

Absorption, lipoprotein transport, and regulation of plasma concentrations of vitamin E in humans

Herbert J. Kayden¹ and Maret G. Traber

New York University Medical Center, 550 First Avenue, New York, NY 10016

I. STRUCTURE AND FUNCTION OF VITAMIN E II. INTESTINAL ABSORPTION AND LIPOPROTEIN TRANSPORT

- A. Intestinal absorption and secretion in chylomicrons
- B. Secretion and catabolism of VLDL
- C. Transport in LDL
- D. Exchange of tocopherol between lipoproteins and transport in HDL

III. DISCRIMINATION BETWEEN TOCOPHEROLS

IV. VITAMIN E DEFICIENCY IN HUMANS WITH LIPID MALABSORPTION

- A. Patients with abetalipoproteinemia or homozygous hypobetalipoproteinemia
- B. Patients with cholestatic liver disease
- C. Patients receiving total parenteral nutrition

V. PATIENTS WITH FAMILIAL ISOLATED VITAMIN E DEFICIENCY

- A. Clinical characteristics
- B. Impaired secretion of α -tocopherol in VLDL
- C. Impaired discrimination between stereoisomers of α -tocopherol

VI. TOCOPHEROL-BINDING PROTEIN

VII. REGULATION OF PLASMA α -TOCOPHEROL

VIII. CONCLUSIONS

This review describes the general characteristics of vitamin E, then focuses on its absorption and transport in lipoproteins in normal subjects and in patients with either genetic abnormalities of lipoprotein metabolism or of vitamin E transport. The basis for the biological preference for *RRR*- α -tocopherol over other forms of vitamin E is then discussed. Finally, the evidence that the hepatic tocopherol-binding protein regulates vitamin E transport and plasma α -tocopherol concentrations is presented.

I. STRUCTURE AND FUNCTION OF VITAMIN E

Vitamin E occurs in nature in eight different forms: α -, β -, γ -, δ -tocopherols (which have a chromanol ring and phytyl tail, and differ in the number and position of methyl groups on the ring) and α -, β -, γ -, δ -tocotrienols (which have unsaturated tails). Synthetic α -tocopherol, sold as vitamin E supplements, contains equal amounts of eight different stereoisomers of α -tocopherol arising from the three chiral centers in the phytyl tail. Tocopherols and tocotrienols are lipid-soluble, sterically hindered phenols, that react more rapidly with peroxy radicals than do polyunsaturated fatty acids (1). Thus, vitamin E is a chain-breaking antioxidant; in vivo α -tocopherol is the major lipid-soluble antioxidant in the plasma (2, 3).

The eight forms of vitamin E differ in their degrees of biologic and antioxidant activities. Biologic activity is assessed by determining the amount of each form of the vitamin that can prevent deficiency symptoms in a defined model system, such as: 1) the rat fetal resorption-gestation assay, 2) the dialuric acid-induced erythrocyte hemolysis test, or 3) the curative myopathy test in experimental animals (4, 5). Antioxidant activity is assessed by measuring the chemical reactivity of the molecules. Examples of these measurements include: 1) oxidation of tocopherols by a phenoxyl radical using stopped-flow spectrophotometry, 2) O_2 consumption during the reaction of tocopherols with polystyryl peroxy radicals in chlorobenzene, or 3) disappearance of tocopherols during oxidation reactions using electron spin resonance, as reviewed (6). The biologic and antioxidant activities of the forms of vitamin E are not identical. For example, γ -tocopherol (a major form of dietary vitamin E in humans) has about one-half the antioxidant activity (1) but only one-tenth the biologic activity

Abbreviations: LDL, low density lipoprotein; HDL, high density lipoprotein; IDL, intermediate density lipoprotein; VLDL, very low density lipoprotein; CETP, cholesteryl ester transfer protein; TGRL, triglyceride-rich lipoprotein; TPGS, tocopherol polyethylene glycol 1000 succinate; TPN, total parenteral nutrition; FIVE, familial isolated vitamin E deficiency.

¹To whom correspondence should be addressed.

of α -tocopherol (4). The source of these differences has been under investigation for decades (7). Our studies of the transport of various tocopherols in lipoproteins, as discussed further in section III, provide an explanation for these differences.

What is the evidence for *in vivo* lipid peroxidation? There have been reports of the presence of lipid hydroperoxides (8) and modified low density lipoproteins (9) in human plasma. As the techniques for measurements improve, undoubtedly the presence of short-lived, reactive species in low concentrations will be detected. At present the most compelling evidence for *in vivo* lipid peroxidation comes from measurements of pentane or ethane in expired breath. These gases are the major volatile hydrocarbons generated through peroxidation of *n*-3 and *n*-6 fatty acids. For example, vitamin E-deficient patients (10, 11), patients on long term total parenteral nutrition (12), or smokers (13) exhale increased amounts of these products of lipid peroxidation; administration of supplemental vitamin E can reduce pentane or ethane excretion to normal levels (10–13). Furthermore, in smokers supplemental vitamin E has been shown to protect low density lipoprotein from lipid peroxidation *in vitro* (14).

The metabolism of vitamin E is not well described. Essentially the function of vitamin E is to trap peroxyl radicals and break the chain reaction of lipid peroxidation (15). Vitamin E, itself, does not prevent the formation of carbon-centered radicals (1). Because α -tocopherol is the most potent antioxidant of the tocopherols, it reacts more quickly with peroxyl radicals than do other tocopherols or polyunsaturated fatty acids, and forms an α -tocopheroxyl radical (1). This radical is resonance-stabilized, therefore, the chain reaction is terminated rather than propagated (1). Subsequently, α -tocopherol is regenerated from the tocopheroxyl radical by ascorbic acid (16–19). Alternatively two α -tocopheroxyl radicals can react together forming a dimer, or the radicals can be completely oxidized to tocopherol quinone.

There is little evidence for tocopherol quinone formation *in vivo*. Current thinking is that once the α -tocopheroxyl radical is formed, then α -tocopherol is immediately regenerated, most likely by ascorbic acid, but glutathione and uric acid are also potential regenerating agents in the plasma (16–19). In this way, vitamin E is not metabolized, but instead is maintained in the body in the unoxidized state. This suggests that plasma and tissue vitamin E are replaced rather than metabolized. This topic will be discussed further in section VII.

II. INTESTINAL ABSORPTION AND LIPOPROTEIN TRANSPORT

Vitamin E because of its hydrophobicity requires special transport mechanisms in the aqueous milieu of the plasma, body fluids and cells. Unlike other fat-soluble

vitamins, vitamin E has no specific plasma transport protein, but rather is transported in plasma lipoproteins and its distribution parallels that of total lipids (20–30). The earliest measurements of the tocopherol contents of lipoproteins were carried out by Lewis, Quaife, and Page in 1954 (31), but more precise measurements and response to supplementation were performed by McCormick, Cornwell, and Brown in 1960 (32).

Studies using deuterium-labeled tocopherols have led to new insights into the absorption and transport of vitamin E (33–41). In humans, orally administered deuterated α -tocopherol is first secreted from the intestine in chylomicrons, then is secreted from the liver in very low density lipoproteins (VLDL) and appears in the plasma simultaneously in low and high density lipoproteins (LDL and HDL, respectively) (36). Each of these aspects will be discussed in more detail below.

