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INVITED REVIEW

ABSORPTION, TRANSPORT AND METABOLISM OF VITAMIN E

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Vitamin E includes eight naturally occurring fat-soluble nutrients called tocopherols and dietary intake of vitamin E activity is essential in many species. α-Tocopherol has the highest biological activity and the highest molar concentration of lipid soluble antioxidant in man. Deficiency of vitamin E may cause neurological dysfunction, myopathies and diminished erythrocyte life span. α-Tocopherol is absorbed via the lymphatic pathway and transported in association with chylomicrons. In plasma α-tocopherol is found in all lipoprotein fractions, but mostly associated with apo B-containing lipoproteins in man. In rats approximately 50% of α-tocopherol is bound to high density lipoproteins (HDL). After intestinal absorption and transport with chylomicrons α-tocopherol is mostly transferred to parenchymal cells of the liver were most of the fat-soluble vitamin is stored. Little vitamin E is stored in the non-parenchymal cells (endothelial, stellate and Kupffer cells). α-Tocopherol is secreted in association with very low density lipoprotein (VLDL) from the liver. In the rat about 90% of total body mass of α-tocopherol is recovered in the liver, skeletal muscle and adipose tissue. Most α-tocopherol is located in the mitochondrial fractions and in the endoplasmic reticulum, whereas little is found in cytosol and peroxisomes. Clinical evidence from heavy drinkers and from experimental work in rats suggests that alcohol may increase oxidation of α-tocopherol, causing reduced tissue concentrations of α-tocopherol. Increased demand for vitamin E has also been observed in premature babies and patients with malabsorption, but there is little evidence that the well balanced diet of the healthy population would be improved by supplementation with vitamin E.

KEY WORDS: Vitamin E, α-tocopherol, absorption, transport, hepatic secretion, ethanol.

ESSENTIALITY OF VITAMIN E

In 1922 Evans and Bishop[1] demonstrated that rats fed a diet deficient in certain lipids developed reproductive failure. The male rats showed loss of spermatogenesis, whereas female rats were unable to retain zygotes. The missing substance was characterized and called vitamin E.[2,3] Since then muscular dystrophy, exudative diathesis, megaloblastosis, pulmonary degeneration, nephrosis and liver necrosis, have been observed experimentally in several animal species as signs of vitamin E-deficiency.[4,6]

Vitamin E comprises eight naturally occurring essential fat-soluble nutrients.[6] The series is made up of four compounds with tocol-structure bearing a saturated phytyl

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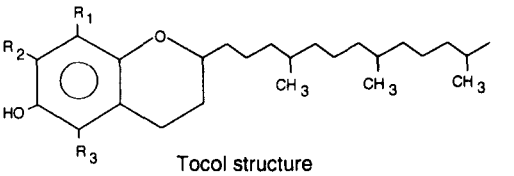
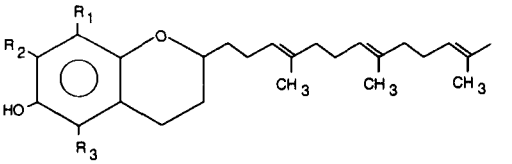
C_{16} sidechain (α-, β-, γ- and δ-tocopherol) and four compounds with tocotrienol structure bearing three double bonds in the phytyl side chain (α-, β-, γ- and δ-tocotrienol) (Figure 1). The term tocopherol is derived from the Greek words tokos and pherein, meaning to bring forth childbirth. Of the eight fat-soluble derivatives, α-tocopherol predominates in many species and has the highest biological activity,[7] the active site being the 6-hydroxyl group.[7,8] The side chain in the 2-position facilitates the incorporation and retention of vitamin E in biological membranes, so that the 6-position is optimal for scavenging free radicals and terminating lipid peroxidation[9] (Figure 2). The relative biological activity of the different vitamers is usually given as: 1 for α-tocopherol, 0.4 for β-tocopherol, 0.1–0.3 for γ-tocopherol, 0.01 for δ-tocopherol and 0.3 for α-tocotrienol based on *in vivo* tests.

All cells exposed to molecular oxygen are at risk of being damaged by O_2-derived free radicals (e.g., superoxide anion radical, hydroxyl radical) and lipid peroxidation products.[10,11] A significant number of complementary antioxidant systems provide an effective defense against oxidative damage.[12,13] This includes superoxide dismutase in mitochondria (Mn-containing), cytoplasm (Cu/Zn-containing) and an extracellular type, glutathione peroxidase (Se-dependent cytoplasmic enzyme), catalase (Fe-containing peroxisomal enzyme), ascorbate, β-carotene, urate and oxidizable peptides like glutathione. Vitamin E is the lipid-soluble antioxidant with the highest molar concentration, which may quench free radicals and act as a terminator of lipid peroxidation.[14,15] The vatamin E radical then formed is fairly stable, because the unpaired electron on the oxygen atom in the C-6 position can be delocalized into the aromatic ring structure, thereby increasing the stability.[8] The vitamin E radical may be reduced back to vitamin E by ascorbate[16,17] and glutathione.[14] Other possible fates of the vitamin E radical are formation of α-tocopheryl quinone, and reaction with another vitamin E radical to form a dimer.[14]

In man, deficiency of vitamin E may produce diminished erythrocyte life span,[18,19] neurological dysfunction and myopathies in patients with different malabsorption syndromes including a-β-lipoproteinemia.[20,23] The most common states of malabsorp-



Tocol structure



Tocotrienol structure

FIGURE 1 Tocol- and tocotrienol structure. R stands for residue and represents for α-tocopherol; R_1, R_2 and R_3 are all methyl (CH_3) groups. β-Tocopherol; $R_1 = CH_3$, $R_2 = H$ and $R_3 = CH_3$. δ-Tocopherol; $R_1 = CH_3$, R_2 and $R_3 = H$. γ-Tocopherol; R_1 and $R_2 = CH_3$ and $R_3 = H$. Corresponding nomenclature is used for the tocotrienols, where three double bonds are located in the side chain.

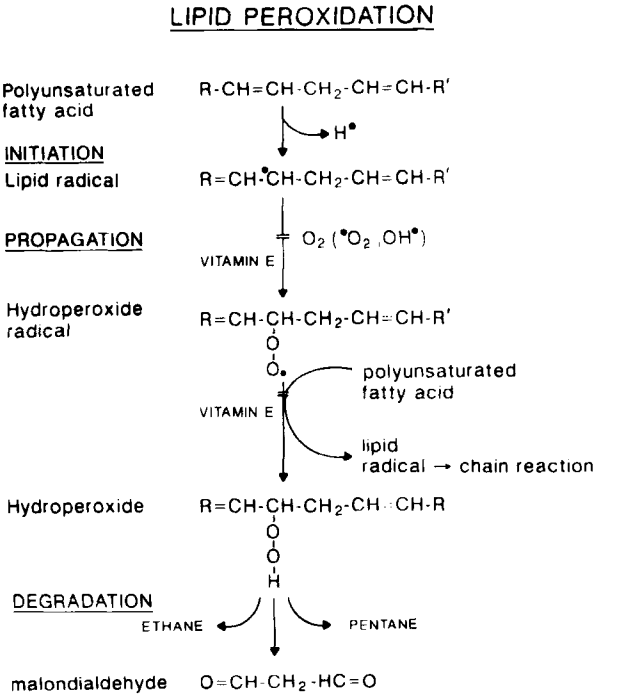


FIGURE 2 Lipid peroxidation is a chain reaction initiated by abstraction of a hydrogen atom from a polyunsaturated fatty acid, leaving behind an unpaired electron on the carbon atom. This lipid radical may react with oxygen (O_2) or oxygen-derived reactive molecules to form a hydroperoxide radical. Hydroperoxide radicals may abstract hydrogen atoms from other lipid molecules to continue the chain reaction, resulting in a variety of products, e.g. ethane, pentane and malondialdehyde. Vitamin E may terminate this chain-reaction at different steps by itself becoming a radical from giving up a hydrogen atom. The vitamin E radical is relatively little reactive compared to other lipid radicals.

tion causing vitamin E deficiency are cholestatic liver disease in children or primary biliary cirrhosis in adults, cystic fibrosis and pancreatic insufficiency. Progressive spinocerebellar degeneration with ataxia, areflexia and impaired vibratory sensation are striking signs of vitamin E deficiency. The most convincing information on the essential role of vitamin E in man was obtained from studies on patients with inborn errors of metabolism, causing defective secretion of chylomicrons. Both with hypo-β-lipoproteinemia and a-β-lipoproteinemia malabsorption of fat may cause deficiency of fat-soluble vitamins.[24]

