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Review article

Vitamins A and E: metabolism, roles and transfer to offspring

C. Debier* and Y. Larondelle

Institut des Sciences de la Vie, Unité de Biochimie de la Nutrition, Université catholique de Louvain, Croix du Sud 2/8, B-1348 Louvain-la-Neuve, Belgium

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Vitamins A and E are essential, naturally occurring, fat-soluble nutrients that are involved in several important biological processes such as immunity, protection against tissue damage, reproduction, growth and development. They are extremely important during the early stages of life and must be transferred adequately to the young during gestation and lactation. The present article presents an overview of their biological functions, metabolism and dynamics of transfer to offspring in mammals. Among other topics, the review focuses on the biochemical aspects of their intestinal absorption, blood transport, tissue uptake, storage and catabolism. It also describes their different roles as well as their use as preventive and therapeutic agents. Finally, the mechanisms involved in their transfer during gestation and lactation are discussed.

Vitamin A: Vitamin E: Role: Metabolism: Transfer

Vitamins A and E are two essential nutrients that play very important roles in numerous biological processes. Because they are fat-soluble, they share several common mechanisms concerning their metabolism and transfer to offspring. They also exhibit some complementary roles. For example, both molecules are commonly used in the prevention or treatment of diseases such as cancer. On the other hand, several molecular aspects of their metabolism and roles are completely different. The present review describes the current knowledge and presents recent findings concerning the metabolism, roles and transfer to offspring of vitamins A and E in mammals.

The generic term 'vitamin A' includes any compound possessing the biological activity of all-*trans*-retinol. The term 'retinoids' includes both the naturally occurring forms of vitamin A and the many synthetic analogues of retinol, with or without a biological activity (Olson, 1984; Blomhoff, 1994*a*). The different forms of vitamin A found in animal tissues are retinol, retinal, retinoic acid (RA) and retinyl esters (RE; Olson, 1984; Blomhoff, 1994*a*; Fig. 1). Among the RE, the predominant one is retinyl palmitate. Retinyl oleate, stearate and linoleate are also found. The general structure of vitamin A is composed of a hydrophobic β-ionone ring and a conjugated isoprenoid lateral chain containing a polar group at its end (Fig. 1). Retinol, like its derivatives, is a hydrophobic compound that is highly unstable in the presence of O_2 (Olson, 1984; Furr *et al.* 1992; Blomhoff, 1994*a*). The main sources of vitamin A in food are dairy products, liver, eggs and fish oils. The carotenoids, or 'pro-vitamin A', can be found in fruits and green or yellow vegetables as well as in several types of oils. β-Carotene is one of the most abundant carotenoids found in the human diet and is the most potent vitamin A precursor of all the pro-vitamin A carotenoids (During & Harrison, 2004). We therefore focus only on this carotenoid molecule in the present paper.

'Vitamin E' is a generic term for compounds that qualitatively exhibit the biological activity of α-tocopherol (Bramley *et al.* 2000). The most biologically active form of vitamin E is *RRR*-α-tocopherol (formerly D-α-tocopherol), which accounts for approximately 90 % of the vitamin E found in animal tissues (Bjorneboe *et al.* 1990; Sheppard *et al.* 1992; Traber *et al.* 1992; Schmidt & Nikoleit, 1993; Bramley *et al.* 2000). The eight naturally occurring vitamin E compounds are α-, β-, δ- and γ-tocopherols and α-, β-, δ- and γ-tocotrienols (Fig. 2). All forms have a 2-methyl-6-chromanol ring attached to a hydrophobic side chain.

Abbreviations: CRABP, cellular retinoic acid-binding protein; CRBP, cellular retinol-binding protein; CYP, cytochrome P450; LPL, lipoprotein lipase; LRAT, lecithin:retinol acyltransferase; PKC, protein kinase C; RA, retinoic acid; RAR, retinoic acid receptor; RBP, retinol-binding protein; RE, retinyl ester; RXR, retinoid X receptor; SR-BI, scavenger receptor class B type I; TG, triacylglycerol; α-TTP, α-tocopherol transfer protein; TTR, prealbumin or transthyretin.

* **Corresponding author:** Dr C. Debier, fax +32 10 473728, email debier@bnut.ucl.ac.be

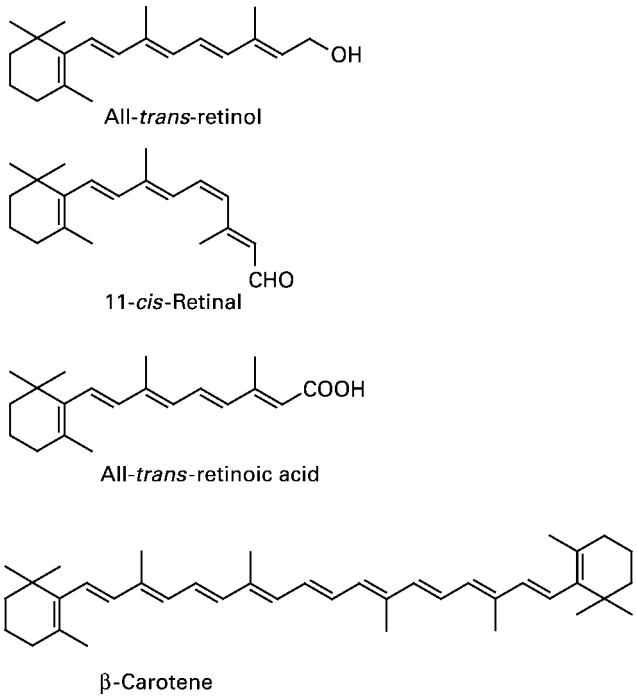
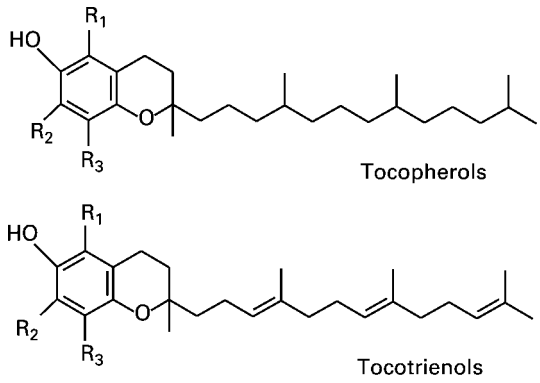


Fig. 1. The chemical structure of different forms of vitamin A.



R_1	R_2	R_3	Description
CH_3	CH_3	CH_3	α
CH_3	H	CH_3	β
H	CH_3	CH_3	γ
H	H	CH_3	δ

Fig. 2. The chemical structure of different forms of vitamin E.

The tocopherols have a saturated 16-C isoprenoid chain. In the tocotrienols, the isoprenoid side chain is unsaturated with double bonds at positions 3′, 7′ and 11′ (Ullrey, 1981; Bjorneboe *et al.* 1990; Sheppard *et al.* 1992; Schmidt & Nikoleit, 1993; Bramley *et al.* 2000). Vitamin E compounds are insoluble in water and soluble in alcohol and other organic solvents (diethyl ether, acetone and chloroform) as well as in oils. Polyunsaturated vegetable oils, cereal seeds, fatty fish, nuts, eggs, liver, dairy products and green vegetables represent dietary sources of vitamin E. As α-tocopherol represents, by far, the most common form of vitamin E in animal tissues, the metabolism detailed later refers mainly to this form.

Metabolism

Vitamin A

Absorption. Vitamin A in the diet originates either in the form of RE from animal tissues or as pro-vitamin A (mainly β-carotene) from plant tissues. Dietary RE are hydrolysed into retinol within the intestinal lumen by pancreatic esterase as well as by enzymes associated with intestinal mucosal cells or enterocytes (Olson, 1984; Blomhoff *et al.* 1991; Blomhoff, 1994*a*,*b*; Ong, 1994; Fig. 3). The uptake of retinol by enterocytes appears to occur by facilitated diffusion (Blomhoff *et al.* 1991; Blomhoff, 1994*a*,*b*). Contrary to previous belief, intestinal transport of β-carotene might be facilitated by the participation of a specific epithelial transporter (During & Harrison, 2004). The absorption efficiency of retinol and β-carotene is largely dependent on the quantity and quality of the fat present in the diet (Olson, 1984; Blomhoff *et al.* 1991; Blomhoff, 1994*a*,*b*).

In the enterocytes, retinol is bound to cellular retinol-binding protein (CRBP) II, an intracellular specific transport protein, which carries one hydrophobic molecule of retinol through the aqueous environment (Blomhoff *et al.* 1991; Blomhoff, 1994*a*,*b*; Ong, 1994). The binding to this protein allows the interaction of retinol with the appropriate enzymes for metabolism and protects it from undesired oxidation or other chemical transformations. It also protects cells from free retinol, which can disrupt the structure and function of the plasma membrane (Blomhoff *et al.* 1991; Blomhoff, 1994*a*,*b*). Retinol undergoes esterification prior to its incorporation into chylomicrons (Fig. 3). This conversion into esters is carried out by two enzymes: lecithin:retinol acyltransferase (LRAT); acyl CoA:retinol acyltransferase. The former esterifies retinol bound to CRBP II, while the latter enables the esterification of free retinol present in the cell, in the case of an important ingestion of vitamin A and saturation of CRBP II. RE can be either stored within lipid droplets inside the enterocytes or packaged into chylomicrons and exocytosed into the lymphatic system (Blomhoff *et al.* 1991; Blomhoff, 1994*b*; Ong, 1994; Fig. 3). A small part of the retinol is also metabolised to its active metabolite, all-*trans*-RA, in the intestinal cells, which is then transferred to the circulation bound to albumin (Kurlandsky *et al.* 1995; Lampen *et al.* 2000).

β-Carotene can undergo a central or a peripheral cleavage in the enterocytes. The first type of cleavage, which is the major pathway, produces two molecules of retinal. Retinal is then bound to CRBP II and reduced to retinol by the enzyme retinol dehydrogenase. The peripheral or eccentric cleavage produces β-apo-carotenals with different chain lengths (Ong, 1994; During & Harrison, 2004). Some intact β-carotene can also be transported to the peripheral tissues via chylomicrons (Blomhoff *et al.* 1991; Blomhoff, 1994*a*; During & Harrison, 2004). The proportion of β-carotene intestinal cleavage varies from one

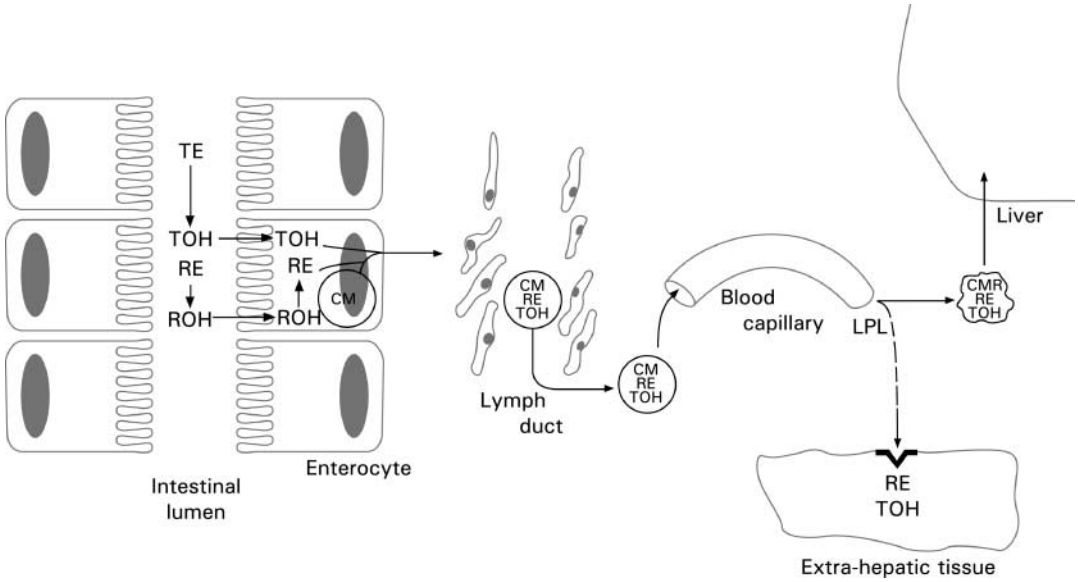


Fig. 3. Intestinal absorption. Tocopheryl esters (TE) and retinyl esters (RE) are hydrolysed in the intestinal lumen and absorbed in the alcohol form (tocopherol, TOH; retinol, ROH) by enterocytes. TOH is incorporated unesterified in chylomicrons (CM) whereas ROH is first esterified to fatty acids before being packed into CM. CM reach the circulation by way of intestinal lymph. Chylomicron remnants (CMR) are formed under the action of lipoprotein lipase (LPL), which hydrolyses triacylglycerols. During that process, some of the TOH and RE associated to CM are transferred to peripheral tissue. However, the majority remains in CMR, which are cleared by the liver.

species to another. In rodents, most of the β-carotene is converted in the intestine; in man, a greater extent of the cleavage takes place in the liver (During & Harrison, 2004).