A. Intestinal absorption and secretion in chylomicrons

The fractional absorption of vitamin E in humans has been estimated to be about 70%, based on the fecal recovery of an oral dose of radioactive α -tocopherol (42, 43). These are likely overestimates because any losses of radioactivity are included in the calculated amount of absorbed material. In thoracic duct-cannulated rats, 65% of unlabeled α - and γ -tocopherols appeared in the lymph after infusion of soybean oil into the duodenum (44). Additional α -tocopherol included in the infusate decreased the efficiency of absorption of α -tocopherol, but had no effect on γ -tocopherol absorption (44), demonstrating that α - and γ -tocopherols do not compete during absorption. It is unknown whether the same phenomenon of decreased vitamin E absorption in response to increasing vitamin E dose occurs in humans.

Bile acids are secreted by the liver into the small intestine where they function to aid in digestion of dietary fat. Micelles composed of products of lipid hydrolysis, dietary fats and biliary secretions form spontaneously in the intestinal lumen (45), which allows hydrophobic lipids to be attacked by pancreatic lipases. Vitamin E absorption requires the presence of bile acids for micelle formation, as demonstrated in bile duct-ligated rats (46) and in children with cholestatic liver disease (47).

Pancreatic enzymes may also aid absorption of vitamin E into enterocytes (48). Patients with cystic fibrosis do not secrete pancreatic enzymes and have been reported to become vitamin E-deficient (49–53). Although most cystic fibrosis patients receive replacement pancreatic enzymes to aid digestion, some continue to malabsorb vitamin E. These latter patients are likely also to have impaired liver function and limited bile flow.

The importance of pancreatic enzymes for cellular uptake of vitamin E was tested *in vitro* using a human intestinal cell line. Uptake of α -tocopherol occurred in the presence of bile acids and fatty acids, but was not further

potentiated by the addition of bile-activated lipase (54), a pancreatic enzyme thought to promote cholesterol absorption (55, 56). Thus, pancreatic enzymes are necessary for lipid hydrolysis, but not specifically to facilitate vitamin E absorption.

It would appear that the absorption of tocopherol into the enterocytes is a passive process with tocopherol moving with the intestinal lipids. Absorption is, therefore, facilitated by a liberal intake of fat. Further evidence of direct absorption is given by the observation that TPGS (tocopheryl polyethylene glycol 1000 succinate), which forms its own micelles at low concentrations, is absorbed in patients with cholestatic liver disease in whom the bile concentration of the intestinal contents is very low (44, 57, 58).

After uptake into intestinal cells, vitamin E is secreted in chylomicrons (44, 59, 60). When chylomicron synthesis is prevented in rats by the administration of puromycin, then vitamin E is not secreted into the lymph (61). Using deuterated tocopherols, studies in humans have demonstrated that the peak in tocopherol secretion in chylomicrons occurs between 6 and 12 h after oral administration of vitamin E (36, 38–40). This value is consistent with the plasma residence time of apolipoprotein B-48 in human chylomicrons of ~5 h, as estimated using amino acids labeled with stable isotopes (62). Although it takes several hours for the newly ingested vitamin E to be secreted in chylomicrons, the estimated half-life of chylomicrons labeled with [^3H] α -tocopherol, when injected into rats, is 12 ± 3 min (60).

Some transfer of vitamin E to tissues takes place during chylomicron catabolism. Chylomicrons are catabolized in the circulation by the endothelial-bound enzyme, lipoprotein lipase, which hydrolyzes triglycerides, releasing free fatty acids (Fig. 1A). Lipoprotein lipase also transfers tocopherols, along with fatty acids, to the tissues during this process (63). This process was demonstrated in vitro using lipid emulsions, fibroblasts as acceptors, and purified bovine lipoprotein lipase (63). Transfer of tocopherol to the cells required binding of the lipoprotein lipase to the cell surface because incubation with heparin, which prevents lipoprotein lipase from binding, prevented the increase in tocopherol content, but it did not alter triglyceride hydrolysis.

Tissues that receive most of their lipids during the delipidation cascade, such as adipose tissue and muscle, perhaps even the brain, probably obtain tocopherols as a result of lipoprotein lipase activity. In patients with lipoprotein lipase deficiency, who have a markedly slowed catabolism of chylomicrons and VLDL (triglyceride-rich lipoproteins, TGRL), ~80% of the plasma tocopherol is transported in TGRL (63, 64). Because of the elevated lipid levels, these patients have plasma α -tocopherol concentrations approximately 10 times normal, but low-normal adipose tissue α -tocopherol concentrations (63).

Thus, the deficiency in lipoprotein lipase does not result in vitamin E deficiency.

During the formation of chylomicron remnants by lipoprotein lipase, excess surface components are transferred to HDL (Fig. 1A). Cholesteryl ester transfer protein (CETP) also takes part in this reaction, exchanging cholesteryl esters from HDL to the remnants and triglyceride to HDL (65). Studies of the transfer of tocopherol between lipoproteins have demonstrated that CETP does not facilitate tocopherol exchange (66); HDL alone can transfer its newly acquired tocopherol by exchange processes to all of the other circulating lipoproteins (66–70). It is thus not surprising that during the first 6–9 h after an oral dose of an equimolar mixture of deuterated tocopherols, as a result of these exchanges, all of the lipoprotein fractions contain equimolar concentrations of the administered labeled tocopherols (36, 38, 40).

Chylomicron remnants, which have acquired apolipoprotein E from HDL, are taken up by the liver, probably by a receptor-mediated process. The LDL receptor, however, is not involved because chylomicron clearance in patients with familial hypercholesterolemia is not impaired, as reviewed (71). Furthermore, chylomicron clearance after duodenal infusion of a cholesterol-rich fat emulsion was similar in subjects expected to have very different LDL receptor levels: 1) normal levels (young and elderly men), 2) elevated levels (men on estrogen therapy), and 3) low levels (patients with heterozygous familial hypercholesterolemia) (72). Thus, most patients with metabolic defects in lipid or lipoprotein metabolism (except patients with lipoprotein lipase deficiency or with an inability to absorb vitamin E or secrete it in chylomicrons) should transport dietary vitamin E to the liver normally.

B. Secretion and catabolism of VLDL

After uptake of chylomicron remnants by the liver, the newly absorbed dietary lipids are secreted by the liver in VLDL (Fig. 1B). Studies using perfused rat livers or isolated rat hepatocytes (73, 74) have demonstrated directly that vitamin E is secreted in VLDL. This is a specific process that results in the preferential incorporation of *RRR*- α -tocopherol into nascent VLDL (Fig. 1B), as demonstrated using deuterated tocopherols in a perfused monkey liver system (39). Cynomolgus monkeys consumed equimolar amounts of 2*R*,4'*R*,8'*R*-, α -(5,7-(C^2H_3)₂)tocopheryl acetate (*d*₆-*RRR*- α -tocopheryl acetate), a source of hexadeuterated- α -tocopherol with natural stereochemistry, 2*S*,4'*R*,8'*R*-, α -(5-(C^2H_3)₂)tocopheryl acetate (*d*₃-*SRR*- α -tocopheryl acetate), a source of trideuterated α -tocopherol with unnatural stereochemistry, and 2*R*,4'*R*,8'*R*- γ -(3,4²H)tocopherol (*d*₂-*RRR*- γ -tocopherol), a source of dideuterated γ -tocopherol with natural stereochemistry. After 24 h the animals were killed, the

