Review

# Vitamin E requirements, transport, and metabolism: Role of α-tocopherol–binding proteins

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*Vitamin E (RRR-α-tocopherol) is a lipid-soluble antioxidant that is present in the membranes of intracellular organelles. There it plays an important role in the suppression of free radical-induced lipid peroxidation. There are eight naturally occurring homologues of vitamin E that differ in their structure and in biological activity in vivo and in vitro. Although γ-tocopherol is a more effective free radical scavenger than α-tocopherol in vitro, the reverse is true in vivo, suggesting that the tocopherol distribution systems favor the localization of α-tocopherol at the sites where it is required. Vitamin E is transported in plasma primarily by lipoproteins, but little is known of how it is transported intracellularly. A 30 kDa α-tocopherol–binding protein in the liver cytoplasm may regulate plasma vitamin E concentrations by preferentially incorporating the vitamin E homologue, RRR-α-tocopherol (α-tocopherol), into nascent very low density lipoproteins. However, this α-tocopherol–binding protein is unique to the hepatocyte, whereas α-tocopherol is present in the cells of all major tissues. Moreover α-tocopherol accumulates at those sites within the cell where oxygen radical production is greatest and thus where it is most required; in the membranes of heavy mitochondria, light mitochondria, and endoplasmic reticulum. This raises the question of how the lipid-soluble α-tocopherol is transported intracellularly in different tissues. We have identified a new α-tocopherol–binding protein of molecular mass 14.2 kDa in the cytosol of heart and liver. This protein specifically binds α-tocopherol in preference to the δ- and γ-homologues but does not bind oleate. Studies on immunoreactivity and ligand specificity of the protein suggest that it is not a fatty acid-binding protein. The 14.2 kDa α-tocopherol–binding protein stimulates the transfer of α-tocopherol from liposomes to mitochondria in vitro by 8 to 10 fold. We suggest that this low molecular mass TBP may be responsible for the intracellular transport and distribution of α-tocopherol in the tissues.* (J. Nutr. Biochem. 5:562–570, 1994.)

**Keywords:** Vitamin E (RRR-α-tocopherol); α-tocopherol–binding protein (TBP); plasma membrane α-tocopherol–binding protein ($TBP_{pm}$); vitamin E transport; lipoproteins (VLDL, HDL, and LDL); fatty acid-binding protein (FABP)

## Introduction

Vitamin E is a lipid-soluble antioxidant that protects the polyunsaturated fatty acids (PUFAs) and other components of cell and organelle membranes from oxidation by reactive free radicals.[1] In addition, vitamin E may have important roles in biological processes that do not necessarily relate to its antioxidant function such as in DNA synthesis, the stimulation of the immune response, and the suppression of inflammation.[2] Consequently, there is growing awareness that vitamin E deficiency contributes to the development of neuropathies and myopathies in animals,[3] and that increased vitamin E intakes may inhibit the progression of many diseases including coronary heart disease, arthritis, malignant hyperthermia, Parkinson's disease, and tardive dyskinesia.[4,5] However, vitamin E deficiency rarely occurs on a dietary basis in developed and most developing countries in older infants, children, and adults because of the ubiquitous distribution of vitamin E in grains, vegetable oil, and animal fats.[6,7] Consequently the most common cause of vitamin E deficiency is malabsorption of dietary vitamin E due to underlying gastrointestinal, pancreatic, and hepatic disorders that interfere with the digestion or absorption of lipid. The

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major exception, however, is the inborn error of metabolism, the isolated vitamin E deficiency syndrome, which leads to symptomatic vitamin E deficiency despite normal intestinal absorption of vitamin E.[8,9] Recently, there are more reports on genetically linked abnormality of vitamin E transport in humans,[10] and the genetic locus of the abnormality is found to be on chromosome 8, as revealed by homozygosity mapping.[11] The other chronic conditions most commonly associated with a symptomatic vitamin E deficiency state include abetalipoproteinemia and other disorders of β lipoprotein secretion, chronic cholestatic hepatobiliary diseases, cystic fibrosis, and other causes of pancreatic insufficiency and short bowel disease.[12]

Despite the increasing interest in the role of vitamin E in maintaining health, little is known about the intracellular transport mechanism that results in the accumulation of vitamin E at those sites in the cells where oxygen radical production is greatest, e.g., in the membranes of mitochondria and endoplasmic reticulum.[13] Translocation of lipophilic compounds through aqueous compartments is often mediated by specific carrier proteins.[14,15] Therefore, this paper considers whether a recently identified 14.2 kDa α-tocopherol–binding protein (TBP) in the cytosol of liver and heart[16,17] could play a role in the intracellular distribution of vitamin E. The relationship of the 14.2 kDa TBP with 30 kDa TBP in hepatocytes and their role in preferential selection of RRR-α-tocopherol from the range of vitamin E homologues in the diet is discussed.