Storage. Chylomicrons containing RE are secreted from enterocytes and move into the general circulation where they are partially degraded by lipoprotein lipase (LPL; Blomhoff *et al.* 1991; Blaner *et al.* 1994; Blomhoff, 1994*a*; Figs 4 and 5). The major part of the RE remains in the chylomicrons during their conversion into chylomicron remnants (Blomhoff *et al.* 1991; Blomhoff, 1994*a,b*). However, during that process, a small amount of RE can also be taken up by extra-hepatic tissues such as the lungs, kidneys, adipose tissue, spleen, skeletal muscle and bone marrow (Blaner *et al.* 1994; Fig. 5). Chylomicron remnants are taken up by the liver, along with RE, through a mechanism

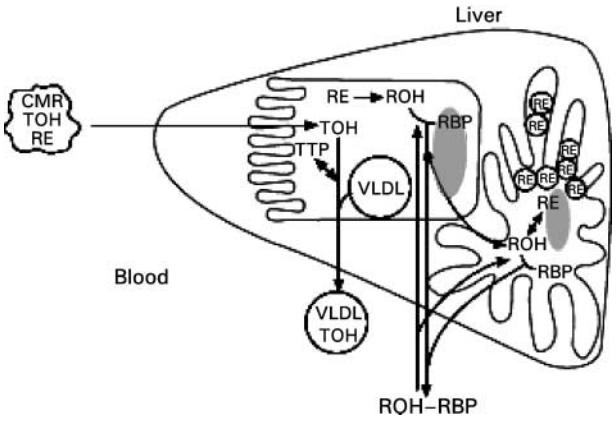


Fig. 4. Storage. Chylomicron remnants (CMR) containing retinyl esters (RE) and tocopherol (TOH) are taken up by liver parenchymal cells. RE are hydrolysed to retinol (ROH), which binds to its transport protein, the retinol-binding protein (RBP). The ROH–RBP complex is secreted into the circulation or transported into stellate cells where it is esterified and stored as RE. Stellate cells may also secrete ROH–RBP into the circulation. Inside the liver, α-tocopherol is preferentially incorporated into VLDL, owing to the selective binding to a specific cellular transport protein, the tocopherol transport protein (TTP).

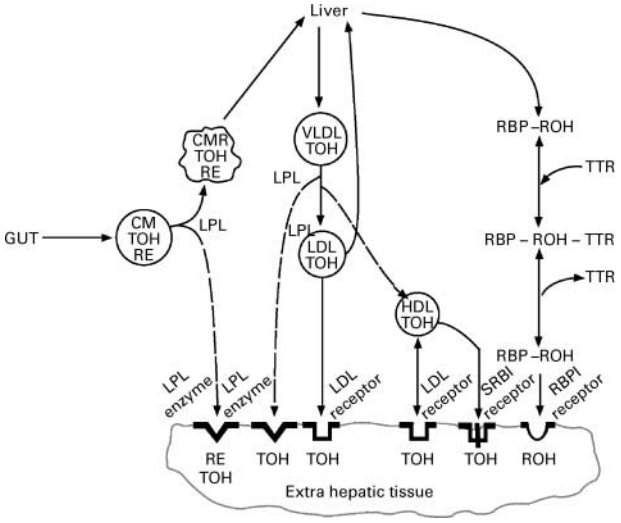


Fig. 5. Blood transport and tissue uptake. Absorbed tocopherol (TOH) and retinyl esters (RE) circulate associated to chylomicrons (CM). A small part is incorporated into extra-hepatic tissue following the action of lipoprotein lipase (LPL), while the liver clears the remaining chylomicron remnants (CMR). Liver α-tocopherol is secreted into the circulation in association with VLDL, which are also metabolised by LPL. During this lipolysis process, some of the TOH is transferred to peripheral tissues or to HDL. The rest of the TOH ends up in LDL and is further transferred to peripheral tissues as well as liver parenchymal cells through the LDL receptor pathway. Liver retinol (ROH) is secreted into the circulation bound to its specific transport protein, the retinol-binding protein (RBP). The RBP–ROH complex is reversibly complexed with transthyretin (TTR). ROH is taken up by tissue by means of cellular surface receptors specific to RBP.

involving LDL receptors and chylomicron remnant receptors as well as LPL (Blomhoff *et al.* 1991; Blomhoff, 1994*a*,*b*).

A great proportion of the RE synthesised in the enterocytes is stored in the liver. In this organ, only the parenchymal and stellate cells are involved in the metabolism of vitamin A. RE are hydrolysed to retinol at the plasma membrane of the parenchymal cells or hepatocytes (Blomhoff *et al.* 1991; Blomhoff, 1994*a*,*b*; Wake, 1994; Fig. 4). Retinol is transferred into the endoplasmic reticulum where it is associated with another specific protein, retinol-binding protein (RBP; Olson, 1984; Blomhoff *et al.* 1991; Blomhoff, 1994*a*,*b*). RBP functions as the plasma transport protein for vitamin A. Retinol bound to RBP, or holo-RBP, can be either secreted directly into the circulation or transferred to liver stellate cells for storage, via a RBP receptor (Blomhoff *et al.* 1991; Blomhoff, 1994*a*,*b*; Wake, 1994; Fig. 4). Under normal conditions (no vitamin A deficiency), stellate cells contain more than 80 % of the vitamin A present in the liver (Blomhoff *et al.* 1985). When retinol enters the stellate cells, it binds to CRBP I and is esterified by LRAT into mostly long-chain RE such as retinyl palmitate, stearate, oleate and linoleate. CRBP I is indispensable for proper delivery of retinol to LRAT. A deficiency in CRBP I results in a sharp decrease of RE accumulation in stellate cells and a waste of retinol (Ghyselinck *et al.* 1999). Also, the expression of LRAT appears to be regulated by all-*trans*-RA (Ross & Zolfaghari, 2004). These esters are then stored in cytosolic lipid droplets (Yost *et al.* 1988; Blomhoff *et al.* 1991; Randolph *et al.* 1991; Blomhoff, 1994*a*,*b*; Fig. 4). More than 98 % of the retinoids recovered from stellate cells are RE (Blomhoff *et al.* 1985). They remain in this state until circulating retinol concentrations begin to decline (Blomhoff, 1994*a*,*b*).

Part of the β-carotene delivered to the liver is incorporated into VLDL that are secreted into the circulation (Blomhoff *et al.* 1991; Blomhoff, 1994*a*,*b*). β-Carotene is further transformed into retinoids in extra-hepatic organs. The other part is directly cleaved in the liver to produce retinol molecules that are introduced in the metabolism of retinoids. As stated earlier, in man, the cleavage of β-carotene takes place mainly in the liver whereas in rodents, only small amounts of β-carotene reach and are cleaved in this organ (During & Harrison, 2004).

Vitamin A is also stored in various extra-hepatic organs. In most mammalian species, the quantity of vitamin A stored in extra-hepatic tissues is much lower than in the liver (Wake, 1994). However, adipose tissue also contains substantial stores of retinoids (retinol and RE). In rats, it has been shown that all adipose depots could account for 15–20 % of the total body retinoid stores (Bonet *et al.* 2003). Marine mammals such as seals also store a large proportion of retinoids in their blubber (40–66 % total body reserves), as a result of the high amount of adipose tissue in these animals as well as its elevated vitamin A content (Schweigert *et al.* 1987; Käkelä *et al.* 1997; Mos & Ross, 2002). Adipose tissue plays an active role in retinoid homeostasis and metabolism. It can take up retinol from the circulation, store it as RE, convert it into RA or mobilise it back to the circulation (Bonet *et al.* 2003). CRBP I is present in most cell types; it serves as an intracellular transporter for retinol and protects it from the cellular environment. At the same time, it also protects the cell membranes from free retinol that could cause cell damage (Bellovino *et al.* 2003).

It is important to note that α-tocopherol is able to affect the metabolism of vitamin A in several tissues and may play a role in tissue retinol homeostatis. It has been shown to modulate the levels of retinol and total vitamin A in tissues such as the liver, kidney and intestine. *In vitro*, α-tocopherol exerts an inhibiting or stimulating action, depending on the tissue, on retinyl palmitate hydrolysis (Napoli *et al.* 1984).

Blood transport. RE stored in the stellate cells of the liver are converted into retinol by retinyl ester hydrolase and retinol is transferred to the parenchymal cells, where it is bound to RBP and released into the circulation. Retinol can also be released directly into the circulation from the stellate cells, which are also able to synthesise RBP (Blomhoff *et al.* 1991; Blomhoff, 1994*a*,*b*; Fig. 4). The mobilisation of vitamin A from the liver is a highly regulated process. Over 90 % of total plasma retinol is transported into the blood circulation bound to RBP (1:1 mol/mol; Olson, 1984; Blomhoff *et al.* 1991; Blomhoff, 1994*a*,*b*; Sivaprasadarao & Findlay, 1994). Liver parenchymal cells are the primary site of RBP synthesis. Approximately 95 % of RBP is associated (1:1 mol/mol) with another transport protein, also produced in the liver, namely, prealbumin or transthyretin (TTR; Raz & Goodman, 1969; Olson, 1984; Blomhoff *et al.* 1991; Blomhoff, 1994*a*,*b*; Sivaprasadarao & Findlay, 1994; Fig. 5). This large protein is involved in the blood transport of thyroxine, a thyroid hormone (Raz & Goodman, 1969; Sivaprasadarao & Findlay, 1994). The interaction of TTR with thyroxine appears to be independent of the RBP–TTR interaction (Raz & Goodman, 1969). The binding to TTR prevents the glomerular filtration of the relatively small RBP–retinol complex (RBP: 19–21 kDa, TTR: 40–60 kDa). It also increases the affinity of RBP for retinol (Raz & Goodman, 1969; Olson, 1984; Noy & Xu, 1990; Blomhoff *et al.* 1991; Blomhoff, 1994*a*,*b*; Green & Green, 1994; Bellovino *et al.* 2003). Both TTR and RBP regulate the concentration of retinol in blood and ensure a constant supply to the dependent cells (Green & Green, 1994).

Accordingly, plasma retinol concentrations usually remain relatively constant over a wide range of dietary intakes and liver stores. If plasma retinol concentration begins to decline, dietary vitamin A absorbed by the liver is secreted directly into the circulation bound to RBP. In the case of an excessive drop in plasma retinol concentrations, vitamin A stored within the liver stellate cells is quickly mobilised.