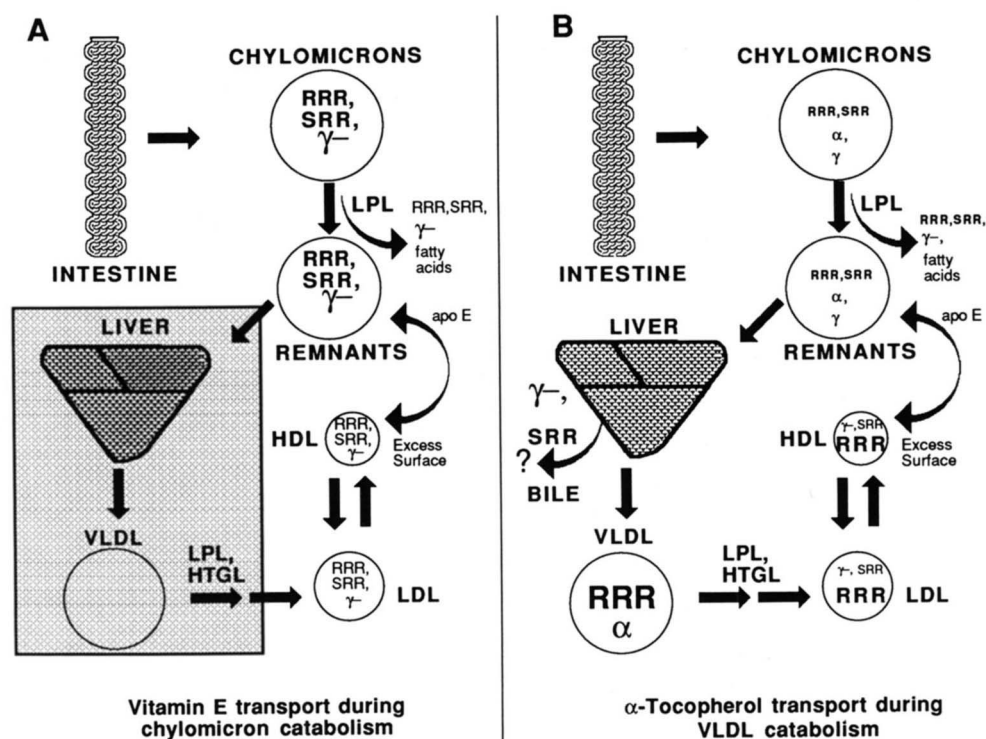


Fig. 1. Schemes of the transport of α -tocopherol in lipoproteins. Shown in A are the transfers of tocopherols during chylomicron catabolism. The intestine secretes chylomicrons containing various forms of vitamin E (RRR - and SRR - α -tocopherols, γ -tocopherol; RRR , SRR , γ , respectively). These chylomicrons are catabolized in the circulation by lipoprotein lipase (LPL), which transfers fatty acids and tocopherols to tissues. The chylomicron remnants can transfer tocopherols to HDL during the exchange of excess surface, and can acquire apolipoprotein E (apoE), which directs the remnants to the liver for catabolism. HDL tocopherols can transfer to other circulating lipoproteins, such as LDL and VLDL (transfer to circulating VLDL is not shown). Shown in B are the events following hepatic uptake of chylomicron remnants, VLDL secretion and catabolism. Following chylomicron remnant uptake, the liver secretes the lipids in nascent VLDL. The hepatic tocopherol-binding protein is thought to preferentially transfer RRR - α -tocopherol to VLDL during its assembly. Other forms of tocopherol (such as γ -tocopherol, or SRR - α -tocopherol) are probably excreted in the bile. High levels of vitamin E are not stored in the liver. Once VLDL is secreted in the circulation, both LPL and hepatic triglyceride lipase (HTGL) participate in the delipidation of VLDL to LDL. Only about half of the VLDL is converted to LDL; the remainder is taken up by the liver (not shown). During triglyceride hydrolysis by the lipases, tocopherol can be transferred to HDL in an analogous manner to transfer during chylomicron catabolism (transfer not shown). The secretion of RRR - α -tocopherol in nascent VLDL by the liver is the mechanism that maintains plasma tocopherol concentrations. The exchange of tocopherols between lipoproteins determines individual lipoprotein concentrations. Figures adapted from ref. 117.

livers were removed and perfused, and lipoproteins were isolated from the perfusate. Of the deuterated tocopherols found in the nascent VLDL, approximately 80% were d_6 - RRR - α -tocopherol, the naturally occurring stereoisomer with the highest biologic activity (39).

The effect of ethanol ingestion on liver α -tocopherol concentrations and lipid levels has been investigated because alcohol may induce hepatic injury via altered lipid metabolism and by free radical-mediated lipid peroxidation (75). Alcohol feeding reduces the α -tocopherol content of rat liver (76); despite doubling the lipid content, the α -tocopherol/lipids ratio is halved (0.11 ± 0.01 compared with $0.25 \pm 0.03 \mu\text{g}/\text{mg}$ lipid in the controls) (75). This could result from an impairment in liver function; therefore, the subcellular distribution of α -tocopherol in rat hepatocytes has also been investigated (77). The light

mitochondrial fraction contained the highest α -tocopherol concentrations, and within this fraction the α -tocopherol concentration was greatest in the lysosomes. The Golgi apparatus contained the highest concentrations of α -tocopherol in the microsomal fraction. Both of these organelles are important in lipoprotein metabolism. Chylomicron remnants and LDL are directed to the lysosomes following receptor-mediated uptake by the liver; the endoplasmic reticulum/Golgi apparatus is involved in the assembly and post-translational processing of nascent VLDL (78–81). Long term administration of ethanol promoted an enrichment of α -tocopherol in the Golgi apparatus, which Hagen et al. (77) suggested might result from a reduced secretion of VLDL-associated α -tocopherol, because ethanol reduces secretion of VLDL in rats (74, 82, 83).

Upon secretion into the plasma, nascent VLDL is catabolized by lipoprotein lipase and hepatic triglyceride lipase (84). As shown by Parhofer et al. (85) using stable isotopes in normal subjects, about half of the VLDL are partially delipidated in the circulation and returned to the liver, while the remainder are converted in the circulation to LDL. Therefore, α -tocopherol, which is secreted from the liver in VLDL, can have alternative fates. Some can be transferred to HDL during lipolysis, some can travel with the VLDL core during the conversion to LDL, and some can return to the liver as VLDL remnants (i.e., intermediate density lipoprotein (IDL)). In this way secretion of α -tocopherol in VLDL can lead to the enrichment of all circulating lipoproteins with α -tocopherol (Fig. 1B).

C. Transport in LDL

LDL are the major transport vehicles in the plasma for cholesteryl ester, and are taken up by cells requiring cholesterol by a high affinity, receptor-mediated process (86). The tocopherol in LDL can be acquired by tissues with LDL receptors, as demonstrated in vitro using fibroblasts with and without LDL receptors (87). Tissues for which this may be an important mechanism for obtaining tocopherol are the adrenal glands and the ovaries, as well as adipose tissue. Tissues with the highest LDL receptor activity in vivo also include the liver and the intestine (88).

LDL uptake is an important mechanism by which tissues obtain α -tocopherol. However, the mechanisms for delivery of α -tocopherol to tissues are redundant because Watanabe rabbits, which have defective LDL receptor activity, have been shown to have normal tissue α -tocopherol levels (89).