Recently, some patients with isolated familial vitamin E deficiency have been described, who have normal fat absorption. Studies with deuterated α-tocopherol suggest that these patients have a defect in the incorporation of α-tocopherol in lipoproteins secreted by the liver.[25]

Vitamin E deficiency has been linked to the development of bronchopulmonary dysplasia,[26] intraventricular hemorrhage in the brain,[26,27] retrolental fibroplasia[25,28] and anemia in premature children.[29] In addition, alterations of vitamin E status have been associated with devleopment of certain forms of cancer,[30–33] cardiovascular diseases,[34,35] and effects on immune functions.[36–39]

INTESTINAL ABSORPTION

Intestinal absorption of lipids and fat-soluble vitamins depends on pancreatic function, biliary secretion, micellar formation and transport across intestinal membranes.[40–42] The absorption of α-tocopherol has been most extensively studied in rats, but data from humans are scarce.[43,44] The major dietary sources of vitamin E for humans are flour and vegetable oils, whereas very little of vitamin E is present in marine lipids.[14]

α-Tocopheryl acetate has been the most frequently used substance to evaluate the extent of α-tocopherol absorption, but the absorption of free α-tocopherol has also been examined. In early reports a variety of methods were employed to study the absorption of α-tocopherol, including measuring the amount of fecal unabsorbed α-tocopherol after oral feeding[44–47] and gut perfusion experiments.[48,49] The methods used were unspecific, and quantitative interpretation of the results is difficult due to a great number of experimental variables used in the different experiments.

By cannulating the main mesenteric lymph duct[50–52] or the thoracic lymph duct,[53–55] it is possible to obtain a more accurate measure of the amount of α-tocopherol absorbed. One major inherent weakness of cannulating the main mesenteric lymph duct is that some mesenteric lymph is drained by accessory ducts. Another point of concern is that the absorption is usually studied in a postoperative state. On the other hand the measured uptake provides minimum estimates for the absorption. Another advantage of using mesenteric lymph duct cannulation is that interference from hepatic and peripheral lymph, also drained by the thoracic duct, can be avoided. When evaluating the results of these experiments, it is also important to keep in mind that the way of administering α-tocopherol may be crucial. Previous studies on absorption of triacylglycerols have revealed that about 50% of the administered dose was absorbed when given as a bolus injection,[56–58] in contrast to an almost complete absorption after a slow, continous infusion at a rate similar to dietary input.[55,59]

When radiolabeled α-tocopherol is administered intraduodenally to rats as a bolus, the absorption of α-tocopherol via mesenteric lymph is approximately 15–20%, as evaluated by cannulation of the main mesenteric lymph duct.[50–52] Bjørneboe *et al.*[52] did not solely rely on measurements of radioactivity in the mesenteric lymph, but the mass of α-tocopherol absorbed was also determined by high performance liquid chromatography in combination with fluorescence detection. When absorption of α-tocopherol and oleic acid was studied during simultaneous duodenal administration of (^{3}H) α-tocopherol and (^{14}C) oleic acid, the latter appeared faster in mesenteric lymph, and total recovery were about 15 and 45%, respectively.[52] Since the absorption of oleic acid under physiological conditions is almost complete, this observation may indicate that the absorption of α-tocopherol is more complete (~40%) than previously suggested.

Traber *et al.*[55] have reported that the absorption of α-tocopherol was approximately 65% when α-tocopheryl acetate was administered as a slow continuous infusion into the duodenum in rats.[55] The amount of α-tocopherol administered was of the same order of magnitude as used in the mesenteric lymph duct studies.[52] The discrepancy between the results from mesenteric lymph duct studies and the findings of Traber *et al.*,[55] with respect to absorption of α-tocopherol, may be due to different ways of administering α-tocopherol and difference in length of time the animals had to recover from surgery.

It is generally accepted that α-tocopheryl esters are hydrolyzed during the absorp-

tive process. Under physiological conditions the hydrolysis occurs in the gut lumen, but a mucosal esterase has also been identified in the endoplasmic reticulum of the enterocytes.[60] Bile salts are required to form micelles, which are necessary for normal absorption of dietary lipids. In patients with malabsorption it is possible to deliver α-tocopheryl esters to the enterocyte by administering α-tocopherol in micellar form.[55,61]

Data based on studies with everted small bowel sacs, with and without metabolic inhibitors, indicate that α-tocopherol is absorbed by a passive diffusion process from the small intestine to the enterocyte.[48] but the transport of α-tocopherol through the epithelial cell is not well understood. In rats it is likely that the area at the junction between the upper and middle thirds of the small intestine is the region with greatest uptake of α-tocopherol.[40,48]

Traber *et al.*[55] have reported that the efficiency of α-tocopherol absorption decreases when the amount of α-tocopherol given to experimental animals increases. The reduced absorption of α-tocopherol with increasing doses may be of relevance when supplementation with α-tocopherol is provided. Traditional bioassays have indicated that α-tocopheryl acetate may be more efficient in relieving signs of vitamin E deficiency than free α-tocopherol.[62,63] When α-tocopherol and α-tocopheryl acetate were dosed twice daily, the net uptake of α-tocopherol from the free form was estimated to be only half that from the ester form.[64] However, from cannulation studies no such difference in rates of absorption between the two forms of α-tocopherol was observed,[55] and with a high intake in man (> 400 IU/day) higher tocopherol levels were obtained with free tocopherol than with tocopheryl ester, suggestive of some limitation in hydrolysis of the ester at this level of intake.[65] The previously observed difference in biopotency between α-tocopherol and its ester form could also be due to oxidation of the free form. Other studies further suggest that medium-chain triglycerides enhance the absorption process,[54] whereas retinoic acid[66] and long-chain polyunsaturated fatty acids[54] may reduce the absorption of α-tocopherol. Long-chain polyunsaturated fatty acids may also enhance the oxidation of α-tocopherol *in vivo*,[5,67] and the observed inhibition of absorption of α-tocopherol may therefore be of particular clinical interest.

About 99% of the α-tocopherol in lymph is transported in association with chylomicrons.[52] Unlike retinol and cholesterol, α-tocopherol is not re-esterified during the intestinal absorption process. In contrast to chickens,[39] it is unlikely that absorption of α-tocopherol by the portal vein makes any significant contribution to the overall absorption of α-tocopherol in rats and humans.[52]

The absorption of tocopherols other than the α-form has not been extensively studied. However, the intake of γ-tocopherol is 2–4 times that of α-tocopherol because of its dietary predominance, and it has been proposed that γ-tocopherol may contribute as much as 20–30% of the total vitamin E activity in the diet.[68] Despite this, several studies indicate that the level of γ-tocopherol averages only 10–15% of the concentration of α-tocopherol in adult human plasma.[68,69] From cannulation studies no major difference in the rate of absorption of α- and γ-tocopherol was observed;[50,55] even excess of α-tocopherol did not reduce the absorption of γ-tocopherol.[55] In man it has been observed that ingestion of high levels of α-tocopherol results in a marked lowering of γ-tocopherol,[70] which may be promoted by preferential secretion of γ-tocopherol to bile due to decreased secretion in lipoproteins released from the liver.[71]

TRANSPORT OF α-TOCOPHEROL

Lipoproteins are plasma lipid transport vehicles consisting of a hydrophobic core, containing triacylglycerol and cholesterol ester, and an amphipatic surface comprised of unesterified cholesterol, phospholipids and a large variety of apolipoproteins. Lipoproteins are heterogeneous in size, chemical composition and function. The difference in density divides the lipoproteins into four major classes, chylomicrons, very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL). Lipoproteins are dynamic structures, and transfer of molecules occurs between the core and surface of lipoproteins, between lipoproteins and between lipoproteins and tissues.[72] The lipoproteins are mainly synthesized in the intestine (chylomicrons and HDL) or liver (VLDL and HDL), or they are formed in plasma (HDL and LDL) during metabolism of triacylglycerol-rich lipoproteins. The