## Antioxidant effectiveness of vitamin E homologues

Dα-tocopherol and dγ-tocopherol are the most common of the eight naturally occurring vitamin E homologues (dα-, dβ-, dγ-, and dδ-tocopherol and dα-, dβ-, dγ-, and dδ-tocotrienol) in the human diet. These two forms of vitamin E have markedly different antioxidant abilities in chemical and biological systems. The most biologically active of these compounds is RRR-α-tocopherol (α-tocopherol).[18] This can be demonstrated using a model system whereby microsomes prepared from the livers of vitamin E-deficient rats are preincubated with ethanolic solutions of the individual tocopherols prior to initiation of peroxidation.[19] The antioxidant effectiveness of the different homologues can then be estimated in relation to their ability to suppress the maximum possible peroxidation. In this model system, dγ-tocopherol has greater antioxidant effectiveness than dα-tocopherol.[19] By contrast, when peroxidation is initiated with microsomal preparations of liver from vitamin E-deficient rats that have been fed equal amounts of either dα- or dγ-tocopherol in the diet, dα-homologue confers greater protection.[20] This suggests that there is a selection process discriminating against the uptake or accumulation of dγ-tocopherol, or that other factors interact with the specific isomers to alter their in vivo effectiveness. Furthermore, the inhibition of protein kinase C and of smooth muscle cell proliferation (induced by oxidised LDL) is reported to be highly specific to dα-tocopherol.[21]

## Assessment of vitamin E requirements

An important new area of vitamin E research concerns the assessment of the need for an antioxidant defense system to protect the body from free radical-induced damage. The assessment of vitamin E requirements in humans is complicated by the infrequent occurrence of clinical signs of deficiency, which usually develop only in premature infants or adults with intestinal malabsorption.[22,23] In the classic deficiency states formerly seen in premature infants there is hemolytic anemia and neurological and retinal damage.[24] The clinical manifestations of vitamin E deficiency vary considerably between species.[12] In general, however, the targets are the neuromuscular, vascular, and reproductive systems. Diverse vitamin E deficiency symptoms have also been described for laboratory and experimental animals.[25–28] The paucity of clinical effects in the human has been taken to signify that modern diets contain sufficient vitamin E to satisfy nutritional needs. The requirement values do not take into account the growing epidemiological evidence that intakes of vitamin E and other antioxidants are beneficial in limiting oxidative damage that may be relevant to the prevention of cataract formation,[29] cardiovascular diseases,[30] and several cancers. Willett's group[31,32] reported a protective effect of vitamin E in prospective studies of adults with large and possibly pharmacological doses. Thus the true needs for vitamin E may be appreciably greater than currently cited and depend on the degree of prevailing oxidative stress in one or more organs.

Little is known of how vitamin E is distributed and metabolized when intakes rise from the current requirement levels to higher intakes and then to pharmacological levels of intake. Clearly, the dietary requirement levels of vitamin E, and perhaps distribution, is affected by the composition of the rest of the diet. High dietary intakes of PUFAs will tend to increase the requirements for vitamin E because of the increased susceptibility of tissues to peroxidation. Should the general population respond to nutritional advice to reduce their total fat intake and increase the proportion of unsaturated fat in the diet, their intake of vitamin E could fall rather than increase. Therefore, it has been proposed by the US Expert Committee that the vitamin E requirement should include a basic requirement plus a factor to take into account PUFA intake[2,33] as shown in Equation 1.

$$\text{Requirement (I.U./day)} = 5.96 + 0.25\ (\%\text{PUFA kcal} + \text{g PUFAs}) \quad (1)$$

To translate vitamin E requirements expressed in international units (IU) into the more conventional mg of equivalent of α-tocopherol, 2 mg must be considered equivalent to 3 IU. A simpler version is then to consider 0.4 mg of α-tocopherol/g PUFA as adequate (Dept. of Health, Dietary Reference Values for food energy and nutrients for the United Kingdom). The Nutrition Working Group of the International Life Sciences Institute of Europe (1990) has similarly suggested that until such time as optimum intakes of vitamin E have been established by long-term intervention trials, the recommended daily allowance is calculated by assuming a daily intake of 14 g PUFA (5% of energy intake) and an energy intake of 2,400 kcal/day, with 40% of the energy derived from fat with a polyunsaturated to saturated ratio between 0.33 and 0.40. This gives a vitamin E requirement of 18 IU/day (12 mg equivalent α-tocopherol per day). The estimates are based on general biological assessments and do not necessarily relate to the amounts of vitamin E needed

to counteract, for example, free radical-induced DNA damage with its multiple role in carcinogenesis or to prevent the development of atherosclerosis and coronary heart disease. It has been suggested that intakes of up to 100 IU/day may be necessary to reduce the susceptibility of individuals to certain diseases.[34] In recent studies, the national diet of Mediterranean countries with low rates of coronary heart disease has been found to contain 15 to 25 mg of α-tocopherol/day.[34] These food supply figures are recognized to overestimate actual intakes by perhaps 28%, so the cross-cultural data may imply a selective average need of α-tocopherol of 15 mg/day.[35]

It is therefore impossible to estimate vitamin E requirements on an objective physiological basis until we have a better understanding of the mechanisms responsible for its extracellular and intracellular transport of various isomers and the importance of discrimination between the homologues of vitamin E. Several nutritional questions clearly demand a biochemical approach. For example, do tissues respond differentially to high levels of vitamin E intake, or is there a mass action effect of vitamin E on all tissues? These issues will affect differential tissue response to oxidative stress. The cellular routing of vitamin E is of fundamental importance to its role in the prevention of disease.