The role of antioxidants in the protection of LDL from autooxidation has been under intense investigation. Epidemiologic studies have shown, in 12 populations of men who have similar plasma cholesterol (5.7–6.2 mmol/l) and blood pressure, that the plasma α -tocopherol levels were negatively correlated with incidence of ischemic heart disease (90). Oxidized LDL may also be an initiating factor in atherosclerosis. Current theories propose that LDL may become oxidized in the interstitial space beneath the endothelial lining of the artery wall as a result of cellular oxidative processes (91, 92). Oxidized LDL may function as a chemotactic agent recruiting monocyte-macrophages from the plasma (93) and, because it can stimulate the synthesis of monocyte chemotactic protein-1 (94, 95), it can promote the recruitment of additional monocyte-macrophages. These cells, which have receptors for modified LDL, can scavenge oxidized LDL and become engorged with cholesteryl ester because this process is not down-regulated by cellular cholesterol concentrations (96). This cycle is especially damaging because macrophages can also oxidize LDL and oxidized LDL inhibits cell mobility, preventing

emigration of cholesteryl ester-loaded macrophages from the subendothelial space (93). Antioxidants, such as vitamin E, can protect LDL by preventing the propagation initiated by free-radical attack (97). However, once the antioxidants are consumed, peroxidation can go on unabated (97). Vitamin E, whether added in vitro to LDL (98) or administered orally in vivo (14, 99, 100), can prolong the lag phase before oxidation of other molecules. Clinical trials to test the efficacy of supplemental vitamin E on prevention of atherosclerosis (101) and studies in experimental animal models are necessary to investigate these interactions.

D. Exchange of tocopherol between lipoproteins and transport in HDL

One important aspect of vitamin E transport is the rapidity with which tocopherols move between lipoproteins. HDL tocopherol readily exchanges with other lipoproteins (66–70); only the tocopherols in TGRL (chylomicrons and VLDL) do not readily exchange (67, 69, 70). However, rapid hydrolysis of TGRL, with production of excess surface, allows transfer of tocopherol from TGRL to HDL and then to other lipoproteins, as discussed in sections IIA and B. In this manner, plasma vitamin E is in a constant state of flux between the lipoproteins.

It is not known if nascent HDL is secreted enriched in α -tocopherol from the liver. It is not likely that this is a quantitatively important mechanism for tocopherol transport from the liver because nascent HDL are secreted without a hydrophobic core (84). Certainly, HDL are important in normal subjects during the catabolism of TGRL, and they are important for transport of tocopherol in patients with abetalipoproteinemia, who lack other lipoproteins (see section IVA). In these patients HDL must deliver tocopherols to tissues, probably by exchange mechanisms, because normal tissue levels of α -tocopherol can be attained (102, 103).

In studies of the transfer of labeled tocopherol between lipoproteins, we found that the HDL tocopherol content was dependent upon the protein ratio of LDL/HDL; as the LDL in the incubation decreased, the labeled tocopherol in HDL increased (70). Furthermore, in lipoproteins isolated from subjects with LDL/HDL protein ratios varying from 0.3 to 1.6 mg/mg, as the percentage of HDL cholesterol in the plasma increased, the amount of tocopherol per protein in HDL also increased. Thus, a person who has a high HDL cholesterol level is likely to retain more tocopherol in the HDL fraction than those with low HDL. Both Behrens and Madere (25) and Clevidence and Lehmann (29) have reported that HDL α -tocopherol is related to HDL protein concentrations. The physiological importance of this observation needs further investigation.

Transport of vitamin E from peripheral tissues to the liver has not been studied extensively. HDL may serve to remove excess tissue tocopherol and return it to the liver, analogous to its role in promoting free cholesterol efflux from tissues, as reviewed (104). Tocopherol efflux from adipose tissue, one of the major stores of tocopherol in the body (105–107), may be important to maintain tissue levels during vitamin E deficiency. Indeed, the α -tocopherol contents of peripheral nerve are correlated with adipose tissue concentrations (109), therefore adipose tissue tocopherol levels have been used as indicators of vitamin E status in patients at risk for vitamin E deficiency (102, 109).

Tocopherol efflux from peripheral tissues does occur in vitamin E-deficient patients. Because adipose tissue tocopherol levels are decreased (102, 109), this demonstrates that there are mechanisms for the mobilization of tocopherols from adipose tissue. Furthermore, in recent studies of adult dogs fed a vitamin E-deficient diet, the time to deplete half ($t_{1/2}$) of the adipose tissue α -tocopherol was approximately 100 days (Pillai, S. R., M. G. Traber, J. E. Steiss, and H. J. Kayden, unpublished observations). This value is similar to the $t_{1/2}$ calculated using previously reported data in mature rats (70 days) (110) and much less than for guinea pigs (600 days) (111). These data suggest that the prolonged time to deplete adipose tissue α -tocopherol in guinea pigs may be unique, and that adipose tissue α -tocopherol may be more readily available than previously thought (105, 111).

III. DISCRIMINATION BETWEEN TOCOPHEROLS

Of the eight forms of dietary vitamin E (α -, β -, γ -, and δ -tocopherols and α -, β -, γ -, and δ -tocotrienols) α -tocopherol has the highest biologic activity, as noted in section I. Although dietary γ -tocopherol is many-fold higher than α -tocopherol (112), plasma and tissues are enriched in α -tocopherol (7, 25, 29, 30, 113, 114). Furthermore, when humans consume vitamin E supplements, either *RRR*- or *all rac*- α -tocopheryl acetates, plasma γ -tocopherol decreases (115, 116) within 24 h of α -tocopherol administration (30). During the first 12 h after a dose containing equal amounts of unlabeled α - and γ -tocopherols, both increased equally in the plasma, but by 24 h only plasma α -tocopherol remained elevated (30, 117). Thus, discrimination does not occur during vitamin E absorption, but is a post-absorptive phenomenon.

Discrimination between stereoisomers of α -tocopherol has also been studied because the commercially available, synthetic vitamin E supplements (*all rac* α -tocopheryl acetate) contain eight stereoisomers, half of which are in the 2*R*-form and half are in the 2*S*-form. The International Unit (IU) of vitamin E has been defined such that one mg of synthetic *all rac* α -tocopheryl acetate equals one IU, and one mg of natural *RRR*- α -tocopheryl acetate equals 1.36

IU. Because the differences in chirality at positions 4' and 8' on the phytyl tail have less effect than those at the 2 position where the phytyl tail joins the ring (118), *RRR*- and *SRR*- α -tocopherol, labeled with different amounts of deuterium, have been used to study discrimination between natural and synthetic α -tocopherols. Tissues from rats fed equimolar amounts of both stereoisomers up to 5 months show a marked preference for the *RRR*-form with only the liver demonstrating a lack of preference up to 30 days of study (33).

To investigate the discrimination between tocopherols, studies of lipoprotein transport of these two deuterium-labeled stereoisomers of α -tocopherol in normal humans were carried out. No discrimination between *RRR*- and *SRR*- α -tocopherols was observed during absorption and chylomicron secretion, but preferential secretion of *RRR*- α -tocopherol in VLDL was observed by 24 h (38).

