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VITAMIN E AS AN ANTIOXIDANT

E VİTAMİNİNİN ANTİOKSİDAN ROLÜ

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ABSTRACT

Antioxidant defences that protect the body from free-radical damage include the enzymes and the antioxidant vitamins. Free radicals are generated endogenously under physiological and pathological conditions but also upon exposure to exogenous challenge. Vitamin E is well accepted as nature's most effective lipid-soluble, chain-breaking antioxidant, protecting cell membranes from free-radical mediated peroxidative damage. Free-radical mediated pathology has been implicated in the development of degenerative diseases, conditions and also aging process. In animals a-tocopherol (the most active form of vitamin E) is membrane bound which was suggested to have a dual role where the phenolic nucleus acts as an antioxidant on the surface of the membrane while the side chain stabilizes the membrane with the lateral methyl groups fitting into gaps caused by cis double bonds in the fatty acids. Thus the molecular mechanism of vitamin E is mediated by the antioxidant function or its membrane stabilizing effect. This paper reviewes the current research on the protective role of vitamin E as an antioxidant.

Key words: *a-* tocopherol, vitamin E, antioxidant.

ÖZET

Viicudu serbest radikal hasarından koruyan antioksidan savunma enzimler ve antioksidan vitaminler< *icermektedir. Serbest radikaller fizyolojik ve patolojik durumlarda ve ayrıca dış kaynaklara maruziyetle uretilmektedir. E vitamini hücre membranlarini serbest radikal aracılı peroksidatif hasardan koruyarak doğanın en etkin lipidde-çözünen zincir-kırıcı antioksidani olarak kabul edilmiştir. Serbest radikal aracılı patoloji dejeneratif durumlar, hastalıklar ve ayrıca yaşlılık gelişimine dahil edilmektedir. Hayvanlarda E vitamininin en etkin sekli olan a-tokoferol membrana bağlıdır. a-Tokoferol için cifte rol ileri sürülmüştür kifenolik çekirdeği membran yüzeyinde antioksidan olarak etki ederken, yan zincirindeki metil grupları, membran yağ asitlerinin çifte bağlarınca oluşturulmuş ceplere girerek membrani stabilize etmektedir. Böylece E vitamininin moleküler mekanizmasına antioksidan etkisi ya da membrane stabilize edici etkisi aracılık etmektedir. Bu makalede E vitamininin antioksidan koruyucu rolü üzerinde hali hazırdaki araştırmalar derlenmiştir.*

Anahtar kelimeler: *a- Tokoferol, E vitamini, antioksidan.*

INTRODUCTION

The discovery of vitamin E

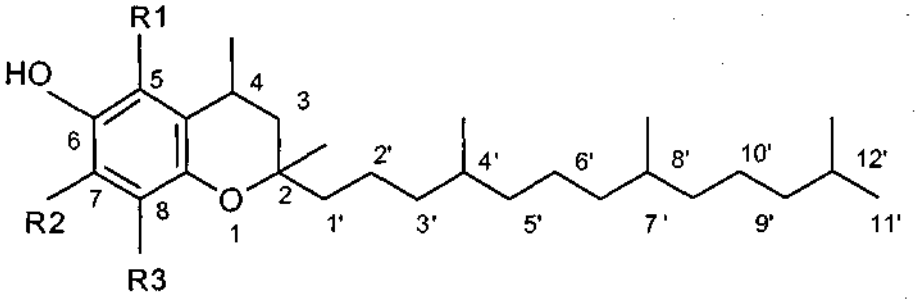
Almost 60 years ago, the nutritional essentiality of vitamin E was recognised for the first time. In 1922, rats given a semi-purified diet containing the then known vitamins, failed to reproduce (1). Two years later an, as yet undescribed dietary factor, was demonstrated and the inadequacy of this factor in the diet resulted in foetal resorption. Wheat germ oil and lettuce were identified as good sources of this factor which was recognized as a vitamin and called vitamin E. In 1931 Pappenheimer and Goettsch (2) described nutritional encephalomalacia (a central nervous system defect, clinically characterized by ataxia, spasms and paralysis) in chicks given semi-purified diets. In 1936 Evans *et al* (3), reported the isolation of a fat-soluble alcohol which had vitamin E activity and named it oc-tocopherol. The following year |3- and y-tocopherols were isolated from vegetable oils (4). Later, another substance named 8-tocopherol was isolated from soybean oil .

Since these discoveries numerous investigators, using several species, have demonstrated nutritionally significant interrelationships between vitamin E and several dietary components (5, 6).