## Intestinal absorption and transport of tocopherols

Although much is known of the mechanisms by which vitamin E is absorbed and transported in plasma,[36,37] its intracellular transport is not well known. Because of its hydrophobicity, vitamin E requires a special transport system in aqueous environments of the plasma, extracellular space, and cell cytoplasm. Unlike other fat-soluble vitamins, it does not have a special carrier protein in plasma and is transported in plasma by lipoproteins such as high density lipoprotein (HDL), low density lipoprotein (LDL), and very low density lipoprotein (VLDL).[37] Dietary studies indicate that there is little or no discrimination between the homologues in the intestine, as tocopherols are absorbed from the gut in micelles whose formation depends on the bile salts and pancreatic lipase.[38,39] Absorption of tocopherol occurs mainly in the upper and middle thirds of the small intestine of animals and is enhanced by medium chain triglycerides but inhibited by long chain PUFAs.[40] Micelles containing tocopherol may passively diffuse through the brush border but the mechanism by which vitamin E is then transported across the intestinal epithelial cells is poorly understood. The vitamin is released from the enterocyte into lymph within chylomicrons, which subsequently appear in the circulation where they are catabolised by lipoprotein lipase (LPL).[41] Some transfer of vitamin E (dα-, dβ-, dγ-tocopherol) to tissues occurs during chylomicron catabolism. This transfer is mediated by LPL, occurs in parallel with fatty acids transfer, and is dependent on binding of LPL to the cell surface.[42] Tissues such as adipose and muscle and, to some extent, brain that receive most of their lipids during the delipidation cascade, also receive tocopherols as a result of LPL activity.

The liver may have an impaired uptake of vitamin E homologues if there is poor intestinal chylomicron production, as in abetalipoproteinemia, or if the actual absorption of vitamin E by the intestine is limited. When there is a reduced catabolism of chylomicrons or of VLDL because of impaired LPL activity, approximately 80% of tocopherols are transported in triglyceride-rich lipoproteins.[42,43] Because of these elevated levels of lipids, these patients have plasma α-tocopherol concentrations approximately 10 to 12 times normal, but with low normal adipose tissue α-tocopherol levels.[42,43] Therefore, the deficiency of LPL activity does not result in vitamin E deficiency in these patients.

LDL uptake is thought to be an important mechanism by which tissues obtain α-tocopherol. However, in homozygous and heterozygous forms of hypercholesterolemia, vitamin E uptake by the liver persists,[44] implying that LDL receptor-mediated mechanisms[45] are not the sole means of hepatic uptake. This is also confirmed by the fact that Watanabe rabbits, which have defective LDL receptor systems, have normal α-tocopherol levels.[46] Once transported into the liver there is a clear differentiation between the homologues, with α-tocopherol being the exclusive isomer incorporated with very low density lipoprotein (VLDL) for secretion into the plasma,[47,48] whereas the other isomers are excreted through the biliary canaliculi.[49]

## Regulation of plasma tocopherol

### *VLDL secretion and chylomicrons*

Plasma levels of dγ-tocopherol in adult Americans average only 10 to 15% of that of the dα-homologue,[48] despite the fact the intake of dγ-tocopherol is two to four times that of dα-tocopherol.[50] Experimental studies[51–53] have shown limited uptake of dγ-tocopherol into peripheral tissues and its rapid clearance from plasma within 2 to 3 days of eating large amounts of dγ-tocopherol. Normally dγ tocopherol, although efficiently absorbed, only accounts for 10 to 15% of plasma tocopherol.[48] From these studies it appears that the liver, but not the intestine, is capable of discriminating between these two tocopherols, as discussed subsequently.