Further studies of the discrimination between γ -tocopherol and the two stereoisomers of α -tocopherol were carried out in normal subjects and patients with genetic abnormalities of lipoprotein metabolism. They consumed an oral dose containing equimolar amounts of $d_2\gamma$ -tocopherol, and d_6RRR - and d_3SRR - α -tocopheryl acetates, then blood samples were obtained at various times up to 72 h (64). In normal subjects the plasma up to 9 h post-dosing contained equal concentrations of the three labeled tocopherols (Fig. 2). This resulted from the catabolism of chylomicrons, which contained equal concentration of the three labels (as illustrated in Fig. 1A). The role of chylomicrons in discrimination between tocopherols was also studied in a patient with lipoprotein lipase deficiency. Here, again, the plasma contained equal concentrations of the labeled tocopherols during chylomicron catabolism, but for a more prolonged period (up to 24 h) due to the slowed TGRL catabolism (not shown) (64). Furthermore, in a patient who had prolonged secretion of chylomicrons and an impaired secretion of lipoproteins containing apolipoprotein B-100, there was no discrimination between these three tocopherols up to 48 h (not shown) (64).

In normal subjects by 24 h after the dose of deuterated tocopherols, the plasma was preferentially enriched in *RRR*- α -tocopherol, resulting from the preferential secretion of *RRR*- α -tocopherol in nascent VLDL (Fig. 2, and as illustrated in Fig. 1B). No significant differences were observed between *SRR*- α - and *RRR*- γ -tocopherol concentrations in plasma lipoproteins or red cells, suggesting that these tocopherols are similarly transported in a nonspecific fashion.

The data from the patients with defined abnormalities of lipoprotein metabolism demonstrated that if the chylomicrons are in the circulation for a prolonged period of time, irrespective of whether this is due to their impaired catabolism or prolonged secretion, then the plasma contained all three of the administered tocopherols in vir-

tually equal concentrations (64). However, if VLDL particles are secreted, albeit abnormal VLDL particles, then the plasma is enriched in *RRR*- α -tocopherol (64). These data suggest that a hepatic protein discriminates between tocopherols and inserts *RRR*- α -tocopherol into VLDL during its assembly. Such a protein has been isolated from rat liver (119, 120) and shown to transfer *RRR*- α -tocopherol in preference to other forms of vitamin E (119), as described further in section VI.

IV. VITAMIN E DEFICIENCY IN HUMANS WITH LIPID MALABSORPTION

Vitamin E deficiency is seen rarely in humans. When it occurs, it is usually a result of lipoprotein deficiencies or lipid malabsorption syndromes. Studies in patients with abetalipoproteinemia or in patients with cholestatic liver disease have been essential to describe the neurologic

disorder resulting from vitamin E deficiency. With this specific description of neurologic abnormalities it has been possible to identify patients who have vitamin E deficiency and no other abnormalities known to cause vitamin E deficiency, as discussed in section V.

A. Patients with abetalipoproteinemia or homozygous hypobetalipoproteinemia

Vitamin E deficiency was first recognized in humans in 1965 when Kayden and Silber (61) proposed that the neurologic abnormalities seen in patients with abetalipoproteinemia, who have virtually undetectable plasma lipoproteins containing apolipoprotein B (chylomicrons, VLDL, LDL) (121), were similar to those observed in monkeys fed vitamin E-deficient diets (122). These patients inefficiently absorb vitamin E, and transport it only in high density lipoproteins (HDL). If small amounts of VLDL are secreted by abetalipoproteinemic patients, or

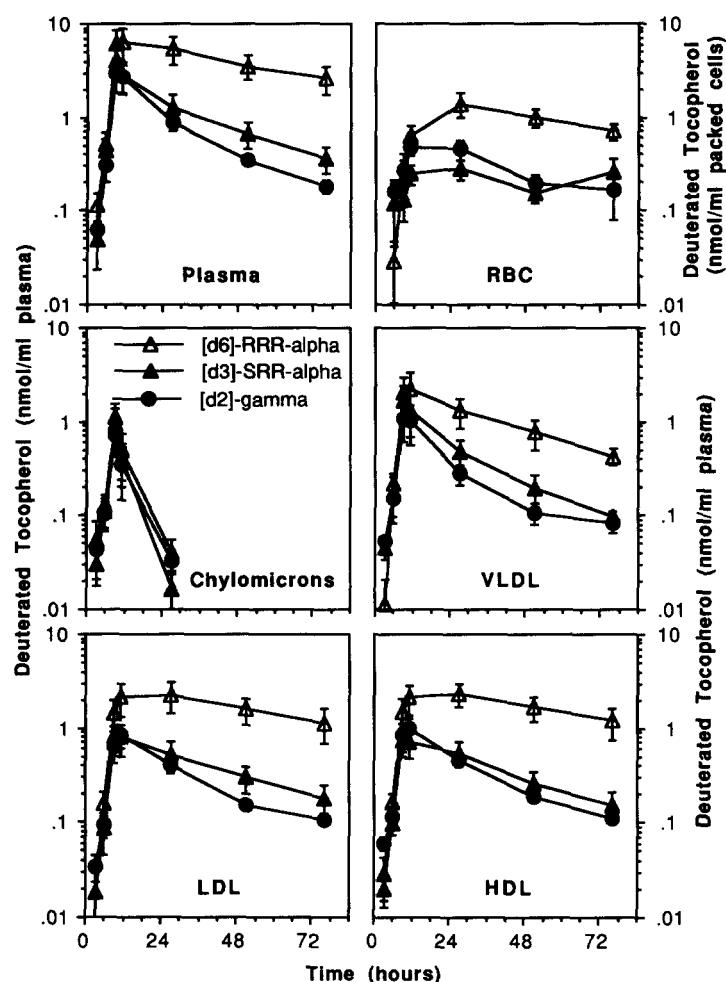


Fig. 2. Concentrations of deuterated tocopherols in plasma, red cells, and lipoproteins. Normal subjects (4) were given an oral dose containing equal amounts (50 or 75 mg) of d_6 -*RRR*- α -tocopheryl acetate, d_3 -*SRR*- α -tocopheryl acetate, and d_2 -*RRR*- γ -tocopherol, then blood samples were obtained at the indicated intervals. The mean \pm SEM of the deuterated tocopherol concentrations (nmol/ml) are shown at each time point in plasma, RBC, chylomicrons, VLDL ($d < 1.006$ g/ml), LDL ($1.006 < d < 1.063$), and HDL ($d > 1.063$). © J. Lipid Res. (64).

if rapidly turning over abnormal lipoproteins containing truncated forms of apolipoprotein B are secreted by patients with homozygous hypobetalipoproteinemia, then minor amounts of vitamin E could be transported in these lipoproteins. However, apolipoprotein B-containing lipoproteins constitute only a tiny fraction of the circulating lipoproteins in these patients.

Patients with abetalipoproteinemia, or homozygous hypobetalipoproteinemia, are aggressively treated with orally administered vitamin E supplements (100–150 mg/kg per day) (121). When provided from infancy, this level of supplementation is sufficient to prevent the occurrence of neurologic abnormalities associated with this disorder. When supplementation is initiated in older patients, who have not previously been treated, it can prevent the further deterioration of neurologic function.