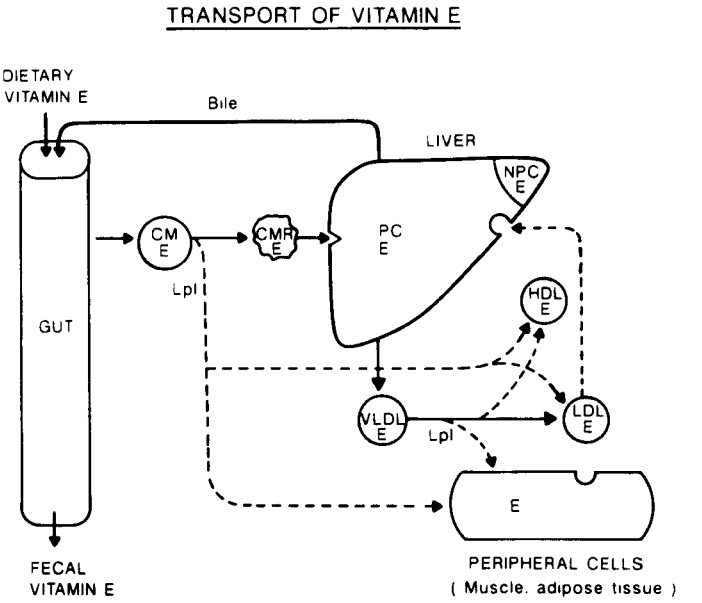


FIGURE 3 Transport of vitamin E between different tissues. Dietary esters of vitamin E are hydrolyzed and the unesterified form is absorbed in the small intestine, incorporated into chylomicrons (CM) and secreted into the intestinal lymph. Lipoprotein lipase (Lpl) is located on the luminal side in capillaries of several tissues and hydrolyses the triacylglycerols in the core of chylomicrons, thereby forming chylomicron remnants (CMR). Along with the free fatty acids some vitamin E might be transferred to different peripheral tissues during lipolysis. CMR are taken up by parenchymal cells (PC) in the liver, via an unregulated CMR-receptor. Most vitamin E is stored in the parenchymal cells, but some is also found in the nonparenchymal cells (NPC). Vitamin E is secreted from the parenchymal cells in association with very low density lipoproteins (VLDL). These particles are metabolized by lipoprotein lipase the same way as chylomicrons. Some vitamin E in the VLDL particles ends up in low density lipoproteins (LDL) as these lipoproteins are transformed in plasma. Vitamin E in LDL may be taken by receptor mediated endocytosis via the LDL-receptor (also called the apoB/E-receptor). Some vitamin E associated with chylomicrons and VLDL are probably transferred to peripheral cells and high density lipoproteins (HDL) during lipolysis by lipoprotein lipase. A significant amount of vitamin E is secreted into the bile as is, or metabolized. A large fraction of vitamin E is not absorbed in the intestine. Pathways of minor or unknown importance are marked with dotted lines.

majority of apolipoproteins are synthesized in the liver and intestine,[73] and they may regulate different enzymes involved in the metabolism of lipoproteins and act as ligands recognized by different cellular receptors.[72,73]

α-Tocopherol is transported in blood associated with lipoproteins (Figure 3) both in humans[74–77] and in rats,[50,52,78–80] and no specific plasma transport protein has been described. In nonfasting rats α-tocopherol is approximately evenly distributed between VLDL ($d < 1.006$ g/ml) and HDL (1.05 g/ml $< d <$ 1.21 g/ml),[52] whereas LDL is the lipoprotein fraction containing most α-tocopherol in fasting humans.[74,76,77] A high correlation exists between the concentration of total lipids and α-tocopherol in serum,[39] but the serum concentration of α-tocopherol might also be correlated with the amount of proteins in the different lipoprotein classes.[77] Dietary supplementation with vitamin E to humans (2 g tocopheryl acetate per day) may result in a rapid and parallel increases in the content of α-tocopherol in blood plasma and erythrocytes.[76] In red blood cells most α-tocopherol is located in the membrane fraction,[81] and a chiral discrimination between different stereoisomers of α-tocopherol has been described, with preferential retention of the natural occurring form, RRR- stereoisomer.[82] Based on studies examining the relative sensitivities of different blood components to changes in dietary intake of vitamin E, it is likely that the most sensitive marker of dietary status of α-tocopherol in blood is the content of α-tocopherol in platelets.[83,84]

The uptake of α-tocopherol in peripheral tissues may occur during catabolism of triacylglycerol-rich lipoproteins by the activity of lipoprotein lipase,[85] via the LDL (apoB/E) receptor or by nonreceptor-mediated uptake.[86]

The fate of pharmacological doses of pharmaceutical preparations of α-tocopherol has been examined after intravenous[87,88] and intramuscular administration,[89] whereas physiological processing of newly absorbed α-tocopherol has been offered little experimental attention. When clearance of (^{3}H)α-tocopherol was studied after intravenous injection of intestinal lymph labeled *in vivo* with radioactive α-tocopherol, the half-life was about 12 min.[79,80] This $t_{1/2}$ is similar to that of retinol and cholecalciferol associated with chylomicrons.[91] The half-life of chylomicrons *in vivo* is in the range of 5–15 min,[92–94] and the chylomicron remnants, formed by the action of lipoprotein lipase, are rapidly taken up via remnant receptors on the surface of hepatocytes.[95–96] These data, along with data on hepatic uptake of radiolabeled vitamin E (see next section on hepatic uptake), suggest that α-tocopherol primarily is cleared from blood in association with chylomicron remnants.

Furthermore, a redistribution of α-tocopherol from chylomicrons/VLDL to HDL was observed in rats with time after intravenous injection of lymph labeled *in vivo* with (^{3}H)α-tocopherol,[79] as also demonstrated *in vitro*.[97] The transfer of α-tocopherol between lipoprotein particles is markedly slower than what is found for cholesterol. After functional hepatectomy, with ligation of the hepatic artery and the portal vein, it was observed that the redistribution of α-tocopherol from chylomicrons/VLDL to HDL was delayed.[79] This finding suggests that the transfer process is at least partly dependent on processing of chylomicron-associated α-tocopherol by the liver. Although a neutral lipid transfer protein has been described in human plasma, it does not regulate α-tocopherol transfer between plasma lipoproteins.[98] The presently available data on transfer of α-tocopherol between lipoproteins do not allow us to make accurate estimations on the relative importance between the redistribution taking place in plasma or via the liver. Another unclear point is by what mechanism the transfer of α-tocopherol takes place between lipoproteins. One possibility is that

α-tocopherol is transferred from one lipoprotein particle to another via the water phase, as has been suggested for cholesterol.

HEPATIC UPTAKE OF α-TOCOPHEROL

The liver consists of several different cell types, the parenchymal cells being larger than the nonparenchymal liver cells and accounting for 85–90% of the total liver mass.[99] The nonparenchymal liver cells represent about 35% of the total number of cells, and may be divided into stellate cells, Kupffer cells and endothelial cells.[99–101]

Studies of hepatic uptake of intravenously injected (^{3}H)α-tocopherol have shown that maximum content in the liver was obtained after about 45 min, when more than 50% of the injected dose of (^{3}H)α-tocopherol was recovered in this organ.[79,80] Hepatic radioactivity then declined, and after 24 h about 11% of the injected dose was retained in the liver. When parenchymal and non-parenchymal liver cells were separated by differential centrifugation, it turned out that α-tocopherol was preferentially taken up by parenchymal cells.[79]

Measurement of α-tocopherol mass have revealed that at least 75% of hepatic α-tocopherol is located in parenchymal cells and that, at most, 25% is confined to nonparenchymal cells as evaluated by differential centrifugation.[52] Thus, transfer of α-tocopherol between parenchymal and nonparenchmal cells may take place after primary uptake in parenchymal cells, even though a direct uptake of α-tocopherol by nonparenchymal cells cannot be excluded.

Centrifugal elutriation makes it possible to obtain a satisfactory separation of different nonparenchymal liver cells.[102] Using this method it was found approximately twofold higher concentration of α-tocopherol per mg protein, in the peroxidase-positive cells (Kupffer cells) as compared to peroxidase-negative cells (stellate cells and endothelial cells), isolated from rats fed an ordinary pellet diet containing about 30 mg vitamin E/kg.[103] In this study the parenchymal cells contained about 90% of hepatic α-tocopherol, which is somewhat higher than previously published.[52] It should be noted, however, that the rats in the latter experiment[103] had been fed a diet containing markedly higher amounts of vitamin E prior to the experiments.

Distribution of α-tocopherol to the different types of liver cells is dependent on the nutritional status of the animals. After dietary supplementation with high amounts of α-tocopherol the content of α-tocopherol in the parenchymal cells increased sixfold, whereas the content α-tocopherol in the nonparenchymal cells was similar to presupplemented values.[103] This finding indicates that parenchymal liver cells have a large storage capacity for dietary surplus of α-tocopherol. When rats were deprived of α-tocopherol for 8 weeks, the parenchymal liver cells had the least reduction of their content of α-tocopherol. In contrast, concentrations of α-tocopherol in nonparenchymal liver cells were greatly reduced, to less than 5% of predepleted values. Accordingly, parenchymal liver cells may have the ability to conserve their content of α-tocopherol when the supply is limited, possibly by mobilization from other tissues.