Structure of vitamin E

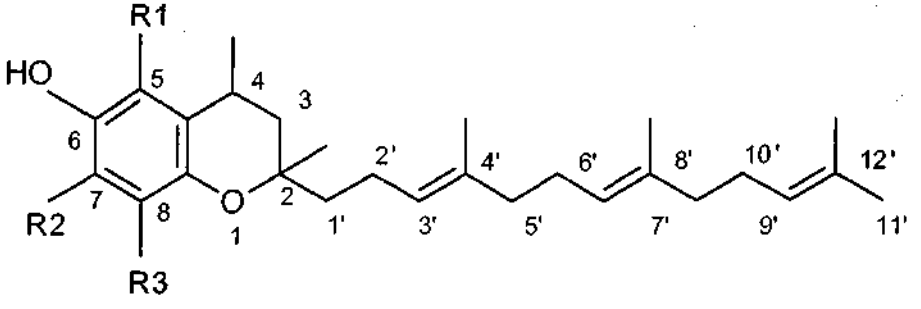
Tocopherols are derivatives of tocol. The multiple nature of tocopherols isolated from plant oils suggested that they were analogues of oc-tocopherol which differed only in the number and arrangement of the methyl groups. The term vitamin E is used as a generic description for all tocol and tocotrienol derivatives qualitatively exhibiting the biological activity of oc-tocopherol. The natural stereo-isomer of oc-tocopherol is (2R, 4 R', 8'R)- oc-tocopherol or RRR-oc-tocopherol.

Later it was shown that some of these substances contained three double bonds in the side chain (7), and the name tocotrienol was proposed for them. What is referred to as vitamin E is a mixture of at least eight related substances (oc-,|3-, y-, 6- tocopherols and a-, (3-, y-, 8-tocotrienols) (8). The structures of natural tocopherols and tocotrienols are shown below.



	R_1	R_2	R_3
α-tocopherol	CH_3	CH_3	CH_3
β-tocopherol	CH_3	H	CH_3
γ-tocopherol	H	CH_3	CH_3
δ-tocopherol	H	H	CH_3

Structure of natural tocopherols



	R_1	R_2	R_3
α-tocotrienol	CH_3	CH_3	CH_3
β-tocotrienol	CH_3	H	CH_3
γ-tocotrienol	H	CH_3	CH_3
δ-tocotrienol	H	H	CH_3

Structure of natural tocotrienols

Tocopherols are widely distributed throughout the plant kingdom, a-tocopherol is the most widespread, being found essentially in the chloroplast. Other tocopherols are located outside the chloroplast The subcellular location of the non-oc-tocopherols is not fully understood. Newton and Pennock (9) worked with green leaves and suggested that y-tocopherol was located outside the chloroplast. Other workers examining plastid fractions from green leaves, have found small amounts of (3- and *y* -tocopherols (10) and 5-tocopherolquinone (11). In the brown algae *Fucus spiralis* (3-, *y*- and S-tocopherols are located outside the plastid (9). No

tocopherols have been found in the nucleus, cell wall or ribosomes of the cell . 8 -Tocopherol is found in the microsomal and soluble fractions whilst y-tocopherol is associated with the cell fraction containing organelles such as mitochondria and golgi.

BIOPOTENCY

The biological activity of the tocopherols varies greatly and this variation is only partly correlated with the antioxidant action (12-16). In 1937, Olcott and Emerson (17) showed that the three naturally occurring forms of vitamin E then known (a-, P- and y-tocopherols) were *in vitro* antioxidants, preventing the oxidation of lard. However, the antioxidant activity found was the reverse of that found for the biological activity; y-tocopherol was the most effective antioxidant, then P- and a- least effective whereas *in vivo* experiments found the activity to be in the order a>P>y. The most biologically potent of the four tocopherols is a-tocopherol (18-20). Diplock *et al,* showed that the higher biopotency of RRR-a-tocopherol was partly related to its increased uptake by the cells whereas all the other tocopherols had a lower uptake level and a lower biopotency than all rac- a-tocopherol. Although the uptake and biopotency of P-tocopherol was not significantly different than a-tocopherol, both paramerters for *y*- and 8-tocopherol were lower (21).