As plasma levels of α -tocopherol in abetalipoproteinemic patients do not reach as much as 15% of normal values, due to the extremely low concentrations of plasma total lipids and absent lipoproteins, measurement of adipose tissue α -tocopherol levels (by needle aspiration biopsy) provides a suitable means of assessment of vitamin E status. Vitamin E-deficient abetalipoproteinemic patients supplemented with the recommended dose of vitamin E will increase their adipose tissue levels over months of treatment, and do reach normal concentrations (102, 109).

Recently, we have carried out studies on the ability of patients with abetalipoproteinemia to discriminate between natural and synthetic vitamin E, using deuterated α -tocopherols (Traber, M.G., D. Rader, R. Acuff, H. B. Brewer, and H. J. Kayden, unpublished observations). We anticipated that these patients might not discriminate between stereoisomers of α -tocopherol because they do not secrete VLDL. The patients' plasma deuterated α -tocopherol concentrations were only 1/10 to 1/100 of that seen in normal subjects, even though the administered dose was much larger in the patients (3.7 g of each isotope) compared with the control subjects (150 mg of each). Three of the patients discriminated normally between the forms of α -tocopherol, suggesting that the hepatic tocopherol-binding protein is present and functional. Two of the patients did not discriminate between stereoisomers of α -tocopherol; this is likely a result of a complete impairment in VLDL secretion. Thus, the ability of abetalipoproteinemic patients to absorb and transport oral α -tocopheryl acetate is markedly impaired and variable among patients. Patients should, therefore, be supplemented with natural *RRR*- α -tocopherol in high dosage.

B. Patients with cholestatic liver disease

Patients with cholestatic liver disease can become vitamin E-deficient if they secrete insufficient amounts of bile acids into the intestinal lumen, which results in limited or nonexistent vitamin E absorption (47). Thus, vitamin E, even when given in very large oral doses, is not absorbed

by these patients. They can be treated with a water-soluble form of vitamin E, TPGS (tocopheryl polyethylene glycol 1000 succinate) (44, 57), which, taken orally, forms micelles at low concentrations, and thus bile acids are not required in the intestinal lumen (44, 58). Alternatively, these patients can be treated with intramuscular injections of vitamin E.

In contrast to patients with abetalipoproteinemia, who take decades to develop neurologic abnormalities, patients with cholestatic liver disease develop severe neurologic abnormalities within a few years of life (123). They are usually infants or young children and because of the combination of their limited vitamin E stores, their inability to absorb vitamin E, and their impaired liver function, they are very susceptible to severe vitamin E deficiency.

The first detectable neurologic abnormality resulting from vitamin E deficiency in humans is decreased sensory perception (124). The severity of the neurologic disease in patients with cholestatic liver disease and in abetalipoproteinemia is correlated with the age at which supplementation is initiated, i.e., the duration of the vitamin E-deficient state. Most children with neonatal cholestasis who have not received supplemental vitamin E by age 4 show evidence of neurologic dysfunction (125). Other abnormalities resulting from vitamin E deficiency include: hyporeflexia or areflexia, truncal ataxia, limb ataxia, ophthalmoplegia, decreased proprioception, decreased vibratory sensation, proximal-muscle weakness, decreased light-touch sensation, decreased pain sensation, dysarthria, pes cavus, and scoliosis (126).

C. Patients receiving total parenteral nutrition

Patients receiving total parenteral nutrition (TPN) depend entirely upon intravenously administered nutrients. Their sources of vitamin E include an intravenously administered, daily multivitamin supplement (10 IU), and a lipid emulsion given every 2 or 3 days. The total average daily intake of α - and γ -tocopherols is approximately 10–12 mg and 40 mg, respectively (127). TPN patients receive lipid emulsions made from soybean oil, therefore, the ratio of polyunsaturated to saturated fatty acids is high. Because polyunsaturated fats are easily oxidized and lipid emulsions have been found to contain pentane, a product of the peroxidation of linoleic acid (128), it is important for TPN patients to achieve an adequate intake of lipid soluble antioxidants, i.e., vitamin E.

The vitamin E status of eight patients receiving TPN for 69 ± 45 (mean \pm SD) months was assessed by measuring plasma and adipose tissue tocopherol concentrations (127). Plasma α -tocopherols were similar to controls; however, adipose tissue α -tocopherol/cholesterol ratios were significantly lower (55 ± 36 versus 106 ± 63 , $P < 0.04$), suggesting that current vitamin E supplementation of TPN patients is insufficient for maintenance of adequate tissue stores. The inadequate antioxidant status

of TPN patients is further substantiated by Lemoyne et al. (12). They have suggested, based on breath pentane measurements (10), that home TPN patients have increased lipid peroxidation (12).

The inadequacy of vitamin E supplementation in TPN patients may be a result of an overestimation of the contribution of γ -tocopherol for antioxidant activity and tissue storage. Assessments of vitamin E intakes of TPN patients based on both α - and γ -tocopherols (7) are overestimates because the lipid emulsions they received were made with soybean oil, which contains 6–10 times more γ -tocopherol than α -tocopherol. Studies of discrimination between tocopherols in humans (see section III) have shown that α -tocopherol is preferentially maintained in the plasma, while γ -tocopherol is not. Discrimination between tocopherols in lipid emulsions has been investigated directly in normal subjects infused with lipid emulsions for 6 h (129). During the infusions plasma γ -tocopherol concentrations increased up to 10-fold, but by 24 h after infusion they returned nearly to baseline. These data suggest that the γ -tocopherol content of the lipid emulsion cannot be used to assess vitamin E intake (129).

Taken together, the studies of the vitamin E status of TPN patients demonstrate that: 1) the patients received limited amounts of α -tocopherol; 2) their γ -tocopherol intakes could not be equated to α -tocopherol; 3) they received primarily polyunsaturated fat, increasing their requirements for lipid-soluble antioxidants; 4) they had increased in vivo lipid peroxidation; and 5) their tissue stores of α -tocopherol were becoming depleted. Therefore, additional supplemental vitamin E should be administered to TPN patients and a long term study of their vitamin E status should be undertaken.

V. PATIENTS WITH FAMILIAL ISOLATED VITAMIN E DEFICIENCY

The recognition of the neurologic disorder associated with vitamin E deficiency has permitted the identification of a group of patients who have these neurologic abnormalities and extremely low plasma α -tocopherol concentrations, and yet have no lipid malabsorption or lipoprotein abnormalities.

A. Clinical characteristics

Familial isolated vitamin E deficiency (FIVE deficiency) has been described in 11 patients worldwide (40, 109, 130–138). These patients have neurologic abnormalities characteristic of vitamin E deficiency. That is, a peripheral neuropathy caused by the dying back of large calibre axons. All have decreased vibration sense; in some the abnormalities have progressed to include areflexia and ataxia. Supplemental vitamin E does halt the progression of this neurologic disorder, and amelioration of symptoms in some patients has been reported.

Patients with FIVE deficiency have nearly undetectable plasma vitamin E levels when consuming a normal diet. They do not have lipid malabsorption syndromes; their gastrointestinal function and lipoprotein metabolism are normal. When given vitamin E supplements (400–1200 IU/day), the patients maintain normal plasma α -tocopherol concentrations, but upon cessation of supplementation the plasma concentrations decrease dramatically within days to deficient levels (< 2 nmol/ml). This syndrome has been termed “familial isolated vitamin E deficiency” (40) because it has been observed in siblings (137) and in families (136); it is not secondary to lipid malabsorption syndromes or any other known cause of vitamin E deficiency; and the syndrome is responsive to the oral administration of vitamin E.