Subcellular distribution of α-tocopherol in rat liver has been reported by several investigators,[104–110] but most authors have presented data on the content of α-tocopherol in one single subcellular compartment. However, the heavy mitochondrial, light mitochondrial and microsomal fractions all contain high concentrations of α-tocopherol, whereas the level is low in cytosol. The light mitochondria has the highest concentration of α-tocopherol as evaluated per mg protein[103,108] and per micromole of

phospholipids.[109] By gradient centrifugation of the light mitochondrial and microsomal fractions, lysosomes and Golgi apparatus were found to be particularly rich in α-tocopherol, whereas the concentration was very low in peroxisomes.[108] The high content of α-tocopherol in the hepatic Golgi fraction is probably due to the high amount of lipoprotein particles (VLDL) being transferred through this organelle before they are released to the sinusoids. It is rather striking that peroxisomes contain very little α-tocopherol, but it is possible that catalase represents the major antioxidant factor in these oganelles. By subfractionation of liver mitochondria it has also been demonstrated that approximately three fourths of mitochondrial tocopherol is located in the outer membrane, whereas one fourth is associated with the inner membrane.[110] Proteins with binding capacity for α-tocopherol have been described in cytosol,[111-114] and the hydroxly group in the chromanol ring may be of importance for the transfer process between cell organelles.[114] A binding protein in the nuclear fraction has also been reported.[115] The binding proteins may facilitate the transport of α-tocopherol intracellularly, but what functions these proteins have compared to lateral diffusion of α-tocopherol in biological membranes remains to be elucidated. Recent findings in patients with clear signs of familial vitamin E deficiency without fat malabsorption, indicates that absorption and lymphatic transport of vitamin E is normal.[25] Traber *et al.*[25] actually suggest that a transfer protein for α-tocopherol might be crucial for the packaging of this vitamin into hepatic lipoproteins.

HEPATIC SECRETION OF α-TOCOPHEROL

Primary cultures of rat hepatocytes are well suited for metabolic studies of cholesterol[116] and triacylglycerol[117] since they synthesize and secrete cholesterol, cholesteryl esters,[118] bile acids[119] and lipoproteins, e.g. VLDL[117,120] at rates in a similar order of magnitude as to what is observed under physiological conditions. The different components of VLDL are synthesized in the endoplasmic reticulum, before they are assembled to lipoprotein particles, transferred to the Golgi complex and then packed in membrane-enclosed vesicles. These secretory vesicles are then moved to the plasma membrane by the microtubular system,[121] where VLDL is released by exocytosis.

Monensin, chloroquine and colchicine have previously been shown to inhibit the secretion of VLDL.[120-121] Eicosapentaenoic acid (20:5, n-3) also reduces the secretion of triacylglycerols from primary cultures of rat hepatocytes, and the stimulatory effect of oleic acid on synthesis and secretion of triacylglycerols by rat hepatocytes is inhibited.[123] Increased intake of eicosapentaenoic acid may also enhance the demand and utilization of α-tocopherol as an antioxidant.[67] The amount of α-tocopherol available for secretion may, accordingly, be reduced in hepatocytes exposed to eicosapentaenoic acid.

To elucidate how α-tocopherol is released from hepatocytes, studies of primary cultures of hepatocytes and rat liver perfusions were performed.[124] Ultracentrifugation of the medium revealed that about 90% of α-tocopherol secreted was associated with VLDL. Monensin, chloroquine and colchicine reduced the hepatic release of α-tocopherol to about 15% of control values during a 20 hour incubation, and eicosapentaenoic acid inhibited the secretion by about 50%. 40 min after intravenous injection of *in vivo* labeled (with tritiated α-tocopherol) intestinal lymph the liver was perfused and 74% of the radioactivity was recovered in the VLDL fraction. Taken together, these findings demonstrate that nascent VLDL is the major secretory vehicle

for α-tocopherol. This observation has been confirmed later on by other investigators in living rats as well as in primary cultures of rat hepatocytes.[125] From the facts that transfer of α-tocopherol *in vivo*[79] as well as *in vitro*[97] is relatively slow compared to clearance of chylomicrons, and that a significant amount of α-tocopherol is associated with LDL in man, it is likely that the secretory pathway via VLDL from the liver, is rather important.

METABOLISM OF α-TOCOPHEROL

Dietary esters of vitamin E are hydrolyzed in the intestine prior to absorption.[60] The major route of excretion is fecal elimination and α-tocopherol is deposited unmodified in tissues. In general vitamin E is biologically metabolized to a small extent, except for oxidation and reduction according to its antioxidant function. The primary hepatic oxidation product of α-tocopherol is α-tocopheryl quinone.[14] This product is further reduced to the hydroquinone, which may be conjugated with glucuronic acid and excreted in the bile, or degraded in the kidneys to α-tocopheronic acid, followed by conjugation and elimination in urine. When bile was collected for 24 hours in the rat, after intravenous injection of lymph labeled *in vivo* with (^{3}H)α-tocopherol, a fairly constant amount of radioactivity was excreted per unit time. The total recovery of radioactivity in the bile was approximately 15% of the injected dose. Of the radioactivity detected in bile 86% was water-soluble metabolites of the injected α-tocopherol.[79]

EXTRAHEPATIC DISTRIBUTION OF α-TOCOPHEROL

Liver, skeletal muscle and adipose tissue have the capacity to accumulate α-tocopherol.[52,126,127] These tissues contribute to about 90% of the total amount of α-tocopherol recovered from 10 different organs in rats.[52] In adipose tissue α-tocopherol is located mainly in the bulk lipid droplet,[126] and the mobilization of α-tocopherol from this depot is slow. The adrenal glands have the highest concentration of α-tocopherol per gram of tissue, which may be due to the specific binding of HDL by rat adrenal glands,[128,129] with subsequent uptake of α-tocopherol. The lungs and the spleen also have relatively high concentrations of α-tocopherol per gram of tissue.[52] This might reflect the importance of α-tocopherol in protection against lung injury due to hyperoxia and in maintenance of the immune response.[36-38] The testes and cerebrum contain less α-tocopherol per gram of tissue than most other organs, which might contribute to the major signs of vitamin E deficiency, such as reproductive failure[1] and serious neurological dysfunction.[20-23] Cerebral cortex has the highest and cerebellum the lowest concentration of α-tocopherol in the rat brian, and an inverse relationship between the concentration of α-tocopherol and activity of glutathione peroxidase has been described.[130]

α-TOCOPHEROL AND ALCOHOL

Chronic ethanol consumption may have significant impact on many organ systems in man. Present knowledge indicate that the liver and brain are the most vulnerable

organs. It is still unclear how ethanol induces hepatic injury, but generation of free radicals and increased lipid peroxidation have been hypothesized as a possible pathogenic mechanism. Enhanced lipid peroxidation observed after acute intake of ethanol, as evaluated by formation of lipid peroxides and pentane,[131–133] and after chronic ethanol ingestion[132–134] supports this hypothesis. Dietary supplement with vitamin E to rats fed ethanol chronically causes a marked reduction in petane production as well as the hepatic concentration of triacylglycerol, whereas cholesterol is relatively unaffected.[135] Chronic administration of alcohol to rats fed a nutritionally adequate diet resulted in a significantly reduced hepatic concentration of α-tocopherol as compared to pair-fed controls.[80] The reduced content of α-tocopherol in liver was traced to the hepatocytes, and the mitochondria had the most marked reduction in their content of α-tocopherol as evaluated by subcellular fractionation. The hepatic uptake of (^{3}H)α-tocopherol was not significantly affected in ethanol-fed rats,[80] whereas the secretion of α-tocopherol from hepatocytes in culture was significantly inhibited after acute and chronic exposure to ethanol.[136]

In man increased activity of free radicals[137] and enhanced lipid peroxidation in the liver[138] of alcoholics have been reported. Also the serum concentration of α-tocopherol was reduced among people with chronically high consumption of alcohol without any liver disease.[139] It is well known that chronic alcoholism is associated with malnutrition and dietary evaluation disclosed that heavy consumers of alcohol ate approximately 40% less vitamin E as compared to controls.[139] Neurological signs of dysfunction like cerebellar ataxia and altered propriceptive sensation, are frequently observed among alcoholics, and a possible link between subnormal serum concentrations of α-tocopherol and increased prevalence of neurological clinical signs and cerebellar atrophy have been observed in alcoholics.[140] It is thus possible that the reduced antioxidant status of alocholics may be of clinical significance for development of neurological dysfunctions. From recent studies it is evident that the concentration of α-tocopherol in liver biopsies from patients with alcholic liver cirrhosis is 45% of what is found in subjects with normal liver histology, whereas there is an insignificant reduction of hepatic concentration of α-tocopherol among patients with alcoholic fatty liver.[141] Taken together these observations may be explained by an increased breakdown of α-tocopherol due to increased generation of free radicals as a consequence of ethanol consumption.