Chylomicrons contain all forms of tocopherol in proportion to their content in the diet, confirming a lack of intestinal discrimination during the absorption process. However, VLDL derived from the liver and plasma lipoproteins (HDL, LDL) are always enriched in α-tocopherol.[47,48] This suggests that a mechanism is present in the liver that preferentially exports α-tocopherol with VLDL after uptake of the various forms of tocopherol with chylomicron remnants. Studies on the absorption and transport of various forms of vitamin E indicate that plasma α-tocopherol is regulated specifically by preferential incorporation into nascent VLDL of α-tocopherol compared with other homologues of vitamin E (tocopherols and tocotrienols).[54,55]

### *Role of the 30 kDa α-tocopherol-binding protein of liver*

An α-tocopherol-binding protein (TBP) of molecular mass 30 kDa, which specifically binds α-tocopherol, has been isolated in the cytosol of liver of different species.[56–61] The 30-kDa TBP is thought to be responsible for specific incorporation of α-tocopherol into nascent VLDL.[54,55] Recently, the cDNA clone for the rat liver protein was isolated, and the amino acid sequence was determined.[62] The 30-kDa TBP was found to have some homology with the retinoladehyde-

binding protein of retina.[62] Both Western and Northern blot analyses revealed that the 30-kDa TBP is expressed exclusively in the hepatocytes and is absent from tissues such as heart, spleen, and lung, etc. and from other cells (endothelial, Kupffer cells).[56–61] Increasing evidence suggests that the 30-kDa TBP appears to be responsible for discrimination between the homologues by incorporating α-tocopherol from lysosomes to the endoplasmic reticulum for VLDL synthesis.[54,55] This could explain why the γ-homologue, although efficiently absorbed, only accounts for 10 to 15% of plasma tocopherol. How the 30-kDa TBP channels α-tocopherol into nascent VLDL awaits further study.

## Intracellular transport and distribution of α-tocopherol in tissues: Role of plasma membrane α-tocopherol-binding protein ($TBP_{pm}$) and cytosolic α-tocopherol-binding protein (14.2 kDa)

Specific mechanisms must also exist for the transfer of α-tocopherol from plasma lipoproteins to peripheral cells and tissues. As described above, LDL receptors are thought to be involved but do not play an obligatory role in the uptake of α-tocopherol bound to lipoproteins because LDL receptor deficiency does not impair α-tocopherol uptake. Other parallel mechanisms must also exist for α-tocopherol uptake by cell membranes. Although the uptake of fatty acids by different tissues is often described as a passive, diffusional process,[63] recent kinetic studies suggest that it may also be carrier mediated.[64] A 43-kDa plasma membrane fatty acid-binding protein ($FABP_{pm}$) is thought to be involved in the transmembrane transport of long chain fatty acids in many cells types.[64] Similarly, a 68-kDa protein in the plasma membrane of the hepatocyte sinusoidal surface is found to be responsible for the uptake of bile acid and other organic anions.[65] The $FABP_{pm}$ functions mainly in the trapping of fatty acids, and their subsequent transmembrane translocation may occur either passively or by carrier-mediated processes. An α-tocopherol–binding protein ($TBP_{pm}$) has been identified and partially characterized from human erythrocyte and adrenal membranes.[66,67] Erythrocyte membrane contains α-tocopherol as the major, if not the only, lipid-soluble chain-breaking antioxidant at concentrations that are much less then those in plasma but are nevertheless sufficient to prevent the formation of lipid peroxides.[68] It is possible that $TBP_{pm}$ is responsible for α-tocopherol uptake in these cell membranes. Binding sites for α-tocopherol ($TBP_{pm}$) have been identified in liver membranes and appear to be proteinaceous because 80% of the total α-tocopherol binding activity is abolished on treatment with trypsin *(Figure 1)*. However, further studies are required to elucidate the significance of $TBP_{pm}$ in vitamin E metabolism.

Once inside a cell, very little information is available about the transport of α-tocopherol from cellular to intracellular membranes. The translocation of hydrophobic compounds through an aqueous environment is often mediated by specific carrier proteins.[14,15] However, the 30-kDa TBP is unlikely to have a general intracellular transfer function because it occurs only in hepatocytes,[56–61] whereas α-tocopherol is widely distributed in organelles of most tissues. Recently we have identified a low molecular weight fraction