B. Impaired secretion of α -tocopherol in VLDL

FIVE deficiency is not the result of impaired absorption of vitamin E, as first demonstrated using an oral vitamin E tolerance test. Sokol et al. (137) administered a large oral dose of vitamin E (100 mg/kg, approximately 5–7 g) to each of four patients. Within the first 12 h the plasma α -tocopherol concentrations in the patients were equal to, or greater than, those in normal subjects. Subsequently, the plasma α -tocopherol concentrations in the patients decreased at a much faster rate and by 72 h had reached < 2 nmol/ml. Because the absorption of a pharmacologic dose of vitamin E was not impaired, the ability of these same four patients to absorb and transport a small oral dose of deuterated α -tocopherol (15 mg) was tested (40). Again, no impairment in α -tocopherol absorption was observed; chylomicrons from patients and controls contained similar concentrations of labeled tocopherol at all times. However, by 24 h plasma-deuterated tocopherol concentrations were significantly lower in patients than in controls. Furthermore, the labeled tocopherol in the plasma and the lipoproteins (except chylomicrons) decreased in the patients at a significantly faster rate. This faster decline in plasma-labeled α -tocopherol was attributed to an impairment in the secretion of deuterated α -tocopherol in VLDL.

These results can explain the response of FIVE deficiency patients to vitamin E supplementation. Because their secretion of α -tocopherol in VLDL is defective, when plasma α -tocopherol returns to the liver during the course of lipoprotein metabolism, α -tocopherol is not effectively re-secreted from the liver, and thus plasma α -tocopherol concentrations fall more rapidly than in normal subjects. When these patients are given vitamin E supplements, α -tocopherol is absorbed and secreted from the intestine in chylomicrons. During chylomicron catabolism, α -tocopherol is transferred to the other circulating lipoproteins (as illustrated in Fig. 1A); in this manner plasma levels are maintained. Upon cessation of supplementation with vitamin E, the input of dietary vita-

min E to the plasma lipoproteins during chylomicron catabolism is insufficient; thus, α -tocopherol levels fall quickly. Effective vitamin E supplementation in FIVE deficiency patients requires multiple dosing throughout the day, i.e., with each meal.

C. Impaired discrimination between stereoisomers of α -tocopherol

The impaired incorporation of α -tocopherol into VLDL seen in patients with FIVE deficiency most likely results from a defective, or absent, hepatic tocopherol-binding protein (40). Because the putative role of this protein is to preferentially incorporate *RRR*- α -tocopherol into VLDL (as discussed in section III), we hypothesized that FIVE deficiency patients should not be able to discriminate between natural *RRR*- and synthetic *SRR*- α -tocopherols (139). Therefore, we tested whether they could discriminate between these two orally administered stereoisomers labeled with different amounts of deuterium (139). The eight patients that we studied segregated into two groups, discriminators ($n=4$) and non-discriminators ($n=4$). Differences in plasma concentrations between the two stereoisomers depended upon the subject's ability to enhance transport of d_6 -*RRR*- α -tocopherol because d_3 -*SRR*- α -tocopherol was transported similarly in patients and controls. The normally enhanced transport of *RRR*- α -tocopherol resulting from the preferential incorporation into nascent VLDL by the hepatic tocopherol binding protein was defective or lacking in the FIVE deficiency patients.

In non-discriminators, plasma lipoprotein concentrations of *RRR*- and *SRR*- α -tocopherols were virtually identical, and decreased rapidly from peak concentrations that were coincident with the chylomicron peak concentrations. We suggest that non-discriminators have normal absorption and secretion of vitamin E in chylomicrons, but either: 1) lack the tocopherol-binding protein, or 2) have a defect in the portion of the protein that recognizes α -tocopherol, and thus does not bind it. Either defect would prevent effective incorporation of *RRR*- α -tocopherol into nascent VLDL for secretion by the liver, and thus result in the rapid disappearance of both labels from the plasma.

In discriminators, plasma d_6 -*RRR*-decreased more slowly than did d_3 -*SRR*- α -tocopherol, but faster than control d_6 -*RRR*- α -tocopherol. Nonetheless, the peak lipoprotein deuterated α -tocopherol concentrations were coincident with those in the chylomicrons. Thus, the discriminator's lipoproteins acquired most of the d_6 -*RRR*- α -tocopherol during chylomicron catabolism with minimal input during VLDL secretion and catabolism. Although some d_6 -*RRR*- α -tocopherol was incorporated in VLDL, it is likely that the tocopherol-binding protein in these patients is unable to insert normal amounts of d_6 -*RRR*- α -tocopherol into nascent VLDL during assembly.

It is evident that controls and patients with FIVE deficiency differed in their abilities to discriminate between stereoisomers of α -tocopherol. Because all subjects transported the unnatural stereoisomer similarly, it is probably transported nonspecifically. In FIVE deficiency patients the normally enhanced transport of d_6 -*RRR*- α -tocopherol via hepatic VLDL secretion is absent or defective, suggesting that the tocopherol-binding protein is absent or defective. Characterization of the precise genetic defect in these patients awaits isolation of the human tocopherol binding protein and cloning of the gene. From the present studies it would appear that there is heterogeneity in the genetic defects of this protein.

VI. TOCOPHEROL-BINDING PROTEIN

We have proposed that the tocopherol-binding protein is responsible for the incorporation of α -tocopherol into nascent VLDL (38-40, 64). In 1977 Catignani and Bieri (140) demonstrated that a partially purified, rat hepatic tocopherol-binding protein discriminated between α - and γ -tocopherols. In 1981 Murphy and Mavis (141) demonstrated that a fraction of rat liver cytosol transferred α -tocopherol between liposomes and microsomes. Recently, the purified rat tocopherol-binding protein was demonstrated to discriminate between α -, β -, δ -, and γ -tocopherols during transfer between liposomes and mitochondria (119).

The tocopherol-binding protein probably has a hydrophobic pocket that recognizes the free hydroxyl group and the three methyl groups on the chromanol ring, as well as the conformation of the 2 position of the phytyl tail. We suggest that after chylomicron remnant uptake by the liver and remnant hydrolysis in the lysosomes, dietary tocopherols become available for secretion. Hypothetically, the tocopherol-binding protein could preferentially transport *RRR*- α -tocopherol from the lysosomes to the endoplasmic reticulum for incorporation into VLDL during assembly.

It is not known if this protein becomes an integral part of the nascent lipoprotein, but studies of α -tocopherol transfer between lipoproteins have not demonstrated any α -tocopherol-binding or transfer proteins in plasma. It is, therefore, more likely that the hepatic tocopherol-binding protein does not remain bound to the VLDL, but releases α -tocopherol into the lipid milieu of the forming VLDL. This would allow the protein to act as a shuttle between the lysosomes and the site of incorporation into VLDL.