FINAL COMMENTS

Although many aspects of the physiological processing of α-tocopherol are well established, many questions still remain unsolved. The absorptive process is not precisely defined, and the function of intracellular binding proteins for α-tocopherol is uncertain. More studies are required to unravel the possible protective effect of α-tocopherol towards lipid peroxidation, and the generation and excretion patterns for metabolites of α-tocopherol.

A beneficial effect of therapeutic supplementation with α-tocopherol is well documented in several malabsorptive states in man, and premature infants may profit from supplementation with α-tocopherol in moderate doses. Furthermore, α-tocopherol may inhibit peroxidation of polyunsaturated fatty acids and LDL, and thereby have a potential in the protection against the development of atherosclerosis.[142] It may turn out that oxidation of LDL is of crucial importance for modification of native

LDL which makes the modified LDL unfit for receptor-mediated uptake via the LDL-receptor (apoB/E-receptor), but prone to be taken up via the scavenger receptor in macrophages. It has been shown that α-tocopherol is totally consumed before LDL is oxidized to such an extent that it is taken up by macrophages, but there are probably also other important antioxidants in LDL in addition α-tocopherol.[143] It is striking, however, that defined oxygen-centered free radicals caused depletion of α-tocopherol in human LDL, decreased the affinity for the apoB/E-receptor but did not cause increased endocytosis in macrophages.[144] These data indicate that more effort should be focused on clearifying the importance of α-tocopherol, and other antioxidants, in protecting native LDL from becoming modified and accessable for vessel wall macrophages. A possible beneficial effect of α-tocopherol in protection against alcohol-induced organ injury also ought to be elucidated. Some major questions about the therapeutic potential of α-tocopherol eventually also should be addressed in properly controlled clinical trials.

References

1. H.M. Evans and K.S. Bishop. (1922) On the existence of a hitherto unrecognized dietary factor essential for reproduction. *Science* **56**, 650–651.
2. H.M. Evans, and G.O. Burr. (1925) The anti-sterility vitamin fat sluble E. *Proceedings of the National Academy of Sciences USA*, **11**, 334–341.
3. O.H. Emerson, G.A. Emerson, A. Mohammed and H.M. Evans. (1937) The chemistry of vitamin E. tocopherols from various sources. *Journal of Biological Chemistry*, **122**, 99–107.
4. K.E. Mason. (1977) The first decades of vitamin E. *Federal Proceedings*, **36**, 1906–1910.
5. M.K. Horwitt. (1962) Interrelation between vitamin E and polyunsaturated fatty acids in adult men. *Vitamins and Hormones*, **20**, 541–558.
6. G.A. Fritsma. (1983) Vitamin E and autooxidation. *American Journal of Medicine Technology*, **49**, 453–456.
7. R.L. Wilson. (1983) Free radical protection: why vitamin E, not vitamin C, β-carotene or glutathione. In: *Biology of vitamin* E (Porter, R. & Whelan, J., eds.), pp. 19–44, Ciba Foundation Symposium 101, Pittman Books Ltd., London.
8. G.W. Burton and K.U. Ingold. (1981) Autoxidation of biological molecules. The antioxidant activity of vitamin E and related chain-breaking phenolic antioxidants *in vitro*. *Journal American Chemical Society*, **103**, 6472–6477.
9. E. Niki, A. Kawakami, M. Saito, Y. Yamamoto, J. Tsuchiya and Y. Kamiya. (1985) Effect of phytyl side chain of vitamin E on its antioxidant activity. *Journal of Biological Chemistry*, **260**, 2191–2129.
10. T.F. Slater. (1984) Free radical mechanisms in tissue injury. *Biochemical Journal*, **222**, 1–15.
11. B. Halliwell and J.M.C. Gutteridge. (1984) Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochemical Journal*, **219**, 1–14.
12. C.D. Puglia and S.A. Powell. (1984) Inhibition of cellular antioxidants: A possible mechanism of toxic cell injury. *Environmental Health Perspectives*, **57**, 307–311.
13. C.W. White and J.E. Repine. (1985) Pulmonary antioxidant defense mechanisms. *Experimental Lung Research*, **8**, 81–96.
14. C.K. Chow. (1985) Vitamin E and blood. *World Rev. Nutr. Diet.*, **45**, 133–166.
15. G.W. Burton, A. Joyce and K.U. Ingold. (1983) Is vitamin E the only lipid-soluble, chain-breaking antixoidant in human blood plasma and erythrocyte membranes? *Archives in Biochemistry and Biophysical*, **221**, 281–290.
16. D.C. Liebler, D.S. Kling and D.J. Reed. (1986) Antioxidant protection of phospholipid bilayers by α-tocopherol. *Journal of Biological Chemistry*, **261**, 12114–12119.
17. P.B. McCay. (1985) Vitamin E: Interactions with free radicals and ascorbate. *Annals of Reviews in Nutrition*, **5**, 323–340.
18. M.S. Losowsky and P.J. Leonard. (1967) Evidence of vitamin E deficiency in patients with malabsorption or alcoholism and the effects of therapy. *Gut*, **8**, 539–543.
19. P.M. Farrell, J.G. Bieri, J.F. Fratantoni, R.E. Wood and P.A. Sant'Agnese. (1977) The occurrence and effects of human vitamin E deficiency. *Journal of Clinical Investigation*, **60**, 233–241.

20. L. Howard, L. Ovesen, S. Satya-Murti and R. Chu. (1982) Reversible neurological symptoms caused by vitamin E deficiency in a patient with short bowel syndrome. *American Journal of Clinical Nutritions*, **36**, 1243–1249.
21. R.J. Sokol, M.A. Guggenheim, S.T. Iannaccone, P.E. Barkhaus, C. Miller, A. Silverman, W.F. Balistreri and J.E.Heubi. (1985) Improved neurologic function after long-term correction of vitamin E deficiency in children with chronic cholestatsis. *New England and Journal of Medicine*, **313**, 1580–1586.
22. D.P.R. Muller, J.K. Lloyd and O.H. Wolff. (1983) Vitamin E and neurological function. *Lancet*, **1**, 225–228.
23. H.J. Kayden and M.G. Traber. (1986) Clinical, nutritional and biochemical consequences of apolipoprotein B deficiency. *Advances in Experimental Medical and Biology*, **201**, 67–81.
24. R.J. Sokol. (1988) Vitamin E deficiency and neurologic disease. *Annual Reviews in Nutr*, **8**, 351–373.
25. M.G. Traber, R.J. Sokol, G.W. Burton, K.U. Ingold, A.M. Papas, J.E. Huffaker and H.J. Kayden. (1990) Impaired ability of patients with familial isolated vitamin E deficiency to incorporate α-tocopherol into lipoproteins secreted by the liver. *Journal of Clinical Investigation*, **85**, 397–407.
26. W.B. Karp and A.F. Robertson. (1986) Vitamin E in neonatology. *Advances in Pediatriatics*, **33**, 127–147.
27. S. Sinha, J. Davies, N. Toner, S. Bogle and M. Chiswick. (1987) Vitamin E supplementation reduces frequency of periventricular haemorrhage in very preterm babies. *Lancet*, **1**, 466–471.
28. H.M. Hittner, A.J. Rudolph and F.L. Kretzer. (1984) Suppression of severe retinopathy of prematurity with vitamin E supplementation. *Opthalmology*, **91**, 1512–1523.
29. A. Zipursky, E.J. Brown, J. Watts, R. Milner, C. Rand, V.S. Blanchette, E.F. Bell, B. Paes and E. Ling. (1987) Oral vitamin E supplementation for the prevention of anemia in premature infants: A controlled trial. *Pediatrics*, **79**, 61–68.
30. N.J. Wald, J. Boreham, J.L. Hayward and R.D. Bulbrook. (1984) Plasma retinol, β-carotene and vitamin E levels in relation to the future risk of breast cancer. *British Journal of Cancer*, **49**, 321–324.
31. W.C. Willet, B.F. Polk, B.A. Underwood, M.J. Stampfer, S. Pressel, B. Rosner, J.O. Taylor, K. Schneider and C.G. Hames. (1984) Relation of serum vitamins A and E and carotenoids to the risk of cancer. *New England Journal of Medicine*, **310**, 430–434.
32. M.S. Menkes, G.W. Comstock, J.P. Vuilleumier, K.J. Helsing, A.A. Rider and R. Brookmeyer. (1986) Serum β-carotene, vitamins A and E, selenium, and the risk of lung cancer. *New England Journal of Medicine*, **315**, 1250–1254.
33. P. Knekt, A. Aromaa, J. Maatela, R.-K. Aaran, T. Nikkari, M. Hakama, T. Hakulinen, R. Peto, E. Saxen and L. Teppo. (1988) Serum vitamin E and risk of cancer among finnish men during a 10 year follow-up. *American Journal of Epidemiology*, **127**, 28–41.
34. K.F. Gey, G.B. Brubacher and H.B. Stahelin. (1987) Plasma levels of antioxidant vitamins in relation to ischemic heart disease and cancer. *American Journal of Clinial Nutrition*, **45**, 1268–1377.
35. J.G. Bieri, L Corash and V.S. Hubbard. (1983) Medical uses of vitamin E. *New England Journal Medical*, **308**, 1063–1071.
36. R.S. Panush and J.C. Delafuente. (1985) Vitamins and immunocompetence. *World Review in Nutrition Dieting*, **45**, 97–132.
37. J. Tanaka, H. Fujiwara and M. Torisu. (1979) Vitamin E and immune response. *Immunology*, **38**, 727–734.
38. L.A. Boxer. (1986) Regultion of phagocyte function by α-tocopherol. *Proceedings at the Nutrition Society*, **45**, 333–344.
39. A. Bendich. (1990) Antioxidant vitamins and their functions in immune responses. In: (Bendich, A, Phillips, M. & Tengerdy, RP. ed.), *Advances in experimental medicine & biology*, **262**, pp. 35–55. Plenum Press, New York.
40. D. Hollander. (1981) Intestinal absorption of vitamins A, E, D, and K. *Journal Laboratory and Clinical Medicine*, **97**, 449–462.
41. D.P.R. Muller, J.A. Manning P.M. Mathias and J.T. Harries. (1976) Studies on the intestinal hydrolysis of tocopheryl esters. *International Journal of Vitamin Research*, **46**, 207–210.
42. D.P.R. Muller, J.T. Harries and J.K. Lloyd. (1974) The relative importance of the factors involved in the absorption of vitamin E in children. *Gut*, **15**, 966–971.
43. R. Blomstrand and L. Forsgren. (1968) Labelled vitamin E in man. Internat. *Z. Vitam. Ernahrung*, **38**, 328–344.
44. J. Kelleher and M.S. Losowsky. (1970) The absorption of α-tocopherol in man. *British Journal of Nutrition*, **24**, 1033–1047.