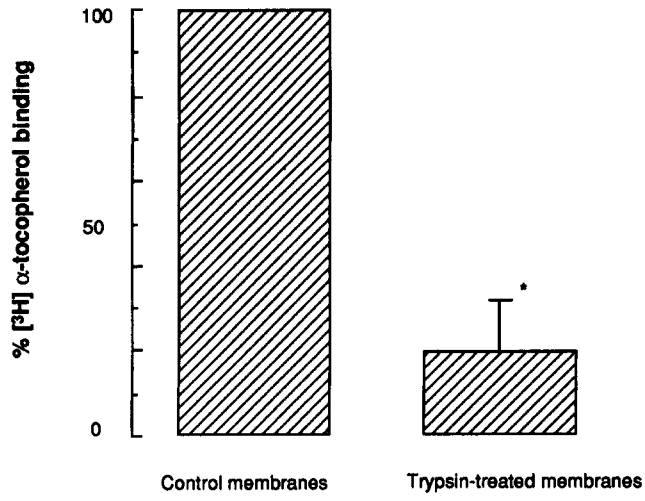


**Figure 1** Binding of [$^3$H]α-tocopherol to rat liver plasma membranes. Rat liver plasma membranes were incubated with 3nM [$^3$H]α-tocopherol in 20 mM Tris-HCl buffer, pH 7.4 for 30 min at 37° C in the presence of 100 μM Triton X-100. After incubation, membranes were centrifuged. The membrane pellet was then washed with the ice-cold incubation buffer to remove unbound [$^3$H]α-tocopherol, and the radioactivity of the membrane pellet was determined. Nonspecific binding was measured in the presence of 50 fold of excess unlabeled α-tocopherol. Trypsin-treated membranes were prepared after treating them with 450 BAEE units of trypsin for 1 hr. Values are the mean of four determinations ± SD (bar) for four animals. *Indicates $P < 0.001$ compared with control.

in the cytosol of both liver and heart of the rat that specifically binds α-tocopherol.[17] *Figure 2* shows the presence of cytoplasmic TBPs in the cytosol of rat heart and liver. Furthermore, a similar 14.2 kDa TBP has been purified and partially characterized from the rabbit heart cytosol.[16] This protein specifically binds α-tocopherol but not the γ or δ homologue and moreover, stimulates by 10 fold its transfer from liposomes to mitochondria. Anti-heart-fatty acid-binding protein (FABP) antibody did not recognize the heart TBP in Western blots, indicating that it is not a heart FABP (~14.8 kDa) that may be exclusively involved in the intracellular transport of α-tocopherol. The 14.2-kDa TBP in the cytosol of liver and heart[16,17] may play a crucial role in these processes. Whether reduced levels of TBP lead to vitamin E deficiency in tissues because of impaired intracellular distribution of α-tocopherol is unknown.

The proposed scheme for the action of FABPs in fatty acid transport suggests that they increase the solubility of fatty acids in the cytoplasm and thereby enhance their net diffusion from the plasma membrane to the intracellular membrane-bound organelles.[69] In the case of unaided cytoplasmic diffusion, there would be a steep concentration gradient from the plasma membrane to cellular sites of metabolic transformation in the membranous organelles. By increasing the solubility of ligands in the aqueous environment, FABP greatly increases their diffusional flux and reduces the intracellular concentration gradient. If metabolic transformation of the ligand is not rate-limiting in the overall process of ligand transcellular movement and elimination, the binding proteins are likely to influence the transport of fatty acids in the cell from the earliest stages of cellular