Recently, Yoshida et al. (142) demonstrated that immunoreactivity to the rat hepatic tocopherol-binding protein was detected in rat liver cytosol and lysate of hepatocytes, but not in the cytosol of rat kidney, spleen, adrenal glands, testes, lung, stomach, intestines, heart, skeletal muscle, and brain, or in the lysate of Ito cells, endothelial

cells, or Kupffer cells isolated from rat liver, or in rat plasma or lipoproteins. They found no evidence that the tocopherol-binding protein is involved in the biotransformation of tocopherol. These authors concluded that the tocopherol binding protein is unlikely to be involved in storage of tocopherol and that a general intracellular transfer function seemed unlikely. They suggested that the tocopherol-binding protein may be critical in the hepatic handling of α -tocopherol by specifically binding and retaining α -tocopherol, as well as possibly transferring α -tocopherol from an endocytic to a secretory compartment (142). Taken together, the available data suggest that the hepatic tocopherol-binding protein is required to discriminate between forms of dietary vitamin E, and is required for insertion of α -tocopherol into VLDL for secretion into the plasma, and thus regulate plasma α -tocopherol levels.

It is also likely that the tocopherol-binding protein functions in determining the fate of tocopherol returned to the liver from the plasma via HDL and LDL (Fig. 3). We have observed that newly ingested deuterated tocopherol replaces circulating plasma tocopherol by as much as 50% after a single dose (and even to higher percentages after 2–3 days of dosing). While there is no precise information on the routes of disappearance, it is likely that the “old” tocopherol is excreted into the bile, which is also the likely route for excretion of excess ingested tocopherol.

Regulation of Hepatocyte Vitamin E

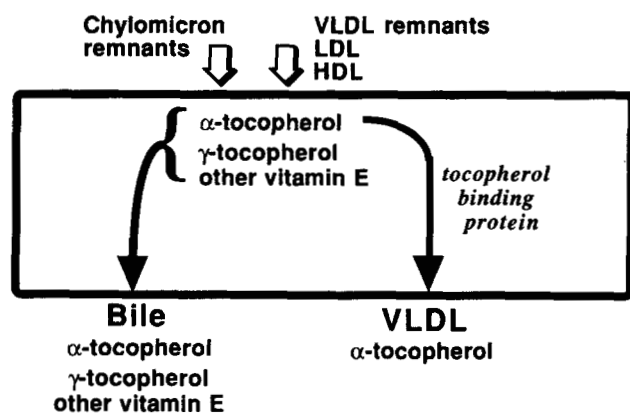


Fig. 3. Hypothetical mechanism for the regulation of plasma vitamin E by the hepatic tocopherol-binding protein. Dietary vitamin E enters hepatocytes in chylomicron remnants. Here the tocopherol binding-protein preferentially transfers *RRR*- α -tocopherol to nascent VLDL, perhaps transferring it from lysosomes to the endoplasmic reticulum. Excess α -tocopherol and other forms of vitamin E are excreted in the bile. The nascent VLDL are secreted into the plasma where they are catabolized—a process that results in the preferential enrichment of LDL and HDL with α -tocopherol. Vitamin E is returned to the liver during the course of lipoprotein metabolism. Thus, plasma concentrations of α -tocopherol are regulated by the secretion of α -tocopherol in VLDL, which is regulated by the hepatic tocopherol binding protein. © Kluwer Academic Publishers (147).

VII. REGULATION OF PLASMA α -TOCOPHEROL

The tocopherol-binding protein appears to be critical for the regulation of plasma α -tocopherol within a narrow range of concentrations. The studies in FIVE patients demonstrated that in the apparent absence of the binding protein, plasma α -tocopherol concentrations fall rapidly. Thus, the protein is necessary to maintain minimal levels of plasma α -tocopherol. In normal subjects during oral supplementation with large amounts of vitamin E (as much as 100 times the daily requirement of 15 mg), plasma α -tocopherol concentrations increase only 2- to 4-fold (21, 100, 143, 144). Thus, excess α -tocopherol does not markedly increase plasma α -tocopherol; certainly a 10-fold increase in plasma α -tocopherol in response to oral vitamin E supplementation has not been observed in humans. By contrast, infusion of lipid emulsions can raise plasma γ -tocopherol concentrations 10-fold, but only during the infusion, and within 24 h these decrease to baseline, about 10–20% of α -tocopherol concentrations (129).

Reports on tissue levels in humans in response to supplemental vitamin E are extremely limited. One adipose tissue sample from a subject who had taken additional vitamin E (1200 IU daily) for several years, but had discontinued for 1 year previously, was only 3 times the normal value (109). Unlike other fat-soluble vitamins, vitamin E is not toxic in high doses (145), perhaps because it is not stored in the liver (103).

If excess supplemental vitamin E is not stored in tissues, what is its fate? There may be limitations on the amounts of vitamin E that can be absorbed from the intestine, but this has not been documented in humans. Excess absorbed tocopherols are readily excreted in bile. After intravenous injection of chylomicrons labeled in vivo with [3 H] α -tocopherol into rats, about 14–20% of 3 H was excreted in the bile within the first 24 h (60, 146). In a preliminary experiment we observed that oral supplementation with α - and γ -tocopherols (300 mg of each) to a patient with an indwelling t-tube in the common bile duct after gall bladder surgery resulted in an increase in the excretion of both in bile with a simultaneous increase in α -tocopherol in the plasma (30). It appears that excess tocopherols are excreted and that the tocopherol-binding protein is necessary to salvage α -tocopherol, to prevent its excretion, and to facilitate its incorporation into VLDL for secretion into plasma.

The high biologic activity of α -tocopherol, compared with other forms of vitamin E, probably results from the activity of the hepatic tocopherol-binding protein. Because this protein salvages α -tocopherol that is returned to the liver during the course of normal lipoprotein metabolism and promotes its resecretion in VLDL, there is a rapid recirculation of α -tocopherol from the plasma to the liver to the plasma (139). Undoubtedly, plasma concentrations of tocopherols determine tissue concentrations, and because plasma α -tocopherol is maintained, α -

tocopherol is the form of vitamin E found in greatest concentrations in the tissues.

Biologic activity is measured by assessing the amount of the various forms of vitamin E necessary to prevent deficiency symptoms. Therefore, the form of vitamin E that is best delivered to susceptible tissues is the one with the highest activity. Due to the action of the hepatic tocopherol-binding protein, α -tocopherol has the highest biologic activity. Limitation of the ability of the tocopherol-binding protein to transfer other various tocopherols results in their lower biologic activities. Similarly, the conformations of the stereoisomers of α -tocopherol must affect their binding and transfer by the tocopherol-binding protein and result in their lower biologic activities.

VIII. CONCLUSIONS

Taken together, the studies on the absorption, transport, and discrimination between forms of vitamin E all lend support to the following concepts. 1) Plasma α -tocopherol levels are regulated; 2) the regulation is quantitative and qualitatively specific with the preferential incorporation into nascent VLDL of *RRR*- α -tocopherol compared with other stereoisomers of α -tocopherol and other forms of vitamin E (tocopherols and tocotrienols) (Fig. 3); 3) the hepatic tocopherol-binding protein is necessary and sufficient to carry out this intrahepatocyte transfer function; 4) the lack of this protein results in vitamin E deficiency due to the rapid removal of α -tocopherol from plasma and excretion, perhaps in bile; and 5) supplemental vitamin E does not markedly increase plasma α -tocopherol levels beyond 2- to 4-fold because of the quantitative limitation of incorporation of α -tocopherol into VLDL by the tocopherol-binding protein. These data also suggest that differences in the biologic activity of the various forms of vitamin E, as discussed in I and IV, result from differences in the affinity of the hepatic tocopherol-binding protein for these compounds. Verification of these hypotheses awaits the purification and characterization of the human tocopherol-binding protein and the characterization of the precise genetic defects in the FIVE patients. ■

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