Although RBP is synthesised abundantly in hepatocytes, its expression has also been detected in other tissues such as the kidney, ovary, testis and adipose tissue (Bellovino *et al.* 2003; Bonet *et al.* 2003). Retinol can also bind to other plasma proteins such as albumin; however, its avidity for retinol is much lower than that of RBP (Noy & Xu, 1990). Albumin rather serves as a transport protein for RA, which circulates in very small levels in the blood (<1 % plasma retinol; Goodman, 1984; Kurlandsky *et al.*

1995). Contrary to other mammals, several carnivore species (especially *Canidae* and *Mustelidae*) transport an important part of vitamin A in their serum in the form of RE (40–90 % of the total; Schweigert *et al.* 1990). In several canine species, these esters are bound to VLDL, LDL and HDL, suggesting that they originate from the liver instead of the intestine. Retinol levels seem, however, to remain stable and RBP is present, which means that these carnivore species are homeostatically regulated as are other species (Raila *et al.* 2000).

No specific transport protein exists for carotenoids in serum. Since they are also fat-soluble, they appear to be carried in the bloodstream in association with VLDL (Blomhoff, 1994*a*,*b*).

Tissue uptake. At the sites of vitamin A uptake, which include virtually every tissue of the body (lung, intestine, adrenal gland, kidney, epithelial tissue, adipose tissue, eye, muscle and testis), retinol is believed to enter the cell by passive diffusion or by way of a RBP receptor (Blomhoff *et al.* 1991; Blomhoff, 1994*b*; Fig. 5). Several tissues are known to express specific cell-surface receptors that recognise RBP. The exact mechanism of transfer of retinol to the cell is still controversial and may vary from one tissue to another. It seems that the binding of RBP to its specific receptor is obligatory for the subsequent delivery of retinol to the cell (Sivaprasadarao & Findlay, 1988*b*). Moreover, several studies have suggested that free RBP, rather than complexed to TTR, binds to the receptor (Sivaprasadarao & Findlay, 1988*a*,*b*). The debate remains concerning the structure of the RBP receptor, as well as whether RBP is endocytosed or delivers retinol via a 'pore-like' receptor (Flower, 2000). Endocytosis of the RBP–receptor complex has been suggested in the liver and the kidney, among others (Flower, 2000). The subsequent release of retinol from the complex would occur in the lysosomes/endosomes. Noy & Xu (1990) also suggested that retinol spontaneously dissociates from its binding site on RBP outside the cell, and is passively transferred through the plasma membrane. Another possibility would be that the RBP receptor acts as a channel receptor, delivering retinol to the cell without RBP. The resulting apo-RBP (retinol-free) would then be recycled or eliminated by the kidneys (Sivaprasadarao & Findlay, 1994; Flower, 2000). Sundaram *et al.* (1998) suggested that the RBP receptor directs the transfer of retinol from extracellular RBP to intracellular CRBP. According to the tissue, retinol is either transformed into RE and stored in lipid droplets (adipose tissue) or activated into RA or retinal (eyes, lungs). Cellular retinoic acid-binding protein (CRABP) types I and II bind RA and mediate its distribution into the cell (see p. 160; Bellovino *et al.* 2003).

As already mentioned, uptake of RE via the chylomicrons is also a way of tissue uptake, both in the liver and in several extra-hepatic tissues (Blomhoff *et al.* 1991; Blomhoff, 1994*a*,*b*). For example, in adipose tissue, which contains substantial stores of retinol and RE, LPL is able to facilitate the delivery of retinol to the cells (Blaner *et al.* 1994; Bonet *et al.* 2003). Two mechanisms have been suggested: LPL increases the internalisation of RE carried in the core of lipoproteins and/or LPL catalyses the hydrolysis of RE into retinol, which is then transferred into the cell (Blaner *et al.* 1994).

Finally all-*trans*-RA, which circulates in the blood in small amounts bound to albumin, can be transferred into the tissues by passive diffusion. This transfer seems, however, to be cell type- and tissue-specific. For example, it appears to be very efficient in the brain and the liver, whereas in the testis, very little RA derives from the circulation. This might possibly result from the presence of enzymes that are able to rapidly metabolise circulating RA and prevent its entry into certain testicular cells (Kurlandsky *et al.* 1995).

Isomerisation and catabolism. A number of metabolic pathways have been described, which may be tissue- or species-specific.

The oxidation of retinol into retinal is a reversible reaction. It is catalysed by three enzyme families: alcohol dehydrogenases; short-chain dehydrogenase; several members of the cytochrome P450 (CYP) family, including CYP1A2, 1B1, 1A1, 3A4, 3A5 and 2D6 (Chen *et al.* 2000; Marill *et al.* 2003). This reaction is considered as being rate-limiting. On the other hand, the conversion of retinal into RA is irreversible and is catalysed by an aldehyde dehydrogenase as well as by members of the CYP family, including CYP1A1, 1A2, 1B1, 2C8, 2C9, 3A4 and 3A7 (Zhang *et al.* 2000; Marill *et al.* 2003).

All-*trans*-RA can then be metabolised by reactions including isomerisation, decarboxylation and glucuronidation. Metabolites of all-*trans*-RA include 13-*cis*-RA, 9-*cis*-RA, retinoyl β-glucuronide, 5,6-epoxy RA, 4-hydroxy RA, 4-oxo RA, 3,4-didehydro RA and 18-OH RA (Clagett-Dame & DeLuca, 2002; Klaassen & Braakhuis, 2002; Marill *et al.* 2003). Some of these metabolites are biologically active while others are just catabolic products (Klaassen & Braakhuis, 2002; Marill *et al.* 2003). An important route of RA metabolism is through hydroxylation of the molecule at position 4 of the cyclohexenyl ring to form 4-hydroxy RA (Klaassen & Braakhuis, 2002). A new member of the P450 family, CYP26, has recently been identified as an RA-inducible enzyme that possesses strong 4-hydroxylation activity for RA (Marikar *et al.* 1998; Lampen *et al.* 2000; Klaassen & Braakhuis, 2002). It is expressed in several tissues, including the liver, heart and intestine (Marill *et al.* 2003). Its expression is regulated by its substrate, all-*trans*-RA, through the activation of RA receptors (see p. 160; Marill *et al.* 2003). This CYP family functions to limit the levels and thus the biological activity of RA (Marikar *et al.* 1998). The CYP26 family has three members: CYP26A1; B1; C1 (Nebert & Russell, 2002; Marill *et al.* 2003). CYP26A1 and B1 are highly specific for the hydroxylation of all-*trans*-RA. On the other hand, they are less effective at metabolising 9-*cis* and 13-*cis* forms. CYP26 appears to play an important role in early development of the embryo (Marill *et al.* 2003). This RA-inducible cytochrome is down-regulated in the liver in the case of a vitamin A deficiency and up-regulated in a dose-dependent manner by dietary vitamin A or exogenous RA (Ross & Zolfaghari, 2004). Other members of the CYP family, among others CYP1A1, 1A2 and 3A, are also involved in the metabolism of RA (Lampen *et al.* 2000; Marill *et al.* 2003). Each RA stereo-

isomer is preferentially metabolised by a specific set of CYP (Marill *et al.* 2003). All-*trans*-RA and its oxidised metabolites can also undergo glucuronidation (Marill *et al.* 2003). It is important to note that many CYP isoforms involved in the metabolism of retinoids are also important enzymes in the metabolism of drugs and contaminants, which may result in significant drug–retinoid or contaminant–retinoid interactions.

Vitamin E

Absorption. Dietary vitamin E is present as tocopherol and tocopheryl esters (Traber *et al.* 1992; Bramley *et al.* 2000; Stahl *et al.* 2002). The most common dietary tocopherols are α- and γ-tocopherol. As for retinyl esters, tocopheryl esters are hydrolysed into tocopherol within the intestinal lumen by pancreatic esterase as well as by intestinal enzymes (Traber *et al.* 1992; Bramley *et al.* 2000; Stahl *et al.* 2002; Fig. 3). The uptake of tocopherol by enterocytes appears to occur by passive diffusion (Bjorneboe *et al.* 1990; Traber *et al.* 1992; Bramley *et al.* 2000; Stahl *et al.* 2002). As for vitamin A, the absorption efficiency of α-tocopherol is largely dependent on the quantity and quality of the fat present in the diet (Bjorneboe *et al.* 1990; Bramley *et al.* 2000). γ-Tocopherol seems to be absorbed with the same efficiency as α-tocopherol (Traber *et al.* 1992; Bramley *et al.* 2000; Stahl *et al.* 2002). However, subsequent loss of γ-tocopherol is observed, apparently due to discrimination by the liver (see p. 158). On the other hand, β- and δ-tocopherol are poorly absorbed (Bramley *et al.* 2000).

Unlike retinol, α-tocopherol is not re-esterified during the absorption process, which does not appear to require any cellular transfer protein. α-Tocopherol is incorporated into chylomicrons within the enterocyte, which are transported from the intestine along the lymphatic pathway to reach the bloodstream (Bjorneboe *et al.* 1990; Traber *et al.* 1992; Bramley *et al.* 2000).

A diet high in vitamin A appears to decrease the bioavailability of vitamin E (Sklan & Donoghue, 1982; Eicher *et al.* 1997; Nonnecke *et al.* 1999; Ametaj *et al.* 2000). According to Sklan & Donoghue (1982), the mechanism may involve enhanced oxidation of dietary tocopherols in the digestive tract. However, these results were found for chicks and should be confirmed in mammals. Dietary vitamin A might also antagonise intestinal absorption of vitamin E (Combs, 1976). Retinol may be in competition with α-tocopherol for either enzymes or mechanisms of cellular uptake (Eicher *et al.* 1994).

Blood transport. In contrast to vitamin A, there is no specific carrier protein in the serum to transport vitamin E. It circulates, in its alcohol form (tocopherol), in serum lipoproteins, together with other lipids, and in erythrocytes. All four main classes of lipoprotein (chylomicrons, VLDL, LDL and HDL) contain α-tocopherol (Bjorneboe *et al.* 1990; Traber *et al.* 1992; Schmidt & Nikoleit, 1993; Bramley *et al.* 2000). However, its distribution among these lipoproteins appears to vary between species (Bjorneboe *et al.* 1990; Schweigert, 1990; Traber *et al.* 1992). In rats, the highest concentrations of α-tocopherol are found in HDL followed by VLDL (Traber *et al.* 1992). In man, LDL and HDL are the main carriers of tocopherol (Traber *et al.* 1992). In cows, HDL followed by LDL is the major lipoprotein involved in α-tocopherol transport (Schweigert, 1990; Herdt & Smith, 1996). The percentage of distribution appears to be VLDL 2, LDL 17 and HDL 77, with only 3 % of the plasma vitamin, which is not associated to lipoproteins (Herdt & Smith, 1996). Plasma α-tocopherol levels are very well correlated with plasma lipid and cholesterol levels. α-Tocopherol and cholesterol are indeed distributed in equal proportions among lipoprotein fractions. Affecting serum lipoprotein concentrations will thus tend to affect serum vitamin E concentration. However, serum lipoprotein concentration is not the only factor dictating vitamin E concentrations. Otherwise, vitamin E supplementation would not be able to raise vitamin E levels in dairy cows, independently of serum lipoprotein concentrations (Herdt & Smith, 1996). α-Tocopherol seems to be localised both at the amphiphilic surface and in the hydrophobic core of the lipoproteins (Traber *et al.* 1992).

Liver α-tocopherol is secreted into the bloodstream within VLDL (Fig. 5). LDL, which are produced during the degradation of VLDL by LPL, still contain the major part of α-tocopherol. They can be taken up both by the liver and also the extra-hepatic tissues via LDL receptors (Bjorneboe *et al.* 1990; Bramley *et al.* 2000; Fig. 5). Endogenous lipoproteins (VLDL and LDL) containing apo-B can exchange α-tocopherol with HDL (Mardones & Rigotti, 2004). HDL and LDL thus ultimately bear the largest proportion of α-tocopherol in plasma, at least in man (Traber *et al.* 1992).