45. S. Krishnamurthy and J.G. Bieri. (1963) The absorption, storage, and metabolism of α-tocopherol-CI4 in the rat and chicken. *Journal of Lipid Research*, **4**, 330–336.
46. J. Kelleher, T. Davies, C.L. Smith, B.E. Walker and M.S.Losowsky. (1972) The absorption of α-tocopherol in the rat. *International Journal of Vitamin Nutrtion Research*, **42**, 394–402.
47. M.S. Losowsky, J. Kelleher and B.E. Walker. (1972) Intake and absorption of tocopherol. *Annauls at the New York Academy of Sciences*, **203**, 212–222.
48. D. Hollander, E. Rim and K.S. Muralidhara. (1975) Mechanism and site of small intestinal absorption of α-tocopherol in the rat. *Gastroenterology*, **68**, 1492–1499.
49. K.S. Muralidhara and D. Hollander. (1977) Intestinal absorption of α-tocopherol in the unanesthetized rat. The influence of luminal constituents on the absorptive process. *Journal Laboratory Clinical Medicine*, **90**, 85–91.
50. I.R. Peake, H.G. Windmueller and J.G. Bieri. (1972) A comparison of the intestinal absorption, lymph and plasma transport, and tissue uptake of α- and γ- tocopherols in the rat. *Biochimica et Biophysical Acta*, **260**, 679–688.
51. P. Johnson and W.F.R. Pover. (1962) Intestinal absorption of α-tocopherol. *Life Sci*, **1**, 115–117.
52. A. Bjørneboe, G.-E. Aa. Bjørneboe, E. Bodd, B.F. Hagen, N. Kveseth and C.A. Drevon. (1986) Transport and distribution of α-tocopherol in lymph, serum and liver cells in rats. *Biochimica et Biophysical Acta*, **889**, 310–315.
53. H.E. Gallo-Torres. (1970) Intestinal absorption and lymphatic transport of dl-3,4- $^{3}H_{2}$-α-tocopheryl nicotinate in the rat. *International Journal of Vitamin Nutrition Research*, **40**, 505–514.
54. H.E. Gallo-Torres, F. Weber and O. Wiss. (1971) The effect of different dietary lipids on the lymphatic appearance of vitamin E. *International Journal of Vitamin and Nutrition Research*, **41**, 504–515.
55. M.G. Traber, H.J. Kayden, J.B. Green and M.H. Green. (1986) Absorption of water-miscible forms of vitamin E in a patient with cholestasis and in thoracic duct-cannulated rats. *American Journal of Clinical Nutrition*, **44**, 914–923.
56. G.V. Vahouny and C.R. Treadwell. (1959) Comparative effects of dietary fatty acids and triglycerides on lymph lipids in the rat. *American Journal of Physiology*, **196**, 881–883.
57. M. Boquillon, R. Paris and J. Clement. (1977) The effect of various dietary factors on the size distribution of lymph fat particles in rat. *Lipids*, **12**, 500–504.
58. M. Boquillon, R. Paris and J. Clement. (1972) Composition, particle size and role in dietary fat transport of two different lipoproteins of the intestinal lymph. *Lipids*, **7**, 409–414.
59. D.M. Sheehe, J.B. Green and M.H. Green. (1980) Influence of dietary fat saturation on lipid absorption in the rat. *Atherosclerosis*, **37**, 301–310.
60. P.M. Mathias, J.T. Harries, T.J. Peters and D.P.R. Muller. (1981) Studies on the *in vivo* absorption of micellar solutions of tocopherol and tocopheryl acetate in the rat. *Journal of Lipid Research*, **22**, 829–837.
61. M.G. Traber, C.A. Thellman, M.J. Rindler and H.J. Kayden. (1988) Uptake of intact TPGS a water-miscible form of vitamin E by human cells *in vitro*. *American Journal of Clinical Nutrition*, **48**, 605–611.
62. T. Leth and H. Sondergaard. (1983) Biological activity of all-rac α-tocopherol and RRR-α-tocopherol determined by three different rat bioassays. *International Journal of Vitamin and Nitritional Research*, **53**, 297–311.
63. H. Weiser, M. Vecchi and M. Schlachter. (1986) Stereoisomers of α-tocopheryl acetate. *International Journal of Vitamin and Nutritional Research*, **56**, 45–56.
64. G.W. Burton, K.U. Ingold, D.O. Foster, S.C. Cheng, A. Webb, L. Hughes and E. Lusztyk. Comparison of free α-tocopherol and α-tocopheryl acetate as sources of vitamin E in rats and humans. *Lipids*, **23**, 834–840.
65. H. Baker, O. Frank, B. DeAngelis and S. Feingold. (1980) Plasma tocopherol in man at various times after ingesting free and acetylated tocopherol. *Nutritus Reporo International*, **21**, 531–536.
66. J.G. Bieri, A.L. Wu and T.J. Tolliver. (1981) Reduced intestinal absorption of vitamin E by low dietary levels of retinoic acid in rats. *Journal of Nutrition*, **111**, 458–467.
67. A. Bjørneboe, E. Søyland G.-E. Aa. Bjørneboe, G. Rajka and C.A. Drevon. (1987) Effect of dietary supplementation with eicosapentaenoic acid in the treatment of atopic dermatitis. *British Journal of Dermatology*, **117**, 463–469.
68. J.G. Bieri and R.P. Evarts. (1974) γ-Tocopherol: metabolism, biological activity and significance in human vitamin E nutrition. *American Journal of Clinical Nitrition*, **27**, 980–986.
69. W.A. Behrens and R. Madere. (1985) Transport of α- and γ-tocopherol in human plasma lipoproteins. *Nutritus Research*, **4**, 167–174.
70. H. Baker, G.J. Handleman, S. Short, L.J. Machlin, H.N. Bhagavan, E.A. Dratz and O. Frank. (1986)