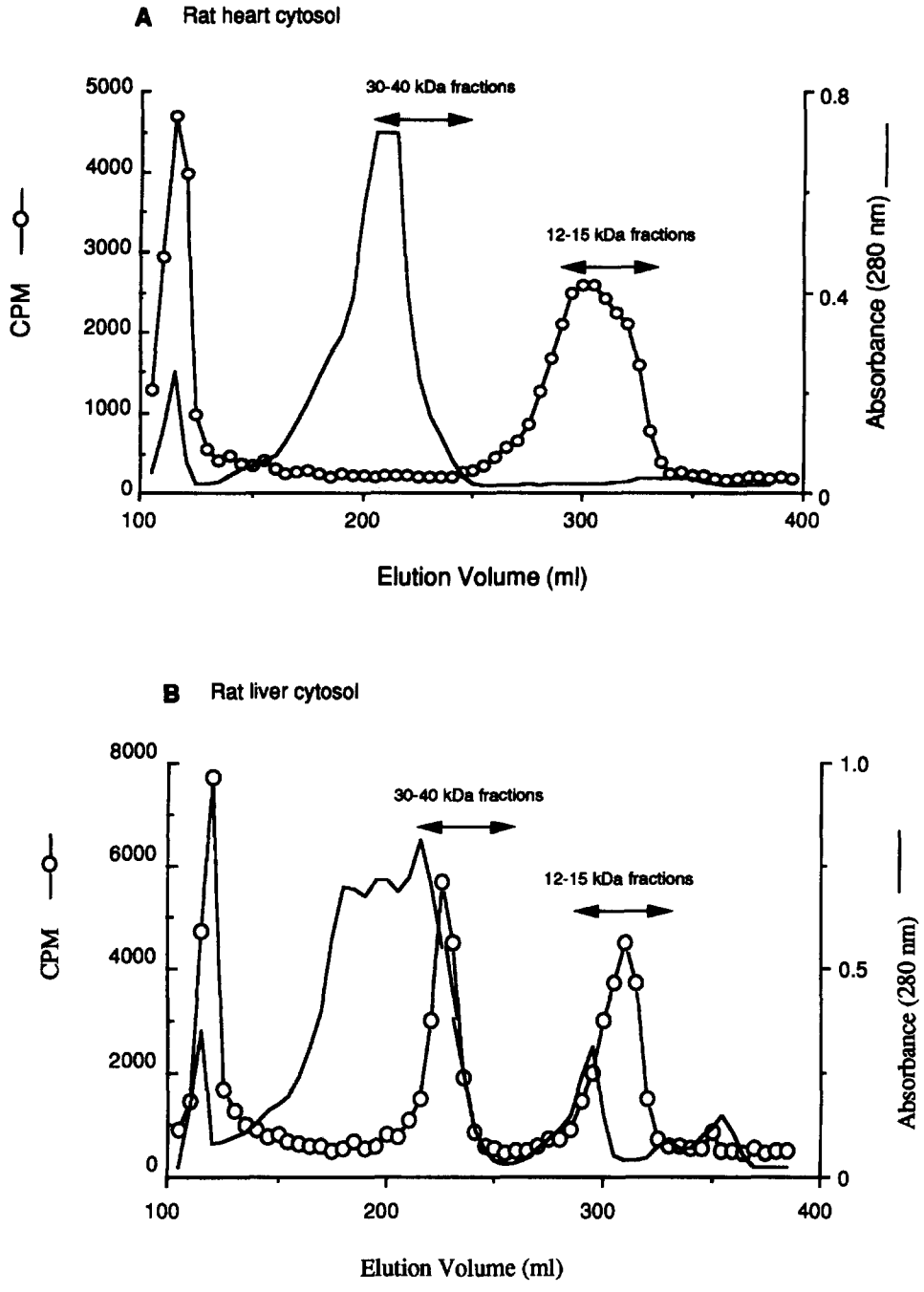


**Figure 2** Gel filtration of rat liver and heart cytosol on Sephacryl S-300 column with [$^3$H]α-tocopherol. [$^3$H]α-Tocopherol (100 nM) was incubated with 1 ml of cytosol of liver or heart (~25 mg protein) for 30 min at 37° C. After the incubation, the mixture was applied to a fast protein liquid chromatography (FPLC) Sephacryl S-300 column (2.6 cm × 60 cm) for gel permeation chromatography. The protein was eluted from the column with 10 mM Tris-HCl buffer, pH 7.4, containing 5 mM β-mercaptoethanol, 100 mM KCl and 5% glycerol at 4° C. Five-mL fractions were collected, and $A_{280}$ and radioactivity were determined. A = heart cytosol; B = liver cytosol.

uptake. It is possible that α-tocopherol binding proteins may function in a similar manner. $TBP_{pm}$ and TBP may be involved in the cellular uptake and transport of α-tocopherol and regulate α-tocopherol levels in the intracellular membranes of the tissue. They may play individual roles or act together in facilitating membrane α-tocopherol uptake, targeting α-tocopherol to organelle membranes, regenerating α-tocopherol, and altering the membrane structure and function. Further studies are required on the interplay between TBP and $TBP_{pm}$ and on their interaction with various membrane structures. Elucidation of the possible coordinated regulation of these proteins should provide insight into their involvement in supplying and/or regenerating α-tocopherol to the various membranes.

## Interplay between the 14.2 kDa and 30 kDa TBPs in the liver to maintain α-tocopherol levels in plasma: Comparative biochemistry with FABP

Discrimination between the various dietary tocopherols occurs principally in the liver (see above). There molecules of both endogenous and exogenous origin are modified before being stored in hepatic tissue, released into the blood stream, or excreted into bile. This would involve initial sequestration of molecules from the systemic circulation and their transport across the cell membrane into cytoplasm. There they may be enzymatically transformed before direction to their ultimate and specific fates, such as deposition in storage compartments within the liver, or transport to the basolateral plasma membrane for eventual release into the sinusoidal circulation, or to the canalicular domain for excretion into bile. The liver discriminates among the tocopherols and tocotrienols by secreting only α-tocopherol in nascent VLDL, despite the presence of other absorbed forms of vitamin E circulating in chylomicrons. Unlike heart, the liver contains two different TBPs, with molecular masses of 30 kDa and 14.2 kDa; the latter only recently described.[16,17,56–61] Because the 30 kDa TBP is a monomeric protein,[56–61] the 14.2 kDa binding protein cannot be its subunit. Both proteins bind α-tocopherol specifically and do not bind other homologues, but their actual roles in hepatic α-tocopherol transport and metabolism have yet to be established. Because the 30 kDa TBP is only selectively found in the liver, it is tempting to speculate that its presence relates to a specific hepatic role, e.g., the secretion of α-tocopherol into nascent VLDL, and thereby maintains plasma levels of α-tocopherol. Because it is a selective binder of the α isomer, it seemingly can have little to do with the excretion of other vitamin E forms into the bile. The 14.2-kDa TBP, however, exists in all the tissues where it has so far been measured. It seems reasonable that it is involved in intracellular distribution and metabolism of α-tocopherol in all tissues including the liver. The presence of more than one TBP within a cell is not unknown as three different proteins (31, 58, and 81 kDa) in the cytosol of cultured smooth muscle cells (Ar75) bind tocopherol.[70]