Some γ-tocopherol can also be found in the blood, usually at a five- to ten-fold lower level than α-tocopherol, despite the fact that several diets are richer in γ-tocopherol. This phenomenon is due to discrimination between the two forms that occur in the liver (see p. 158; Bjorneboe *et al.* 1990; Traber *et al.* 1992; Traber & Arai, 1999). In marine mammal species, such as seals, only α-tocopherol has been found in serum (Engelhardt *et al.* 1975).

Storage. A cellular binding protein seems to exist in the liver and some other tissues. It has been suggested that this protein, called α-tocopherol transfer protein (α-TTP), enables the transfer of α-tocopherol between membranes and is involved in the incorporation of α-tocopherol into nascent VLDL (Sato *et al.* 1991; Traber *et al.* 1992; Schmidt & Nikoleit, 1993; Hosomi *et al.* 1997; Traber & Arai, 1999; Bramley *et al.* 2000; Stahl *et al.* 2002). It therefore plays an important role in determining plasma vitamin E level. It appears that only *RRR*-α-tocopherol is able to bind this protein with high affinity (Traber *et al.* 1992; Schmidt & Nikoleit, 1993; Traber & Arai, 1999; Bramley *et al.* 2000). γ-Tocopherol has much less affinity for α-TTP (9 % *v.* 100 % for *RRR*-α tocopherol) while α-tocopheryl acetate (2 %) and quinone (2 %) are almost ineffective (Hosomi *et al.* 1997). By means of α-TTP, the liver thus appears to make the discrimination between α- and γ-tocopherols and other forms of vitamin E, which is not the case in intestine cells (Traber *et al.* 1992; Schmidt & Nikoleit, 1993; Traber & Arai, 1999; Bramley *et al.* 2000). γ-Tocopherol is thus excreted in bile while α-tocopherol is preferentially retained. This is probably one of the reasons why *RRR*-α-tocopherol is

the most biologically active form of vitamin E (Schmidt & Nikoleit, 1993; Hosomi *et al.* 1997; Traber & Arai, 1999; Bramley *et al.* 2000; Stahl *et al.* 2002). Indeed, the biological activity of vitamin E is dependent on its delivery to the tissues. Reductions in the binding capacity or affinity of α-TTP will limit the secretion of the various forms of vitamin E into lipoproteins and the subsequent delivery of vitamin E by these lipoproteins to tissues (Hosomi *et al.* 1997). It has been shown in rats that the expression of α-TTP is influenced by dietary α-tocopherol (Azzi *et al.* 2002; Stahl *et al.* 2002).

About 75 % of the hepatic α-tocopherol content is stored in parenchymal cells whereas only 25 % is confined in non-parenchymal cells (stellate cells, Kupffer cells and endothelial cells; Bjorneboe *et al.* 1990). Skeletal muscle and adipose tissue have the capacity to accumulate α-tocopherol. Together with the liver, these tissues contribute 90 % of the total amount of α-tocopherol in the body (Bjorneboe *et al.* 1990). Adipose tissue has been found to contain the majority of the tocopherols in the body (Bramley *et al.* 2000). The mobilisation of α-tocopherol from this tissue in response to a dietary deficiency is, however, very slow (Bjorneboe *et al.* 1990).

In addition to α-TTP, a family of cellular tocopherol-associated proteins has been identified recently and appears to be present in all cell types. Tocopherol-associated proteins seem to be involved in the transport of tocopherol inside the cell. These proteins may possibly also play a role in the transport of a number of other lipophilic molecules in competition with α-tocopherol (Azzi *et al.* 2002).

Tissue uptake. Because it is fat-soluble and carried by lipoproteins, α-tocopherol can be transferred to the tissues through pathways by which lipids are delivered. The uptake varies considerably among tissues; it is very rapid in the liver, lung, spleen, kidney and erythrocytes. On the other hand, it occurs at very slow rates in the brain and adipose tissue. Several mechanisms of transfer have been identified.

Some exchange mechanisms can occur in the circulation. This pathway concerns mainly erythrocytes, which lack both lipoprotein receptors and the LPL pathway. They thus depend on spontaneous transfer from lipoproteins for adjustment of their α-tocopherol concentration (Traber *et al.* 1992; Bramley *et al.* 2000). However, the transfer of vitamin E between lipoproteins is not mediated by neutral lipid (triacylglycerols (TG) and cholesterol esters) transfer, which occurs through the action of the neutral lipid transfer protein (Granot *et al.* 1988). The phospholipid transfer protein, which facilitates the movement of phospholipids between HDL and lipoproteins containing apo-B, is also capable of mediating the transfer of α-tocopherol between lipoproteins as well as its uptake by endothelial cells (Mardones & Rigotti, 2004).

LPL is involved in the cellular uptake of α-tocopherol. This enzyme catalyses the hydrolysis of TG in chylomicrons and VLDL into glycerol and fatty acids, which are then transferred to the cell. LPL also facilitates the exchange of lipids between different lipoprotein classes. In addition, LPL can act as a cell-surface bridge for lipoproteins and promote the lipoprotein receptor-mediated process. Traber *et al.* (1985) have shown that tocopherol is transferred to cells during the hydrolysis of TG by the action of LPL *in vitro*. The enzyme must bind to the cell membrane to allow proper intracellular tocopherol transfer, acting as a 'bridging' molecule. Quite surprisingly, the uptake of α-tocopherol from HDL seems to be significantly improved in the presence of LPL, although this lipoprotein class is very poor in TG. This phenomenon supports the fact that it is mainly the 'bridging function' of LPL rather than its TG lipolysis activity that is important for α-tocopherol transfer (Mardones & Rigotti, 2004). Tissues capable of synthesising and secreting LPL, such as the skin, adipose tissue and muscle, may thus obtain tocopherol by this mechanism (Traber *et al.* 1992; Schmidt & Nikoleit, 1993; Bramley *et al.* 2000; Mardones & Rigotti, 2004). Moreover, the catabolism of chylomicrons through the LPL pathway represents the most likely way for extra-hepatic tissues to take up other forms of vitamin E. Thus, this pathway may explain why adipose tissue contains a high proportion of γ-tocopherol (Stahl *et al.* 2002). A novel role for LPL has also been recently discovered, concerning the delivery of α-tocopherol at the blood–brain barrier. An *in vitro* study using porcine brain capillary endothelial cells has shown that the uptake of LDL–α-tocopherol at this barrier can be selectively enhanced in the presence of LPL, revealing a relevant role for the enzyme in vitamin E supply to the brain (Goti *et al.* 2002).

α-Tocopherol can also be transferred to tissues through the LDL receptor pathway, which binds lipoproteins containing apo-B and apo-E (Traber & Kayden, 1984; Thellman & Shireman, 1985; Traber *et al.* 1992; Schmidt & Nikoleit, 1993, Bramley *et al.* 2000). Several studies have, however, shown that LDL receptor-deficient animals do not exhibit signs of vitamin E deficiency. This suggests that, even if the LDL receptor is important for α-tocopherol uptake, it is not essential, probably because of its redundancy with the other pathways (Mardones & Rigotti, 2004).

HDL, which play a central role in reverse cholesterol transport from peripheral tissues to the liver, can also enable the uptake of α-tocopherol by tissues (Traber *et al.* 1992; Bramley *et al.* 2000). A receptor called the scavenger receptor class B type I (SR-BI) has been identified in mice and seems to play an important role in the cellular uptake of α-tocopherol through its selective binding to HDL. The expression of this receptor in certain types of pneumocyte indicates that HDL is the main source of α-tocopherol in pulmonary tissues (Kolleck *et al.* 1999). The high α-tocopherol concentrations in the adrenal gland may also be due to specific binding of HDL and subsequent uptake of α-tocopherol (Stahl *et al.* 2002). Other studies suggest that HDL, by means of this selective receptor pathway, provide a significant source of α-tocopherol for the central nervous system. The occurrence and significance of this receptor in other mammal species, such as man, remain to be investigated (Mardones & Rigotti, 2004).

Finally, the VLDL receptor, which binds lipoproteins containing apo-E, is expressed in various tissues, such as adipose tissue, muscle, heart, kidney and placenta (Gafvels *et al.* 1994; Wittmaack *et al.* 1995). Its significance regarding vitamin E tissue uptake has never been investigated. However, this receptor may perhaps play a role in α-tocopherol tissue uptake in mammals such as rodents, for which

a significant part of vitamin E is transported in the circulation bound to VLDL.

Catabolism. It appears that a considerable amount of the vitamin E ingested (varying between 30 and 70 %) is not absorbed and is excreted in the faeces (Bramley *et al.* 2000). Moreover, when large amounts of vitamin E are administered, much is excreted in bile, which accounts for its relative safety in the case of excess as compared with vitamins A and D (Bjorneboe *et al.* 1990; Schmidt & Nikoleit, 1993; Bramley *et al.* 2000). Via the antioxidant function of vitamin E (see p. 163), it is transformed into a hydroquinone that can be conjugated with glucuronic acid, secreted into the bile and excreted in the faeces (Bjorneboe *et al.* 1990; Schmidt & Nikoleit, 1993). The urinary route, with the excretion of α-tocopheronic acid and α-tocopheronolactone, represents less than 1 % of the absorbed vitamin E (Bramley *et al.* 2000).

Roles

Vitamin A

Vitamin A plays a central role in many essential biological processes such as vision, immunity, reproduction, growth and development (Olson, 1984; Blomhoff, 1994*a*; Blomhoff & Smeland, 1994; Napoli, 1999). The main active form of vitamin A is RA. It is involved in immunity and reproduction as well as in growth and development (Eskild & Hansson, 1994; Maden, 1994; Ross & Gardner, 1994; Napoli, 1999). Retinol seems to have an important role to play in reproduction while retinal is essential for vision (Thompson *et al.* 1963; Olson, 1984; Chew, 1993; Blomhoff, 1994*a*; Eskild & Hansson, 1994).

Mechanism of action of RA. Cellular RA can be obtained from either the conversion of retinol to retinal and then to RA or direct uptake from the blood circulation (Ross & Gardner, 1994; Napoli, 1994, 1999; Kurlandsky *et al.* 1995; see p. 157). There are two biologically active isomers of RA: all-*trans*-RA and 9-*cis*-RA. RA binds to the specific cytosolic proteins CRABP I and II (Blomhoff, 1994*a*; Ross & Gardner, 1994; Napoli, 1999). CRABP I is expressed almost ubiquitously whereas the expression of CRABP II occurs almost exclusively in the skin (Li & Norris, 1996). The RA–CRABP complex is then translocated to the nucleus where RA binds to retinoid-dependent nuclear receptors. Two types of these receptors exist: retinoic acid receptors (RAR) and retinoid X receptors (RXR). These receptors belong to the superfamily of nuclear hormone receptors and act as ligand-activated transcription factors (Clagett-Dame & DeLuca, 2002; Bonet *et al.* 2003; Marill *et al.* 2003). These receptors present a specific affinity for some isomeric forms of RA; RAR is responsive to all-*trans*-RA and 9-*cis*-RA, while RXR is responsive only to 9-*cis*-RA (Clagett-Dame & DeLuca, 2002; Bonet *et al.* 2003; Marill *et al.* 2003). Three subtypes of RAR and RXR (α, β, δ) have been described in mammalian tissues. They are encoded by different genes and show different developmental expressions (Bonet *et al.* 2003). Once activated by their ligands, RAR bind, as RAR–RXR heterodimers, to specific DNA target sequences called retinoic acid response elements, which are located in the promoter region of target genes. This binding leads to changes (activation or inhibition) in gene expression that mediate biological effects (Kastner *et al.* 1994; Ross & Gardner, 1994; Napoli, 1999; Clagett-Dame & DeLuca, 2002; Bonet *et al.* 2003; Marill *et al.* 2003). Although most of the effects of RAR are thought to be mediated mainly by RAR–RXR heterodimers, when activated by 9-*cis*-RA, RXR can also act as a homodimer on transcription activation via the retinoic X response element. RXR can also act as a heterodimer with other nuclear receptors such as the thyroid hormone receptor or the vitamin D receptor on their specific response elements (Clagett-Dame & DeLuca, 2002; Bonet *et al.* 2003; Marill *et al.* 2003). Several hundred genes are induced or repressed by retinoids. It is through this alteration of gene expression that RA regulates a variety of essential functions such as growth, development, immune function and reproduction. In general, experiments with RAR and RXR knockout mice have been very useful to bring to the fore the many roles played by vitamin A.

