A may act mechanistically in mediating its many physiologic effects. These investigators proposed that blood retinoic acid concentrations provide a feedback control mechanism that, in the absence of dietary retinoic acid, regulates mobilization and/or release of retinol bound to RBP from endogenous stores (Keilson et al., 1979). Although the specifics of this hypothesis are incorrect, the notion that retinoic acid is centrally involved in the control of vitamin A metabolism and mobilization in the whole body, we believe, is not. We would propose that the central rule governing all of vitamin A metabolism, storage, and mobilization in the body is to keep cellular retinoic acid concentrations constant at a level that is required by the specific cell type. We propose that all of vitamin A metabolism is controlled in a manner that complies with this rule, whether this be the accrual of more vitamin A stores or the mobilization of stores.

While the specific biochemical details regarding how peripheral tissues/cells communicate with the liver and other tissues where vitamin A is stored to facilitate vitamin A storage or vitamin A mobilization remain to be elucidated, the goal of this control is to assure that cellular retinoic acid concentrations remain appropriate. Within quiescent cells, the biochemical machinery focused on vitamin A is completely geared towards maintaining appropriate retinoic acid concentrations. Within proliferating or differentiating cells, the biochemical machinery is completely geared
towards generating retinoic acid at concentrations appropriate to the cell’s changing needs. At the molecular and cellular levels, these are the cardinal considerations underlying all of vitamin A metabolism.

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