It is of interest to compare the metabolic handling of vitamin E and fatty acids in the liver. There are clear similarities as well as major differences in the metabolic handling of fatty acids and vitamin E. One such difference is the presence of two TBPs (14.2 kDa and 30 kDa) in the liver,[17,56–61] but only one FABP (14 kDa).[14,15] Liver FABP binds fatty acid, prostaglandins, and hem but not α-tocopherol.[14,15,71–73] Fatty acids are abundant, an important source of energy, and are essential components of cell membrane phospholipids. They are released by hydrolysis of dietary glycerolipids and are taken up into enterocytes, re-esterified to triglycerides, and then transported to peripheral tissues in chylomicrons. Hydrolysis of chylomicron triglycerides by LPL releases free fatty acids that are taken up by the tissues (mainly adipose) and esterified. Fatty acids are released from adipose tissue triglyceride by hormone sensitive lipase and transported in the plasma bound to serum albumin. The liver produces fatty acids through de novo synthesis from acetate. Hepatic fatty acids are either oxidized for energy or esterified mainly to phospholipids, triglycerides, or cholesteryl ester that either remain in the liver or are secreted predominantly in VLDL. FABP is assumed to be involved in the intracellular transport and metabolism of long chain fatty acids.[14,15,71,73–76]

The various dietary forms of vitamin E are processed in a manner similar to fatty acids from their absorption at the intestine to chylomicron synthesis. Thereafter there are many differences. Once vitamin E enters in the liver, only α-tocopherol is secreted from the liver in nascent VLDL, as already noted. The 30-kDa TBP is thought to be involved in the transfer of α-tocopherol from lysosomes to the endoplasmic reticulum for preferential incorporation of α-tocopherol into nascent VLDL.[54–61] Thus, the intracellular hepatic routing of the vitamin E analogues must be very different from that of fatty acids. No such discrimination of individual fatty acids occurs in the liver.[63] It seems reasonable to infer that the 14.2 kDa TBP, like FABP, is probably involved in the intracellular transport and metabolism of α-tocopherol in the liver as well as in the heart.

## Conclusions

Interaction between lipids and proteins is of fundamental importance in the organization and function of living entities. An integral aspect of this interaction is the translocation of lipids through aqueous compartments in association with specific proteins.[14,15] These nonenzymatic, nonstructural intracellular lipid-binding proteins are not involved in the simple binding and diffusional transport of lipids, but there is still considerable uncertainty regarding the exact function. The kinetics of lipid binding to these proteins within the cytoplasm and the nature of structure and diffusion properties of the cytoplasmic compartment itself remains to be established. Nevertheless, the translocation in plasma or in intracellular space needs a protein vehicle for the effective transport of hydrophobic compounds, such as vitamin E. *Figure 3* shows a schematic diagram of vitamin E transport from intestine to liver and then from lipoproteins to various tissues.

In vitro data clearly suggest that vitamin E has anti-atherogenic properties, which include its ability to inhibit endothelial injury and LDL oxidation and proliferation of smooth muscle cells and to reduce monocyte/macrophage cytotoxicity.[21,77–79] Data from human studies suggest an inverse correlation between plasma levels of vitamin E and mortality from ischemic heart disease.[67,80] However, several studies[81–85] failed to find any relationship between plasma vitamin E levels and heart disease. One of the reasons could be the fact that tissue levels of vitamin E are not well correlated with plasma vitamin E levels. This is to be expected when the actual levels in plasma depend on the combination of different tocopherols in different lipoprotein pools *(Figure 3)* and on the selectivity of processes for generating and secreting both the lipoprotein complexes and tocopherols in different organs. It could be argued that it is the α-tocopherol associated with LDL that will modulate the effect of LDL induction of atherosclerosis, whereas in platelet membranes it may affect the thrombotic component accompanying a carotid or coronary occlusion.[86–88] Only when the importance of these distinctive processes and the metabolic roles of vitamin E are established will vitamin E requirements be properly understood.