Retinoids not only regulate transcription via the activation of specific retinoid receptors. They can also form a covalent bond with some proteins (retinoylation), which can modify the properties of the target protein and thus its activity (Marill *et al.* 2003). For example, they appear to suppress the activity of activator protein 1, which mediates the signal for growth factors and tumour promoters (Klaassen & Braakhuis, 2002).

Vision. The active form of vitamin A in vision is retinal, which is derived from circulating retinol and RE. RA is inactive in the vision process (Zile & Cullum, 1983). Retinal is essential for vision in darkness as well as for colour perception. It is situated in the photoreceptors of the retina. Two types of photoreceptor are present in the retina: rhodopsins and iodopsins (Olson, 1984). The former are situated in the rods and are involved in vision in dim light. The latter are present in cones and are involved in colour perception and vision in bright light. These two receptors are composed of a molecule of 11-*cis*-retinal covalently bound to a protein, opsin (Zile & Cullum, 1983; Pepe, 1999). In the presence of light, 11-*cis*-retinal is converted to all-*trans*-retinal (Olson, 1984). This conformational change of the vitamin A molecule leads to hydrolysis of the linkage between retinal and opsin, causing catalytic activation of the apoprotein (Pepe, 1999). Its photoexcited state (metarhodopsin II) can activate the GTP-binding protein transducin, which in turn activates a phosphodiesterase specific for cGMP. This enzyme hydrolyses cGMP into 5′-GMP, causing closure of the ionic channels. This phenomenon ends with a hyperpolarised electrical signal on the plasma membrane that leads to neurotransmission (Lamb, 1986; Pugh & Lamb, 1990; Pepe, 1999). All-*trans*-retinal is then reduced to all-*trans*-retinol, which is isomerised to 11-*cis*-retinol by a retinol isomerase. A retinol dehydrogenase finally oxidises 11-*cis*-retinol to the corresponding retinal isomer. Once in darkness, 11-*cis*-retinal combines again to opsin and the complex is regenerated (Zile & Cullum, 1983; Olson, 1984; Pepe, 1999; Kuksa *et al.* 2003; Lamb & Pugh, 2004). Some vitamin A molecules are lost during this cycle. It is therefore necessary to have a continuous supply of all-*trans*-retinol via the blood to generate

a sufficient amount of pigments. Night blindness and double vision are precursory signs of hypo- and hypervitaminosis A, respectively (Basu & Dickerson, 1996).

Reproduction. An adequate supply of vitamin A is essential to ensure good functioning of the reproductive system. Vitamin A deficiency causes infertility or impaired reproduction (Zile & Cullum, 1983; Eskild & Hansson, 1994; Clagett-Dame & DeLuca, 2002). The oestrus cycle appears to be disrupted and the vagina becomes permanently keratinised (Bates, 1983). The most characteristic results of vitamin A deficiency are fetal resorption, stillbirth and congenital malformations (Bates, 1983; Clagett-Dame & DeLuca, 2002). Vitamin A plays an essential role in female reproduction from implantation of the embryo to birth of a viable neonate. Even though RA is necessary for proper offspring development, retinol appears to be required, in addition to RA, for successful gestation and to avoid fetal resorption (Thompson *et al.* 1963; Chew, 1993; Wellick & DeLuca, 1995; Wellick *et al.* 1997; Clagett-Dame & DeLuca, 2002). RA and retinol also appear to have a stimulating effect on the production of progesterone (Bates, 1983; Chew, 1993). In males, retinol and RA are involved in the regulation of testicular function; a deficiency induces cessation of spermatogenesis and adversely affects testosterone production (Thompson *et al.* 1963; Chew, 1993; Eskild & Hansson, 1994; Livera *et al.* 2002). The effect of RA on spermatogenesis appears to occur mainly through the binding to RAR (Akmal *et al.* 1997; Gaemers *et al.* 1998). Retinoids seem to exert an action on the three main types of testicular cell (Sertoli, germinal and Leydig) (Livera *et al.* 2002). The effects of retinoids on fetal and adult testis appear to be opposed. Indeed, retinol and RA play a negative role in steroidogenic activity during the differentiation of rat fetal Leydig cells (Livera *et al.* 2002).

Growth and development. During gestation, vitamin A is transferred to the fetus through the placenta. This supply is essential, as retinoids are involved in growth and cellular differentiation of the fetus (Zachman, 1995). Several experiments using retinoid receptor (RAR or RXR) knockout mice as well as RA receptor antagonists or dietary manipulations have provided a better understanding of when and for what developmental processes vitamin A is needed. Even though, as mentioned earlier, retinol is the only form of vitamin A that supports reproduction in full, all-*trans*-RA appears to be the most important form for proper embryonic development (Clagett-Dame & DeLuca, 2002). Vitamin A has an essential role in the development of organs such as the lungs (Zachman, 1995; Massaro *et al.* 2000; Mendelson, 2000; Cardoso, 2001; Biesalski & Nohr, 2003), heart (Mendelsohn *et al.* 1999; Ross *et al.* 2000) and skeleton (Yamaguchi *et al.* 1998). Among others, the surprisingly high content of retinyl palmitate in fetal lung and its depletion after birth testify to the high retinol need during lung development (Zachman *et al.* 1984). RA also enables the settting up of vascular and nervous systems (Ross *et al.* 2000; Maden, 2001; Colbert, 2002). Both cytoplasmic and nuclear classes of retinoid-binding protein (CRBP, CRABP and RAR, RXR) are expressed early in development and are proposed to control the concentration of RA and the transcription of retinoid responsive genes, respectively (Ross & Gardner, 1994). The control occurs by differential expressions of retinoid receptors as well as by time-dependent changes of vitamin A metabolism via cellular vitamin A-binding proteins such as CRBP and CRABP (Biesalski & Nohr, 2003). RA is involved as a morphogenic agent during embryonic development. It has been proposed to act as a signalling molecule in limb development and anteroposterior body axis patterning. The concept of an asymmetric distribution of RA appears to be responsible for establishing positional information (Maden, 1994; Ross & Gardner, 1994; Means & Gudas, 1995; Stoilov, 2001). The molecular mechanism seems to be based on the expression of RA-synthesising and RA-degrading enzymes. During the development of the anteroposterior axis in the early chicken embryo, the posterior region, which exhibits high concentrations of RA, expresses retinal dehydrogenase (which catalyses the irreversible oxidation of retinal to RA). On the other hand, the anterior region, which is characterised by low RA concentration, expresses a CYP enzyme from the CYP26 family that is capable of degrading RA to oxidised metabolites (Stoilov, 2001). RAR regulate many developmental control genes, including homeobox genes and growth factor genes (Means & Gudas, 1995). Multiple fetal anomalies occur in vitamin A-deficient animals as well as in RAR knockout mice. Transplacental transfer is tightly regulated by the homeostasis of the mother. Hypovitaminosis A may occur in the case of maternal deficiency. Congenital abnormalities of the limbs, vertebral column, heart, ocular tissues, respiratory and cardiovascular systems, and abnormal segmentation of the embryo, can be observed (Ross & Gardner, 1994; Zachman, 1995; Ross *et al.* 2000). An excess of vitamin A also induces this type of abnormality. The importance and location of the problem depend on the period of gestation and the duration of the excessive or deficient supply (Bates, 1983; Ross *et al.* 2000; Stoilov, 2001). RXRα appears to mediate the teratogenic effects of RA in several parts of the body. Indeed, RXRα knockout mice given a teratogenic dose of RA during gestation exhibit a decreased rate of teratogenicity compared with wild-type animals (Stoilov, 2001).

Immunity. The interest in vitamin A as an immunoregulator has followed the observed susceptibility of vitamin A-deficient animals to infection, resulting from depressed humoral and cellular immunity (Blomhoff & Smeland, 1994). The immune regulation function is carried out by all-*trans*- and 9-*cis*-RA (Semba, 1998). These molecules play a central role in regulating the development, differentiation and apoptosis of immune cells, which is required for the good functioning of innate and adaptive immunity. In the skin, these RA maintain the integrity of keratinocytes and determine the number of Langerhans cells. They also modulate the expression of lactoferrin (Semba, 1998). Moreover, RA play a role in the regeneration of mucosal barriers damaged by infection (Stephenson, 2001). Vitamin A deficiency impairs both humoral and cell-mediated immunity (Blomhoff & Smeland, 1994; Semba, 1998). The different symptoms of a deficiency are, on the one hand, impairment in the functioning of lymphocytes, natural killer cells and neutrophiles and, on the other, decrease

in cell proliferation and antibody production (Blomhoff & Smeland, 1994; Semba, 1998; Stephenson, 2001). All this results in an increased risk of infection, longer diseases, as well as higher child mortality (Semba, 1998). The molecular mechanisms by which vitamin A affects immune function have not been carefully examined (Stephensen, 2001). Supplementation of the elderly with vitamin A and β-carotene (among others) may be indicated to improve their immune function (Mitchell *et al.* 2003).

Epithelial cells. Vitamin A is essential for cellular differentiation, which explains its role in maintaining the integrity of the genital, gastrointestinal and respiratory epithelia (McLaren, 1984; McCullough *et al.* 1999; Biesalski & Nohr, 2003). It also plays a major role in the regulation of keratinocytes from the eye (McLaren, 1984; McCullough *et al.* 1999). RA controls the regular differentiation of epithelial cells as a ligand for RAR and RXR (Biesalski & Nohr, 2003). In general, vitamin A deficiency induces problems for epithelial integrity of the eye, respiratory tract, gastrointestinal tract and reproductive tract (McCullough *et al.* 1999). It is responsible for a loss of ciliate cells and a stop on the production of mucus. A simple columnar epithelium will become pseudostratified (trachea), stratified (vagina) or stratified keratinising (epidermis; Rosenthal *et al.* 1994; McCullough *et al.* 1999). Pulmonary infections will be more frequent due to keratinisation of the mucous cells. In the small intestine, a loss of cells and microvilosities can be observed. Ulcerations and scars on the cornea, as well as eye keratinisation, are also among the observed symptoms (McLaren, 1984).

Antioxidant function. Carotenoids do seem to have a separate function as antioxidants in addition to their provitamin A function (Stahl *et al.* 1994). Recently, antioxidant activity has also been attributed to retinol (Palace *et al.* 1999). It therefore shares, to some extent, a common role with vitamin E. The antioxidant activity of vitamin A and carotenoids is conferred by the hydrophobic chain of polyene units that can quench singlet O and stabilise peroxyl radicals (Stahl *et al.* 1994; Palace *et al.* 1999). In general, the longer the polyene chain, the greater is the polyene's radical stabilising activity. The antioxidising activity of the different forms of vitamin A has been ranked as retinol = retinal >> retinyl palmitate > RA (Palace *et al.* 1999). The secret of extreme longevity appears to be due, in part, to the preservation of normal vitamin A and vitamin E serum values, combined with a positive correlation between these two nutrients that seems to protect centenarians against oxidative stress (Basile *et al.* 2003).