Comparison of plasma α and γ-tocopherol levels following chronic oral administration of either all-rac-α-tocopheryl acetate or RRR-α-tocopheryl acetate in normal adult male subjects. *American Journal of Clinical Nutrition*, **43**, 382–387.
71. M.G. Traber and H.J. Kayden. (1989) preferential incorporation of α-tocopherol vs γ-tocopherol in human lipoproteins. *American Journal of Clinical Nutrition*, **49**, 517–526.
72. K.R. Norum, T. Berg, P. Helgerud and C.A. Drevon. (1983) Transport of cholesterol. *Physiology Review*, **63**, 1343–1419.
73. R.W. Mahley, T.L. Innerarity, S.C. Rall and K.H. Weisgraber. (1984) Plasma lipoproteins, apolipoprotein structure and function. *Journal of Lipid Research*, **25**, 1277–1294.
74. E.C. McCormick, D.G. Cornwell and J.B. Brown. (1960) Studies on the distribution of tocopherol in human serum lipoproteins. *Journal of Lipid Research*, **1**, 221–228.
75. D. Lambert and J. Mourot. (1984) Vitamin E and lipoproteins in hyperlipoproteinemia. *Atherosclerosis*, **53**, 327–330.
76. L.K. Bjornson, H.J. Kayden, E. Miller and A.N. Moshell. (1976) The transport of α-tocopherol and β-carotene in human blood. *Journal of Lipid Research*, **17**, 343–352.
77. W.A. Behrens, J.N. Thompson and R. Madere. (1982) Distribution of α-tocopherol in human plasma lipoproteins. *American Journal of Clinical Nitrition*, **35**, 691–696.
78. L.K. Bjornson, C. Gniewkowski and H.J. Kayden. (1975) Comparison of exchange of α-tocopherol and free cholesterol between rat plasma lipoproteins and erythrocytes. *Journal of Lipid Research*, **16**, 39–53.
79. A. Bjørneboe, G.-E. Aa. Bjørneboe and C.A. Drevon. (1987) Serum half-life, distribution hepatic uptake and biliary excretion of α-tocopherol in rats. *Biochimica et Biophysical Acta*, **921**, 175–181.
80. G.-E. Aa. Bjørneboe, A. Bjørneboe, B.F. Hagen, J. Morland and C.A. Drevon. (1987) Reduced hepatic α-tocopherol content after long-term administration of ethanol to rats. *Biochimica et Biophysical Acta*, **918**, 236–241.
81. C.K. Chow. (1975) Distribution of tocopherols in human plasma and red blood cells. *American Journal of Clinical Nutrition*, **28**, 756–760.
82. S.C. Cheng, G.W. Burton, K.U. Ingold and D.O. Foster. (1987) Chiral discrimination in the exchange of α-tocopherol stereo isomers between plasma and red blood cells. *Lipids*, **22**, 469–473.
83. J. Lehmann, D.D. Rao, J.J. Canary and J.T. Judd. (1988) Vitamin E and relationships among tocopherols in human plasma, platelets, lymphocytes, and red blood cells. *American Journal of Clinical Nutrition*, **47**, 470–474.
84. S.T. Omaye and F.I. Chow. (1986) Distribution of vitamins A and in blood and liver of rats depleted of vitamin A or vitamin E. *Lipids*, **21**, 465–469.
85. M.G. Traber, T. Olivecrona and H.J. Kayden. (1985) Bovine milk lipoprotein lipase transfers tocopherol to human fibroblasts during triglyceride hydrolysis *in vitro*. *Journal of Clinical Investigation*, **75**, 1729–1734.
86. M.G. Traber, H.J. Kayden. (1984) Vitamin E is delivered to cells via the high affinity receptor for low-density lipoprotein. *American Journal of Clinical Nutrition*, **40**, 747–751.
87. H.E. Gallo-Torres and O.N. Miller. (1971) Tissue uptake and metabolism of d, 1-3,4-$^{3}H_2$-α-tocopheryl nicotinate and d,*l*-α-tocopheryl- 1,2-$^{3}H_2$-acetate following intravenous administration. *International Journal of Vitamin Nutrition Research*, **41**, 339–354.
88. M.E. Knight and R.J. Roberts. (1986) Disposition of intravenously administered pharmacologic doses of vitamin E in newborn rabbits. *Journal of Pediatrics* **108**, 145–150.
89. A. Nishida and H. Togari. (1986) Effect of vitamin E administration on α-tocopherol concentrations in the retina, choroid, and vitreous body of human neonates. *Journal of Pediatrics*, **108**, 150–153.
90. T. Shiratori. (1974) Uptake, storage and excretion of chylomicrabound ^{3}H-α-tocopherol by the skin of the rat. *Life Science*, **14**, 929–935.
91. R. Blomhoff, P. Helgerud, S. Dueland, T. Berg, J.I. Redersen, K.R. Norum and C.A. Drevon. Lymphatic absorption and transport of retinol and vitmin D_3 from rat intestine. *Biochimica et Bipohysica Acta*, **772**, 109–116.
92. T. Olivecrona. (1962) Metabolism of chylomicrons labeled with C^{14} glycerol-H^{3}-palmitic acid in the rat. *Journal of Lipid Research*, **3**, 439–444.
93. O. Faergeman and R.J. Havel. (1975) Metabolism of cholesteryl esters of rat very low density lipoproteins. *Journal of Clinical Investigation*, **55**, 1210–1218.
94. M.H. Green, E.R. Massaro and .B. Green. (1984) Multicompartmental analysis of the effects of dietary fat saturation and cholesterol on absorptive lipoprotein metabolsim in the rat. *American Journal of Clinical Nutrition*, **40**, 82–94.
95. C.H. Floren and A. Nilson. (1977) Binding, interiorization and degradation of cholesteryl ester-

labelled chylomicron-remnant particles by rat hepatocyte monolayers. *Biochemical Journal*, **168**, 483–494.
96. B.C. Sherrill, T.L. Innerarity and R.W. Mahley. (1980) Rapid hepatic clearance of the canine lipoproteins containing only the E apoprotein by a high affinity receptor. *Journal Biological Chemistry*, **255**, 1804–1807.
97. J.B. Massey. (1984) Kinetics of transfer of α-tocopherol between model and native plasma lipoproteins. *Biochimica et Biophysica Acta*, **793**, 387–392.
98. E. Granot, I. Tamir and R.J. Deckelbaum. (1988) Neutral lipid transfer protein does not regulate α-tocopherol transfer between human plasma lipoproteins. *Lipids*, **23**, 17–21.
99. E. Wisse and D.L. Knook. (1979) The investigation of sinusoidal cells: A new approach to the study of liver function. In: Progress in Liver Diseases (Popper, H. & Schaffner, F., eds.), Vol. VI, pp. 153–171, Grune & Stratton, New York.
100. D.L. Knook and E.C. Sleyster. (1976) Separation of Kupffer and endothelial cells of the rat liver by centrifugal elutriation. *Experiments in Cell Research*, **99**, 444–449.
101. D.L. Knook and E.C. Sleyster. (1977) preparation and characterization of Kupffer cells from rat and mouse liver. In: Kupffer Cells and Other Liver Sinusoidal Cells. (Wisse, E. & Knook, D.L., eds.), 273–288. Elsevier/North Holland Biomedical Press, Amsterdam.
102. R. Blomhoff, B. Smedsrød, W. Eskild, P.E. Granum and T. Berg. (1984) Preparation of isolated liver endothelial cells & Kupffer cells in high yield by means of an enterotoxin. *Experiments in Cell Research*, **150**, 194–204.
103. A. Bjørneboe, M. Nenseter, B.F. Hagen, G.-E. Aa. Bjørneboe, K. Prydz and C.A. Drevon. (1991) Distribution and subcellular localization of α-tocopherol in rat liver cells. *Journal of Nutrition*. In Press
104. D.J. Murphy and R.D. Mavis. (1981) A comparison of the in vitro binding of α-tocopherol to microsomes of lung, liver, heart and brain of the rat. *Biochimica et Biophysica Acta*, **663**, 390–400.
105. K.E. Hill and R.F. Burk. (1984) Influence of vitamin E and selenium on glutathione-dependent protection against microsomal lipid peroxidation. *Biochemical Pharmacology*, **33**, 1065–1068.
106. D.J. Kornbrust and R.D. Mavis. (1980) Relative susceptibility of microsomes from ling, heart, liver, kidney, brain and testes to lipid peroxidation: Correlation with vitamin E content. *Lipids*, **15**, 315–322.
107. E. Cadenas, M. Ginsberg and H. Sies. (1984) Evaluation of α-tocopherol antioxidant activity in microsomal lipid peroxidation as detected by low level chemiluminescence. *Biochemical Journal*, **223**, 755–759.
108. B.F. Hagen, A. Bjørneboe, G-E. Aa. Bjørneboe and C.A. Drevon. (1989) Effect of chronic ethanol consumption on the content of α-tocopherol in subcellular fractions of rat liver *Alcoholism (NY)*, **13**, 246–251.
109. J.L. Buttriss and A.T. Diplock. (1988) The relationship between α-tocopherol and phospholipid fatty acids in rat liver subcellular membrane fractions. *Biochimica et Biophysica Acta*, **962**, 81–90.
110. S.M. Thomas, J.M. Gebicki and R.T. Dean. (1989) Radical initiated α-tocopherol depletion and lipid peroxidation in mitochondrial membranes. *Biochimica et Biophysica Acta*, **1002**, 189–197.
111. G.L. Catignani and J.G. Bieri. (1977) Rat liver α-tocopherol binding protein. *Biochimica et Biophysica Acta*, **497**, 349–357.
112. D.J. Murphy and R.D. Mavis. (1981) Membrane transfer of α-tocopherol. *Journal of Biological Chemistry*, **256**, 10464–10468.
113. H. Mowri, Y. Nakagawa, K. Inoue and S. Nojima. (1981) Enhancement of the transfer of α-tocopherol between liposomes and mitochondria by rat liver protein(s). *European Journal of Biochemistry*, **117**, 537–542.
114. C.P. Verdon and J.B. Blumberg. (1988) An assay for the α-tocopherol binding protein mediated transfer of vitamin E between membranes. *Analyital Biochemistry*, **169**, 109–120.
115. P.P. Nair, R.N. Patnaik and J.W. Hauswirth. (1978) Cellular transport and binding of α-tocopherol. In: Tocopherol, Oxygen and Biomembranes (deDuve, C. & Hayaishi, O., eds.), pp 121–130. Elsevier/North-Holland Biomedical Press, Amsterdam.
116. C.A. Drevon, D.B. Weinstein and D. Steinberg. (1980) Regulation of cholesterol esterification and biosynthesis in monolayer cultures of normal adult rat hepatocytes. *Journal of Biological Chemistry*, **255**, 9128–9137.
117. R.A. Davis, S.C. Engelhorn, S.H. Pangburn, D.B. Weinstein and D Steinberg. (1979) Very low density lipoprotein synthesis and secretion by cultured rat hepatocytes. *Journal of Biological Chemistry*, **254**, 2010–2016.
118. C.A. Drevon, S.C. Engelhorn and D. Steinberg. (1980) Secretion of very low density lipoproteins enriched in cholesteryl esters by cultured rat hepatocytes during stimulation of intracellular cholesterol esterification. *Journal of Lipid Research*, **21**, 1065–1071.