The newly identified intracellular TBP and $TBP_{pm}$ provide

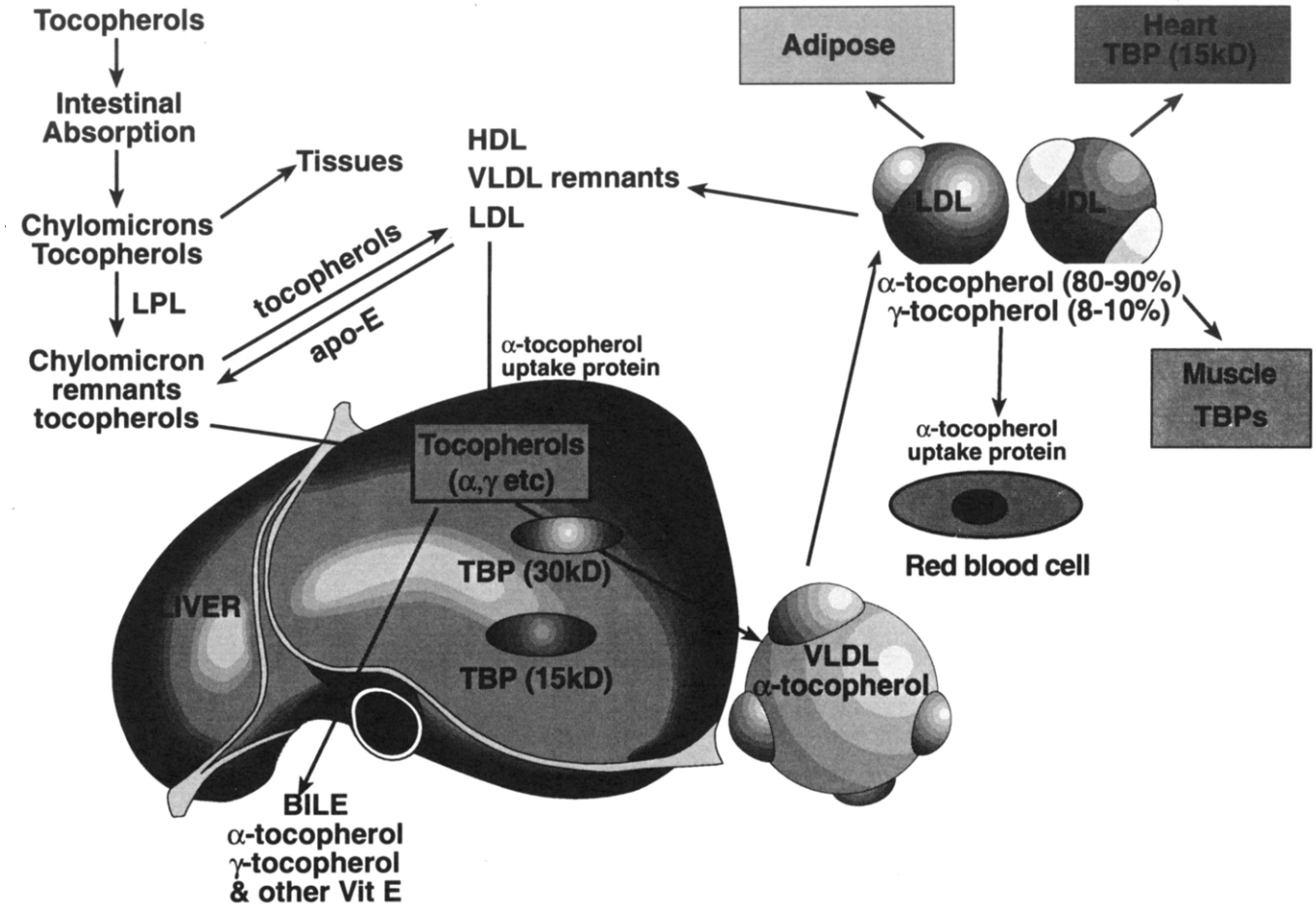


**Figure 3** Proposed pathway of vitamin E transport. Transport of tocopherols from intestine to other tissues. Tocopherols are absorbed from diet in chylomicrons and then mainly transported to liver where α-tocopherol is usually secreted in nascent VLDL. The other vitamin E homologues, as well as excess α-tocopherol, are excreted in the bite. The 30-kDa TBP is thought to be responsible for specific incorporation of α-tocopherol into VLDL. The low molecular weight TBP both in the liver and heart is thought to be responsible for intracellular distribution and transport of α-tocopherol. $TBP_{pm}$ and LDL receptors may be responsible for the uptake of α-tocopherol from lipoproteins in tissues.

some explanation for isomeric vitamin E uptake and intracellular distribution. As TBP is thought to be responsible for intracellular transport as well as the retention of α-tocopherol in the tissue, expression and function of TBP may be crucial for the regulation of α-tocopherol levels in the tissues. The activity of the binding proteins may therefore be crucial for effective regulation of α-tocopherol levels in plasma, membranes, and cellular organelles. Thus the cytoplasmic 14.2-kDa TBP may function as an intermembrane carrier of water-insoluble α-tocopherol and may be a new member of the intracellular lipid-binding protein family.[14,15,71,73–76] Although not yet established, the principal function of the TBP may be to facilitate the uptake and intracellular trafficking and retention of α-tocopherol in tissues. The presence of the 14.2-kDa TBP in the cytosol of hepatocytes and cardiomyocytes supports the conventional view that it plays a key role in the delivery of α-tocopherol to microsomal membranes over and above its ability to enhance the retention of α-tocopherol (decrease efflux) within these cells after its uptake across the plasma membrane. If reduced activity of the 30-kDa TBP in the liver leads to signs of vitamin E deficiency because of impaired incorporation of α-tocopherol into nascent VLDL,[54,55] abnormalities in the function of the 14.2 kDa TBP may have similar outcome, despite the normal plasma levels of vitamin E.

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