Effect on lipid metabolism. RA enhances whole-body thermogenic capacity through the transcriptional activation of uncoupling proteins, expressed in brown adipocytes and involved in heat generation (Villarroya *et al.* 1999; Bonet *et al.* 2003). It also plays a role in the development and function of adipose tissue, by reducing adipogenesis and promoting apoptosis of fat stores (Bonet *et al.* 2003). For these reasons, retinoids offer potential for research on the treatment of obesity and type II diabetes (Villarroya *et al.* 2004). However, other studies have shown that retinoids may cause hyperlipidaemia. Indeed, the levels of plasma vitamin A appear to be positively correlated to those of circulating TG, especially in hyperlipidaemic subjects (Ulukaya & Tokullug, 1999). The mechanisms seem to occur through a decrease of lipoprotein catabolism that ensues from a decrease in LPL activity. RA is also involved in regulation of the expression of fatty acid desaturases, which are involved in a wide range of physiological processes including fetal growth and development, reproduction, cell differentiation and immune and inflammatory responses (Zolfaghari & Ross, 2003).

Because of their various properties, vitamin A and its derivatives are used in the prevention or treatment of several pathologies.

(1) Through the regulation of cell growth and apoptosis. Because of their effects on cell growth regulation and differentiation, retinoids are frequently and successfully used as therapeutic agents for the treatment of several cancers (Yang *et al.* 1999; Hansen *et al.* 2000; Schwartz, 2000; Zhang *et al.* 2000; Hammond *et al.* 2002; van Moorselaar & Voest, 2002; Kalvakolanu, 2004). The greatest success with RA treatment has been obtained with acute promyelocytic leukaemia, for which complete remission is observed for the majority of patients because of the differentiation of leukaemic blasts (Huang *et al.* 1988; Parthasarathy & Mehta, 1998; Hansen *et al.* 2000; Costoya & Pandolfi, 2001; Zhu *et al.* 2001; Salih & Kiener, 2004). The inhibitory action of RA on carcinogenesis appears to be exerted through the inhibition of cell proliferation and migration and the induction of cell death by apoptosis (Yang *et al.* 1999; Hansen *et al.* 2000; Zhang *et al.* 2000; Simoni & Tolomeo, 2001; van Moorselaar & Voest, 2002; Kalvakolanu, 2004).
(2) Through antimutagenic effects. Retinol and retinal have been shown to cause a decrease in the mutagenicity of genotoxic compounds. The mechanism is believed to result from the non-selective inhibition of separate cytochrome isoforms (Odin, 1997; Edenharder *et al.* 1999).
(3) Through anti-inflammatory effects. Vitamin A also plays a role as an anti-inflammatory agent through the action of RA. Its supplementation may be beneficial in several inflammatory conditions such as skin disorders and bronchopulmonary dysplasia. The mechanisms by which RA regulate the expression of genes involved in the inflammatory process remain to be determined (Reifen, 2002).
(4) Through antioxidant properties. Because of their antioxidant properties, retinoids may have a protective effect on the oxidant-mediated mechanisms of some pathological processes (Morcos & Tomita, 1996). For example, retinol, in association with α-tocopherol, exerts a protective effect against the toxicity of ochratoxin A. Indeed, the cytotoxicity of this mycotoxin is a result mainly of the oxidative stress it induces (Baldi *et al.* 2004). Also, β-carotene and retinoids can inhibit hepatocarcinogenesis through the selective modulation of the antioxidant defence system combined to xenobiotic detoxification in the liver (Bishayee *et al.* 2000). However, some caution should be taken concerning the use of retinoids or β-carotene as preventing

or therapeutic agents. Indeed, when retinol is used at concentrations greater than the physiological limit, it can induce oxidative stress (Gimeno *et al.* 2004). Likewise, β-carotene may also cause problems. This nutrient is one of the most studied cancer chemopreventive agents because of either its antioxidant activity or its ability to be converted into vitamin A. However, recent studies in man have shown unexpected and paradoxical results. Supplementation with high doses of β-carotene resulted in increased lung cancer incidence in several populations, mainly those exposed to environmental mutagens in industrial settings and carcinogens in, for example, cigarette smoke (Paolini *et al.* 2003; Russell, 2004). The exact mechanism is not completely known, but it seems to occur through the induction of several CYP enzymes, resulting in increased bioactivation of procarcinogens, the destruction of RA and the enhancement of cell proliferation (Paolini *et al.* 2003; Russell, 2004). The β-carotene metabolites resulting from its eccentric cleavage may also facilitate the binding of smoke-derived carcinogens to DNA (Russell, 2004).

Vitamin E

The principal form of vitamin E, α-tocopherol, accounts for most of the fat-soluble chain-breaking antioxidant activity in mammalian tissues and plasma (Packer, 1991; Bramley *et al.* 2000). It prevents or minimises specific diseases associated with free radical damage such as CVD, chronic inflammation and cancer (Packer, 1991). Like vitamin A, vitamin E also plays an important role in the development of the immature immune system of the newborn (Peplowski *et al.* 1980; Hayek *et al.* 1989; Babinszky *et al.* 1991; Nemec *et al.* 1994; Rajaraman *et al.* 1997; Kolb & Seehawer, 1998), as well as in maintaining the immune system in general (Meydani & Tengerdy, 1992; Bramley *et al.* 2000). Novel properties of vitamin E, which are independent of its antioxidant function, have also been discovered recently (Azzi *et al.* 2002).

Antioxidant property. The biological activity of vitamin E is generally believed to be due to its antioxidant function. α-Tocopherol indeed accounts for most of the lipid-soluble, chain-breaking antioxidant activity in mammalian tissues and plasma, and protects PUFA in cellular membranes and lipoproteins from oxidation into hydroperoxides (Ullrey, 1981; Packer, 1991; Liebler, 1992; Niki & Matsuo, 1992; Serbinova *et al.* 1992; Bramley *et al.* 2000). Vitamin E inhibits lipid peroxidation primarily by trapping propagating peroxyl radicals ($LOO^{\cdot}$) through the following reactions:

$$LOO^{\cdot} + LH \rightarrow LOOH + L^{\cdot} \quad \text{(chain propagation)} \qquad (1)$$

$$LOO^{\cdot} + \alpha\text{-}TH \rightarrow LOOH + \alpha\text{-}T^{\cdot} \quad \text{(chain inhibition)} \qquad (2)$$

The antioxidant function of vitamin E is located in the chromanol nucleus, where the phenolic hydroxy group donates an H atom to quench lipid radicals. It thus becomes an α-tocopheroxyl radical (α-$T^{\cdot}$; reaction (2)). However, contrary to fatty acids, the α-tocopheroxyl radical is fairly stable, because the unpaired electron of the O atom is delocalised throughout the aromatic ring. The rate constant of reaction (2) is indeed up to three orders of magnitude higher than that of the peroxyl radical propagation (reaction (1)). Thus, the α-tocopheroxyl radical does not react with membrane PUFA, and propagation of the chain reaction is inhibited.

The presence of at least one methyl group on the aromatic ring is also critical. The more methyl groups, the higher is the biological activity. α-Tocopherol, which contains three methyl groups, is the most biologically active of all homologues, followed by β-, γ- and δ-tocopherols. The lateral chain is also very important for the dynamics of transport and retention of the molecule within the membranes; consequently, any modification induces an important decrease in biological activity. α-Tocopherol can be regenerated from the tocopheroxyl radicals by cellular reductants, including ascorbic acid, via the reaction:

$$\alpha\text{-}T^{\cdot} + \text{electron donor}_{red} + H^{+} \rightarrow \alpha\text{-}TH + \text{electron donor}_{ox} \qquad (3)$$

Tocopheroxyl radicals that do not complete this redox reaction may also react instead with peroxyl radicals to form other-non radical products, such as the tocopheryl quinone or the epoxy quinone:

$$LOO^{\cdot} + \alpha\text{-}T^{\cdot} \rightarrow \text{non-radical products} \qquad (4)$$

The hydroperoxides produced during the propagation phase can finally break down to aldehydes, ketones, alcanes or other products that may bind to or disrupt cellular macromolecules such as DNA and proteins or induce inflammatory reactions.

By preventing free radical-mediated tissue damage to cellular lipids, vitamin E is believed to play a key role in delaying the pathogenesis of a variety of degenerative diseases such as CVD, cancer, inflammatory diseases, neurological disorders, cataract and age-related cell degeneration (Packer, 1991; Borek, 1992; Bramley *et al.* 2000).

Effect on immunity. Vitamin E appears to be essential for normal function of the immune system (Meydani & Tengerdy, 1992; Bramley *et al.* 2000). Vitamin E deficiency has been shown to impair immune response and supplementation with higher than recommended dietary levels enhances humoral and cell-mediated immunity. It also plays an important role in the development of the immature immune system of the newborn (Peplowski *et al.* 1980; Hayek *et al.* 1989; Babinszky *et al.* 1991; Wuryastuti *et al.* 1993; Nemec *et al.* 1994; Rajaraman *et al.* 1997; Kolb & Seehawer, 1998). Leucocytes are very rich in polyunsaturated phospholipids prone to oxidative destruction. Tocopherol could affect the immune system through its antioxidant function either by decreasing reactive oxygen metabolites and/or by altering the formation of arachidonic acid metabolites, both of which have been shown to suppress immune responsiveness (Meydani & Tengerdy, 1992). The immunostimulatory effect of tocopherol cannot however be fully explained by its antioxidant function, because other antioxidants do not produce similar actions (Meydani & Tengerdy, 1992). Similar to vitamin A, supplementation of vitamin E may have a boosting effect on the immune function of the elderly (Knight, 2000; Mitchell *et al.* 2003). On the other hand, some antagonistic effects

between vitamin A and vitamin E in terms of their effects on the immune system have been described (Kubena & McMurray, 1996). This phenomenon may occur through the suppression of gastrointestinal absorption of vitamin E by vitamin A.

Regulation of cell proliferation and gene expression. New, non-antioxidant roles of vitamin E have recently been brought to the fore. Among others, α-tocopherol appears to play a role in the inhibition of cell proliferation in several cell types as well as in gene expression. Cell proliferation inhibition seems to result from the inhibiting effect that vitamin E exerts on protein kinase C (PKC). α-Tocopherol is able to activate protein phosphatase 2A, which causes the dephosphorylation and inactivation of PKC (Azzi *et al.* 2002). It is through this action that tocopherol is able to modulate the cellular transport of RA. Indeed, Gimeno *et al.* (2004) have shown that the treatment of human cultured fibroblasts with tocopherol (α, β and δ), in the presence of retinol, induces increased expression of CRABP II. The mechanism seems to be as follows. RXRα is involved in the induction of CRABP II and can be phosphorylated by PKC. Through the inactivation of PKC, tocopherols induce a dephosphorylation of RXRα and thus an increase in the amount of CRABP II. α-Tocopherol can also inhibit the aggregation of human platelets by a mechanism dependent on PKC inhibition. α-Tocopherol is able to modulate the expression of several proteins such as collagenase and liver α-TTP (Azzi *et al.* 2002). In smooth muscle cells as well as in monocyte/macrophages, it can down-regulate the expression of the oxidised LDL scavenger receptors. These effects occur in a PKC-independent way (Azzi *et al.* 2002).

Like vitamin A, vitamin E is regularly used as a chemopreventive and chemotherapeutic agent.

(1) In vascular diseases. Vitamin E plays a role in the prevention of CVD by preventing the oxidative modification of LDL (Packer, 1991; Frei & Ames, 1992; Bramley *et al.* 2000). It has a pronounced stimulatory influence on the formation of prostaglandin E from arachidonic acid. In particular cases, vitamin E supplementation decreases the progression of established atherosclerosis by suppressing oxidative and inflammatory reactions (Morcos & Tomita, 1996; Cyrus *et al.* 2003). However, it seems that the role of vitamin E against atherosclerosis is not only due to its antioxidant function (Ozer & Azzi, 2000). Indeed, as just described, α-tocopherol also exerts an antiproliferative effect on smooth muscle cells as well as an inhibiting action on human platelet aggregation through the inhibition of PKC. The fact that β-tocopherol, which is almost as potent an antioxidant as α-tocopherol, does not have any inhibiting effect on PKC activity and smooth cell proliferation shows that the mechanism involved is not related to the radical scavenging properties of these two molecules (Ozer & Azzi, 2000; Azzi *et al.* 2002). Some tocotrienols appear to be very effective hypocholesterolaemic agents and are also very effective in decreasing atherogenic apo-B as well as lipoprotein plasma levels (Qureshi & Qureshi, 1992; Theriault *et al.* 1999).