119. R.A. Davis, P.M. Hyde, J-C.W. Kuan, M. Malone-McNeal and J. Archambault-Schexnayder. (1983) Bile acid secretion by cultured rat hepatocytes. *Journal of Biological Chemistry*, **258**, 3661–3667.
120. J.Ø. Nossen, A.C. Rustan, T. Barnard and C.A.Drevon. (1984) Inhibition by chloroquine of the secretion of very low density lipoproteins by cultured rat hepatocytes. *Biochimica et Biophys Acta*, **803**, 11–20.
121. O. Stein and Y. Stein. (1973) Colchicine-induced inhibition of very low density lipoprotein release by rat liver in vivo. *Biochimica et Biophysica Acta*, **306**, 142–147.
122. A.C. Rustan, J.Ø. Nossen, T. Berg and C.A. Drevon. (1985) The effects of monensin on secretion of very-low-density lipoprotein and metabolism of asialofetuin by cultured rat hepatocytes. *Biochemical Journal*, **227**, 529–536.
123. A.C. Rustan, J.Ø. Nossen, E.N. Christiansen and C.A. Drevon. (1988) Eicosapentaenoic acid reduces hepatic synthesis and secretion of triacylglycerol by decreasing the activity of acyl coenzyme A: 1,2-diacylglycerol acyltransferase. *Journal of Lipid Research*, **29**, 1417–1427.
124. A. Bjørneboe, G-E. Aa. Bjørneboe, B.F. Hagen, J.Ø. Nossen and C.A. Drevon. (1987) Secretion of α-tocopherol from cultured rat hepatocytes. *Biochimica et Biophysica Acta*, **922**, 199–205.
125. W. Cohn, F. Loechleiter and F. Weber. (1988) α-Tocopherol is secreted from rat liver in very low density lipoproteins. *Journal of Lipid Research*, **29**, 1359–1366.
126. L.J. Machlin and E. Gabriel. (1982) Kinetics of tissue α-tocopherol uptake and depletion following administration of high levels of vitamin E. In: Vitamin E, Biochemical, Hematological, and Clinical Aspects (Lubin, B. & Machlin, L.J., eds.), vol. 393, pp 48–59. Ann. N.Y. Acad. Sci.
127. M.G. Traber and H.J. Kayden. (1987) Tocopherol distribution and intracellular localization in human adipose tissue. *American Journal of Clinical Nutrition*, **46**, 488–495.
128. P.J. Hornsby and J.F. Crivello. (1983) The role of lipid peroxidation and biological antioxidants in the function of the adrenal cortex. *Molecular Cell Endocrinology*, **30**, 123–147.
129. J.T. Gwynne and B. Hess (1980) The role of high density lipoproteins in rat adrenal cholesterol metabolism and steroidogenesis. *Journal of Biological Chemistry*, **255**, 10875–10883.
130. M.A. Goss-Sampson and D.P.R. Muller. (1987) Studies on the neurobiology of vitamin E and some other antioxidant systems in the rat. *Neuropathological Applicator on Neurobiology*, **13**, 289–296.
131. R.E. Litov, D.H. Irving, J.E. Downey and A.L. Tappel. (1978) Lipid peroxidation: A mechanism involved in acute ethanol toxicity as demonstrated by in vivo pentane production in the rat. *LipidS*, **13**, 305–307.
132. N.R. Di Luzio and A.D. Hartman. (1967) Role of lipid peroxidation in the pathogenesis of the ethanol-induced fatty liver. *Federal Proceedings*, **26**, 1436–1442.
133. N.R. Di Luzio. (1973) Antioxidants, lipid peroxidation and chemical-induced liver injury. *Federal Proceedings*, **32**, 1875–1881.
134. H. Remmer, D. Albrecht and H. Kappus. (1977) Lipid peroxidation in isolated hepatocytes from rats ingesting ethanol chronically. *Archives Pharmacology*, **298**, 107–113.
135. R.E. Litov, D.L. Gee, J.E. Downey and A.L. Tappel. (1981) The role of lipid peroxidation during chronic and acute exposure to ethanol as determined by pentane expiration in the rat. *Lipids*, **16**, 52–57.
136. A. Bjøneboe, G-E. Aa. Bjørneboe, B.F. Hagen and C.A. Drevon. (1987) Acute and chronic effects of ethanol on secretion of a α-tocopherol from primary cultures of rat hepatocytes. *Biochimeca et Biophysical Acta*, **922**, 357–363.
137. R. Fink, M.R. Clemens, D.H. Marjot, P. Patsalos, P. Cawood A.G. Norden, S.A. Iversen and T.L. Dormandy. (1985) Increased free-radical activity in alcoholics. *Lancet*, **II**, 291–294.
138. T. Suematsu, T. Matsumura, N. Sato, T. Miyamoto, T. Ooka, T. Kamada and H. Abe. (1981) Lipid peroxidation in alcoholic liver disease in humans. *Alcoholism, (NY)*, **5**, 427–430.
139. G.-E. Aa. Bjørneboe, J. Johnsen, A. Bjørneboe, J.E. Bache-Wiig, J. Mørland and C.A. Drevon. (1988) Diminished serum concentration of vitamin E and selenium in alcoholics. *Annals in Nutrition Metabolism*, **32**, 56–61.
140. G-E. Aa. Bjørneboe, J. Johnsen, A. Bjøorneboe, S.L. Marklund, N. Skylv, A. Høiseth, J.E. Bache-Wiig, J. Mørland and C.A. Drevon. (1988) Some aspects of antioxidant status in blood from alcoholics. *Alcoholism (NY)*, **12**, 806–810.
141. H. Bell, A. Bjørneboe, B. Eidsvoll, K.R. Norúm, N. Raknerud, K. Try, Y. Thomassen and C.A. Drevon. Reduced concentration of hepatic vitamin E in patients with alcoholic liver cirrhosis. Submitted.
142. D. Steinberg, S. Parthasarathy, T.E. Carew, J.C. Khoo and J.L. Witztum. (1989) Beyond cholesterol. Modifications of low density lipoprotein that increase its atherogencity. *New England Journal of Medicine*, **320**, 915–924.

143. W. Jessup, S.M. Rankin, C.V. De Whalley, R.S. Hoult, J. Scott and D.S. Leake. (1990) α-Tocopherol consumption during low-density-lipoprotein oxidation. *Biochemical Journal*, **265**, 399–405.
144. S. Bedwell, R.T. Dean and W. Jessup. (1989) The action of defined oxygen centered free radicals on human low-density lipoprotein. *Biochemical Journal*, **262**, 707–712.

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