(2) In cancers. Vitamin E may have different roles in the prevention and treatment of cancer. Its roles in cell cycle control, differentiation and DNA damage repair are not clearly understood (Sung *et al.* 2003). Its inhibiting effect on PKC in many cells may explain some of its anti-tumour effects (Azzi *et al.* 2002). Vitamin E seems to be useful in protecting the liver and possibly other organs against the toxicity and carcinogenicity of several compounds, such as *N*-nitrosamines and polycyclic aromatic hydrocarbons, through the inhibition of CYP (Sheweita *et al.* 2001). α-Tocopherol also exhibits antimutagenicity properties, especially in cases where the mutagenic agent is a free radical. As an example, α-tocopherol is very effective against radiation, which acts by generating free radicals from water or biomolecules (Odin, 1997). α-Tocopheryl succinate can be very useful as an adjunct to standard cancer therapy by increasing tumour responses and possibly decreasing some of the toxicity to normal cells (Prasad *et al.* 2003). Vitamin E can protect the organism against carcinogenesis and tumour growth and decrease the toxicity of several anticancer therapies, probably through its antioxidant properties and immunomodulating functions (Das, 1994). However, inconsistent findings have been shown regarding the role of antioxidants in cancer prevention and some epidemiological studies have been inconclusive (Kimmick *et al.* 1997). Several authors have pointed out that the protective effect of a combination of many nutrients found in fruits and vegetables may be different from the administration of one or two single nutrients. Therefore, a diet rich in fruits and vegetables appears to be better than the consumption of specific antioxidant supplements (Lee, 1999). However, contrary to vitamin A, which can exert adverse affects if administered in high doses, vitamin E supplementation is safe (Pryor, 2000).

Transfer to offspring

Vitamins A and E are of utmost importance during the early stage of life. Both nutrients are transferred to the offspring by limited placental transfer during gestation as well as by the ingestion of milk produced by the lactating mother during the neonatal period. Despite their paramount importance, little is known concerning the placental transfer and mammary gland uptake of these essential nutrients.

Vitamin A

Gestation. Vitamin A is provided to the fetus through a limited and tightly controlled placental transfer (Moore, 1971; Ismadi & Olson, 1982; Bates, 1983; Ross & Gardner, 1994). The amount of retinol provided to the fetus is usually maintained constant until maternal stores are almost completely depleted (Vahlquist & Nilsson, 1979; Ismadi & Oslon, 1982; Pasatiempo & Ross, 1990; Ross & Gardner, 1994).

The exact mechanism of transfer remains unknown but it seems to involve RBP receptors. Indeed, a receptor for RBP has been characterised in the human placenta (Sivaprasadarao & Findlay, 1994). However, some studies have recently suggested that RBP might be dispensable for retinol transfer, as homozygous RBP null mutant mice are viable and fertile (Clagett-Dame & DeLuca, 2002). Moreover, contrary to what was suggested earlier, maternal RBP does not cross the placenta and cannot enter the fetal circulation (Quadro *et al.* 2004). In man, apo-RBP (retinol-free) concentration appears to be elevated during pregnancy, suggesting that pregnancy may alter the affinity of RBP for retinol or induce the binding of the vitamin to other, still uncharacterised proteins (Sapin *et al.* 2000). Moreover, in man and sheep, maternal and fetal RBP–retinol seem to be complexed to a protein other than TTR (Donoghue *et al.* 1982; Sklan *et al.* 1985).

Vitamin A can also be taken up at the placental barrier in the form of RE packed in lipoproteins (Quadro *et al.* 2004). In the case of RBP null mutant mice, RE may compensate for the absence of retinol–RBP during gestation (Quadro *et al.* 2004). RA is also transferred from the maternal to the fetal compartment. All-*trans*-RA reaches the embryo to a much greater extent than does any of its *cis* isomers (Tzimas *et al.* 1994; Nau, 1995; Sass *et al.* 1999). The reason for this is not clear. Differential binding affinities to proteins may exist and influence the transport of the RA forms into the embryonic compartment (Nau, 1995). It is important to note that the placental transfer of RA isomers varies from one species to another. For example, 13-*cis*-RA exhibits very limited placental transfer in rats and mice whereas the transfer is much higher in primates. This is one reason why 13-*cis*-RA appears to be a more potent teratogen in monkeys and man than in rodents (Hummler *et al.* 1994; Nau, 1995, 2001).

At the end of gestation, the fetus starts to synthesise RBP and accumulate more vitamin A in its liver (Bates, 1983; Vahlquist & Nilsson, 1984; Sklan *et al.* 1985; Ross & Gardner, 1994; Böhles, 1997). Mammalian fetuses are, however, characterised by lower liver stores than adults because placental transfer as well as liver vitamin A storage starts only during the last third of gestation (Bates, 1983; Davila *et al.* 1985; Ross & Gardner, 1994; Cardona-Perez *et al.* 1996).

Initiation of all-*trans*-RA synthesis starts early during gestation. The enzyme catalysing the oxidation of retinal into RA, aldehyde dehydrogenase, plays an important role in generating all-*trans*-RA as null mutant embryos die *in utero*. However, the development of null mutant embryos can be rescued by the inclusion of large amounts of all-*trans*-RA in the maternal diet (Clagett-Dame & DeLuca, 2002).

Because β-carotene is transported by lipoproteins as is vitamin E, the mechanism of its placental transfer may be compared with that of α-tocopherol (see p. 166). Its transfer efficiency through the placental membrane appears to be much less effective than that of retinol (Sapin *et al.* 2000). This may be due to differences in the mechanisms of transfer, which are related to lipoprotein metabolism in the case of β-carotene (Sapin *et al.* 2000). The importance of vitamin A during fetal development may explain its relatively high transfer rate from maternal to fetal blood as compared with β-carotene (Böhles, 1997; Sapin *et al.* 2000).

Neonatal period. Because of the limited transplacental transfer, mammal newborns have low stores of vitamin A (Ismadi & Olson, 1982; Davila *et al.* 1985; Pehrson *et al.* 1990; Hidiroglou *et al.* 1993*a*,*b*; Njeru *et al.* 1994; Olivares, 1995; Chen *et al.* 1996; Böhles, 1997; Mahan & Vallet, 1997; Léger *et al.* 1998; Oostenbrug *et al.* 1998; Kiely *et al.* 1999; Sapin *et al.* 2000; Debier *et al.* 2002*a*). The neonate thus relies strongly on milk consumption to establish proper tissue stores, maintain rapid growth and harmonious development, and develop its immune system (Blomhoff, 1994*a*).

Tissue levels change greatly after colostrum and milk consumption. For example, in rats, liver concentrations, which are very low at birth, increase significantly after milk ingestion and are greatly affected by maternal dietary supplementation (Davila *et al.* 1985). However, the timing of colostrum ingestion seems to play a role in the efficiency of intestinal vitamin absorption. In calves, it has been shown that colostrum must be ingested very quickly after birth adequately to increase circulating concentrations of retinol and β-carotene (Blum *et al.* 1997; Zanker *et al.* 2000), suggesting that colostrum feeding on the day of birth is essential for the establishment of absorptive mechanisms allowing intestinal transport of fat-soluble vitamins (Blum *et al.* 1997). However, although delaying colostrum ingestion for 12 h impairs plasma retinol and β-carotene concentrations, it does not appear negatively to influence the hepatic vitamin A concentration of the young (Zanker *et al.* 2000).

Because of the low vitamin A status at birth (Moore, 1971; Ismadi & Olson, 1982; Ross & Gardner, 1994; Chen *et al.* 1996; Oostenburg *et al.* 1998; Debier *et al.* 2002*a*), colostrum has an important role to play in providing initial protection to the newborn against deficiency. It usually contains higher vitamin A concentrations than milk (Stowe, 1982; Chappell *et al.* 1985; Syvaoja *et al.* 1985; Loudenslager *et al.* 1986; Ostrea *et al.* 1986; Donoghue, 1988; Hidiroglou, 1989; Schweigert, 1990; Boersma *et al.* 1991; Meneses *et al.* 1994; Böhles, 1997; Schweigert & Gottwald, 1999; Zanker *et al.* 2000; Macias & Schweigert, 2001; Schweigert *et al.* 2004). The same is true for β-carotene (Chappell *et al.* 1985). A higher vitamin A concentration in colostrum is not encountered in all mammal species, however. In marine mammals such as the grey seal, milk vitamin A levels increase at the end of lactation, which may be due to the fact that, unlike most other mammals, seal females fast during lactation (Debier *et al.* 2002*a*). The increase appears to result from the concentration of vitamin A in lipid body stores and from a higher mobilisation rate of blubber vitamin A at late lactation (Debier *et al.* 2002*a*).

Contrary to what could be expected, fat-soluble vitamin A does not follow the milk lipid profile, which increases as lactation progresses (Macias & Schweigert, 2001; Debier *et al.* 2002*a*). Differences in blood transport and mammary gland uptake may be at the origin of these discrepancies.

Whereas blood contains 95 % of vitamin A in the RBP–retinol form, milk is characterised by 95–100 % of its

vitamin A in the RE form, with retinyl stearate, palmitate and oleate being the most important ones (Ross, 1982). Free retinol is esterified inside the mammary gland before secretion of RE into the milk (Tomlinson *et al.* 1974; Vahlquist & Nilsson, 1979; Ross, 1982; Bates, 1983). Retinol esterification seems to be regulated by a different mechanism than in the liver. Indeed, neither CRBP nor LRAT is present in substantial levels in the mammary gland; rather acyl CoA:retinol acyltransferase may be the most important enzyme involved in the process of retinol esterification in the mammary gland (Randolph *et al.* 1991; Blomhoff, 1994*a*,*b*).

Vitamin A sources of the mammary gland comprise RE delivered by lipoproteins (chylomicrons) and blood retinol. However, RBP–retinol seems to be transferred preferentially from blood to milk compared with circulating RE. As RBP–retinol does not vary over a wide range of vitamin A ingestion, uptake by the mammary gland does not vary with higher ingestion. By contrast, RE packed in chylomicrons act directly on the vitamin A content of the milk, as they are taken up by the mammary gland (Moore, 1971; Pasatiempo & Ross, 1990; Ross & Gardner, 1994; Green *et al.* 2001; Ross *et al.* 2004). LPL in lactating mammary tissues may be responsible for hydrolysis of chylomicron-derived RE and allow uptake of retinol by the gland (Green *et al.* 2001). Green *et al.* (2001) speculate that, during lactation, a large proportion of dietary vitamin A is directed to the mammary gland rather than to the liver, as opposed to what occurs in the non-lactating state. The uptake of vitamin A from chylomicrons into mammary tissues via the action of LPL thus explains why milk vitamin A concentrations may vary even when plasma retinol levels are unchanged (Davila *et al.* 1985; Green *et al.* 2001; Ross *et al.* 2004).

A slight decrease in maternal circulating vitamin A levels is usually noticed at late gestation–early lactation (Stowe, 1982; Bates, 1983; Dostalova, 1984; Goff & Stabel, 1990; Schweigert, 1990; Jensen *et al.* 1999, Debier *et al.* 2002*a*). This decrease is likely to be the consequence of a higher uptake of circulating vitamin A by the mammary gland (probably due to an increase of mammary receptors for RBP–retinol) for the formation of colostrum (Schweigert, 1990). β-Carotene concentrations in maternal serum also decrease at late gestation. Its transfer into colostrum may occur through a specific transport system for LDL into the secretory cells, as described later for vitamin E (Schweigert, 1990).

Vitamin E

Gestation. As for vitamin A, placental transfer of vitamin E is limited, and the fetus is characterised by low circulating vitamin E concentrations (Sinha & Chiswick, 1992; Hidiroglou *et al.* 1993*a*,*b*; Njeru *et al.* 1994; Olivares, 1995; Böhles, 1997; Mahan & Vallet, 1997; Léger *et al.* 1998; Kiely *et al.* 1999). The transfer efficiency and fetal levels remain low even if the dietary vitamin E intake by the gestating mother becomes higher and increases her serum vitamin E levels (Sinha & Chiswick, 1992; Pazak & Scholz, 1996; Mahan & Vallet, 1997; Léger *et al.* 1998). It has been suggested that this low placental transfer may be due to the inefficient transfer of plasma lipids (Pazak & Scholz, 1996).

A steady increase in the level of α-tocopherol is observed throughout fetal life; the younger the fetus, the lower the tissue levels. Like vitamin A, vitamin E also accumulates in the fetus mainly throughout the last third of pregnancy (Böhles, 1997; Chan *et al.* 1999). This accumulation seems to correlate with the increase in fetal lipid mass during that period (Böhles, 1997). Indeed, in man, the amount of adipose tissue increases from less than 1 % of body weight during the first two trimesters to approximately 16% of body weight at term. The vitamin E body status of the fetus is therefore expected to be higher at late pregnancy (Sinha & Chiswick, 1992). Both LPL activity and VLDL and LDL receptor pathways in placental cells are involved in placental lipid transport especially at late pregnancy (Bonet *et al.* 1992, 1995; Wittmaack *et al.* 1995). They may play a role in the transfer of vitamin E. In rodents, both the LDL receptor and the HDL receptor SR-BI (see p. 7 of proofs) may be involved in the transfer of vitamin E to the fetus (Wyne & Woollett, 1998).

The fetal accumulation of vitamin E seems to vary according to the tissue. For example, in rats, net placental transfer of vitamin E to the fetal liver is very low. By contrast, vitamin E is preferentially incorporated into the heart and lung (Pazak & Scholz, 1996). The placental barrier also appears able to discriminate between the different forms of vitamin E: *RRR*-α-tocopherol is preferentially transferred compared with synthetic all-racemic α-tocopherol (Acuff *et al.* 1998; Schenker *et al.* 1998; Hidiroglou *et al.* 2001; Lauridsen *et al.* 2002). Also, the alcohol form is transported better than the ester acetate form (Schenker *et al.* 1998). α-TTP, the cytosolic protein that specifically binds α-tocopherol in the liver (see p. 6 of proofs), is also expressed in the placenta. This protein may play a major role in supplying vitamin E to the fetus throughout gestation (Jishage *et al.* 2001; Kaempf-Rotzoll *et al.* 2003; Jauniaux *et al.* 2004). α-TTP knockout mice are indeed infertile (Muller-Schmehl *et al.* 2004). α-TTP most probably plays a role in the stereoselective transport of *RRR*-α-tocopherol from maternal to fetal plasma (Muller-Schmehl *et al.* 2004).

It is, however, important to point out that the low placental transfer and tissue levels of vitamin E, which are noticed in most species investigated, have not been observed in the guinea pig, in which fetal liver vitamin E concentrations are high at late gestation. The nutrient is then released into the blood after birth. It is suggested that the liver serves as a storage site during gestation and that plasma vitamin E does not reflect tissue stores (Kelly *et al.* 1992; Hidiroglou *et al.* 2001).

Neonatal period. As in the case of vitamin A, circulating vitamin E levels at birth are very low as a consequence of the limited transplacental transfer (Ismadi & Olson, 1982; Pehrson *et al.* 1990; Hidiroglou *et al.* 1993*a*,*b*; Njeru *et al.* 1994; Olivares, 1995; Chen *et al.* 1996; Böhles, 1997; Mahan & Vallet, 1997; Léger *et al.* 1998; Oostenbrug *et al.* 1998; Kiely *et al.* 1999; Sapin *et al.* 2000; Debier *et al.* 2002*b*; Lauridsen *et al.* 2002). In pigs and rats, low levels at birth are also observed in other tissues, such as the muscle, liver, brain, heart and kidney

(Hidiroglou *et al.* 1993*b*; Pazak & Scholz, 1996). In most mammals, vitamin E intake through milk is therefore of the utmost importance to supply the newborn with an essential defence against oxygen toxicity and to stimulate the development of its immune system (Ostrea *et al.* 1986; Babinszky *et al.* 1991; Eicher *et al.* 1994; Nemec *et al.* 1994; Rajaraman *et al.* 1997). Haemolytic anaemia in the premature human neonate, which corresponds to lipid peroxidation in the erythrocyte membranes, is one of the symptoms of vitamin E deficiency at the very early stage of life. The erythrocyte membrane of newborns is particularly susceptible to oxidation damage, presumably due to the low vitamin E and reduced activities of antioxidant enzymes (Jain, 1989; Inanami *et al.* 1999). This phenomenon is even increased if the diet becomes richer in PUFA (Olivares, 1995). The quinone form of vitamin E is significantly elevated in newborns, suggesting an increased oxidative stress and vitamin E utilisation compared with their mother (Jain *et al.* 1996). A good supply of vitamin E to the offspring is also particularly critical for those who are characterised by poor and uncertain post-weaning feeding (Ross & Gardner, 1994; Debier *et al.* 2002*b*).

The dramatic increase in vitamin E content in body tissues of the offspring following birth is attributed to the ingestion of colostrum and milk, emphasising the limited placental vitamin E transfer and the importance of milk consumption (Pazak & Scholz, 1996; Debier *et al.* 2002*b*; Lauridsen *et al.* 2002). For example, liver vitamin E concentrations appear to increase by approximately thirty times in rats within 4 d postpartum (Pazak & Scholz, 1996). In pigs, the liver also accumulates α-tocopherol at a very fast rate: concentrations increase by nearly three-fold within 2 weeks following birth. This organ accumulates α-tocopherol at a much faster rate than other tissues (Hidiroglou *et al.* 1993*b*). The liver is indeed the main storage organ after ingestion in various mammal species and serves as a major source of vitamin E postnatally for mobilisation to extra-hepatic tissues (Engelhardt, 1977; Schweigert *et al.* 1990; Hidiroglou *et al.* 1993*b*; Pazak & Scholz, 1996). An eighty-fold increase in α-tocopherol concentrations has been observed in the plasma of piglets within 4 d following birth (Lauridsen *et al.* 2002). A few days to a few weeks, depending on the species, are generally necessary for serum vitamin E to attain levels comparable to those of adults (Loudenslager *et al.* 1986; Ostrea *et al.* 1986; Schweigert, 1990; Hidiroglou *et al.* 1993*a*; Swanson *et al.* 2000; Debier *et al.* 2002*b*).

As is the case for vitamin A and β-carotene, the timing at which colostrum is fed appears to be very important. Calves receiving colostrum after 24 h exhibit lower α-tocopherol concentrations, suggesting a reduced efficiency of intestinal absorption (Blum *et al.* 1997; Zanker *et al.* 2000).

Colostrum contains higher vitamin E concentrations than milk (Stowe, 1982; Chappell *et al.* 1985; Syvaoja *et al.* 1985; Harzer *et al.* 1986; Loudenslager *et al.* 1986; Ostrea *et al.* 1986; Donoghue, 1988; Hidiroglou, 1989; Schweigert, 1990; Boersma *et al.* 1991; Hidiroglou *et al.* 1993*a*; Meneses *et al.* 1994; Böhles, 1997; Schweigert & Gottwald, 1999; Zanker *et al.* 2000; Macias & Schweigert, 2001; Debier *et al.* 2002*b*; Hidiroglou *et al.* 2001, 2003; Gay *et al.* 2004). A progressive increase in the diameter of fat globules as milk matures has been suggested to explain the decrease in vitamin E between colostrum and milk (Ruegg & Blanc, 1981; Barbas & Herrera, 1998). This size change could lead to an increase in the relative percentage of core components of milk fat globules, such as TG and cholesterol esters, and a decrease in the relative percentage of the membrane components, such as α-tocopherol and cholesterol (Bitman *et al.* 1986; Boersma *et al.* 1991).

Like vitamin A, fat-soluble vitamin E does not follow the milk lipid profile, which increases as lactation progresses (Harzer *et al.* 1986; Macias & Schweigert, 2001; Debier *et al.* 2002*b*). The reason for this may result from selective mechanisms related to mammary uptake or milk secretion, which could involve different lipoprotein fractions at specific times of lactation (Schweigert *et al.* 2004).

The high concentrations of vitamin E in colostrum imply an active uptake by the mammary gland in compensation for limited placental transport (Chappell *et al.* 1985). As occurs for vitamin A, a drop in maternal circulating vitamin E levels is usually noticed at the end of gestation or at early lactation (Vrzgula *et al.* 1979; Schweigert, 1990; Goff & Stabel, 1990; Mahan & Vallet, 1997; Debier *et al.* 2002*b*; Lauridsen *et al.* 2002; Gay *et al.* 2004). In some species, such as man and horse, this decrease occurs after a gradual increase during gestation (Dostalova, 1984; von Mandach *et al.* 1994; AlSenaidy, 1996; Oostenbrug *et al.* 1998; Schweigert & Gottwald, 1999). The decrease in plasma α-tocopherol concentration from late gestation to parturition may be the consequence of a considerable amount of α-tocopherol partitioned into the colostrum (Lauridsen *et al.* 2002). The mechanism of transfer from blood into milk is not really known. However, a passive transfer along with TG seems to be excluded because, in all species investigated, the lipid profile of milk totally differs from that of vitamin E (Schweigert, 1990; Schweigert & Gottwald, 1999). Moreover, in cows, daily secretion of α-tocopherol in milk appears to be limited in quantity and is independent of the amount of milk and milk fat yield. This phenomenon also supports the hypothesis that the transfer of vitamin E into milk may occur through a protein-mediated transport (Jensen *et al.* 1999).

Herdt & Smith (1996) have shown that, in cows, gestation and lactation stage affect serum vitamin E concentrations by influencing the concentration of both lipoprotein particles and vitamin E within the individual particles. The vitamin E concentration change seems similar to the cholesterol change, suggesting that much of the variation of vitamin E levels during lactation can be attributed to changes in serum lipoprotein concentrations (Herdt & Smith, 1996). The LDL fraction plays a crucial role for cholesterol and vitamin E metabolism (Schweigert, 1990; Herdt & Smith, 1996). In cows, this fraction appears to decrease sharply before birth in the plasma of gestating females and increase again after parturition, following the dynamics observed for vitamin E and cholesterol (Schweigert, 1990). This phenomenon might be due to an increase in activity of the mammary LDL receptors and thus to an important uptake of LDL by the mammary gland around parturition. Such a mechanism could explain the high concentration of vitamin E found in the colostrum of cows compared with mature milk. LPL also seems to play a role by modulating the

uptake of α-tocopherol during pregnancy and lactation (Martinez *et al.* 2002).

Contrary to the placental transfer, which remains low even with increased maternal serum levels, the transfer through colostrum and milk can be increased via a higher vitamin E ingestion by the mother (Pehrson *et al.* 1990; Mahan & Vallet, 1997; Focant *et al.* 1998; Léger *et al.* 1998). As in the case of placental transfer, there is also segregation in favour of the natural *RRR*-α-tocopherol form compared with the synthetic form. The presence of an α-TTP like mechanism in the mammary gland cannot be excluded. Mammary α-TTP could facilitate α-tocopherol secretion into milk (Lauridsen *et al.* 2002). The presence of an SR-BI receptor (see p. 159) at the mammary gland should also be investigated, as it could be involved in the uptake of HDL–α-tocopherol.

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