Previous studies (22-23) have shown that -a-tocopherol has the highest biologic activity and it is generally accepted to be the most important antioxidant. However, recent studies have demonstrated that y-tocopherol is a more effective free radical scavenger than -a-tocopherol (24-25). Several clinical trials have failed to demonstrate any beneficial effects of commercial -a-tocopherol preparations in prevention of cardiovascular death, meanwhile tocopherols in the food seem to be beneficial. Liu *et al* (26) have shown that the mixed tocopherol preparation has a more potent protective effect than a-tocopherol alone in preventing lipid peroxidation. They found that 3- and 8-tocopherol in mixed tocopherol are taken up by erythrocytes more readily than a-tocopherol and the use of mixed tocopherol protects erythrocytes more efficiently from lipid peroxidation than a-tocopherol alone. This observation may explain why the administration of a-tocopherol in clinical trials have failed. Mixed tocopherols may thus be important in the suppression of free radical-induced lipid peroxidation.

Although it is generally accepted that the relative antioxidant activities of the tocopherols *in vivo* is in the order a > P > *y* > 8 (27-30) there appears a general confusion for their relative potency *in vitro* (31). The chemical structures of the tocopherols and tocotrienols gives a hydrogen donating power in the order a > P > *y* > 8 (32). This order of acivity was also observed in a homogenous solution in dichlorobenzene (31) but the order was reverse in fats, oils and lipoproteins *in vitro* (33-39). The reason for this reversed order is not yet clearly understood. However it is now realized that *in vitro* activities of tocopherols are not only dependent on their absolute chemical reactivities toward free radicals, but also on other possible

side reactions. These side reactions , that are dramatically affected by tocopherol concentrations (40,41, 35), by light and temperature (42,43), solvent (44), type of substrate (42,45,46) and by prooxidants and synergists in the system, may be highly propagative. Thus all the chemical and physical parameters of the system significantly affects the reaction mode of chromanols.

The presence of the phenolic hydroxyl in tocopherols and tocotrienols is critical for activity, but there is no agreement on its role. When the hydroxyl group is masked a total loss of activity is seen. On the other hand, replacement of the hydroxyl by an amino group or esterification of the hydroxyl have no significant effect on the activity (12). Probably esterified tocopherol is cleaved to the free phenol before absorption. It has been accepted that the phytyl side chain of vitamin E at the 2-position facilitates the incorporation and retention of vitamin E in biomembranes (47, 48). In order to understand the role of phytyl side chain of vitamin E in the biological membranes effects of vitamin E ant its analogue 2,2,5,7,8-pentamethyl-6-chromanol (PMC) (vitamin E without side chain) on the inhibition of the oxidation of methyl linoleate and soybean phosphatidylcholine in homogenous solution and in aqueous dispersion have been studied (47). These antioxidants showed similar antioxidative activities. It was concluded that the antioxidant activities of vitamin E and its analoque without the side chain were quite similar within liposomes, micelle and in homogenous solution whereas the phytyl side chain enhanced the retainment of vitamin E in liposomes (49) and suppress the transfer of vitamin E between liposomal membranes.

The presence of three methyl groups in the aromatic ring is required for the optimal activity. A sharp reduction in the activity takes place when a methyl group is lost. a-Tocopherol is shown to be the most active form of all tocopherols toward peroxyl radicals (50, 51) in the resorption gestation (52) and in the muscular dystrophy test (53). p-Tocopherol has 25-40 % and y-tocopherol has 8-19 % of a-tocopherol activity when measured by gestation resorption assay (52). Furthermore, they showed that synthetic 5,7 dimethyl tocol has 60 % of the *a*-tocopherol activity which suggested that the presence of methyl groups in the positions ortho to the hydroxyl group is important for the biological activity of the tocopherol. They also showed that synthetic tocol, synthetic 5-methyl tocol and 7-methyl tocol had negligible activities. Among the various methods used to assess the biological activity of the different tocol derivatives, the most commonly accepted being the rat fetal resorption assay (54). Authors ascribed relative biopotencies of 100, 56, 16 and 0.5 % to a-, (3-, *y*- and 5- tocopherols respectively. Tocotrienols, generally show very low biological activity and only a -tocotrienol has a significant activity in the haemolysis test being about 25 % of the activity of a -tocopherol (19). The overall activity of this family is only 16 % that of tocopherols (54).

The results show that all three methyls are important for maximal activity. Since (3-, *y*-tocopherol and 5,7-dimethyl tocol all show some activity while 5-, 7- and 8- methyl tocols have negligible activity it can be seen that two methyls can endow some biological activity. Comparing the relative activities of the dimethyl tocols shown above it would appear that the

importance of the three methyls is 5>7>8. The relative biopotencies of tocopherols and tocotrienols are shown below (table 1).

Table 1 Relative biopotencies of tocopherols and tocotrienols (55)

Compound	Foetal resorption % in rat	Haemolysis % in rat
a-tocopherol	100	100
(3-tocopherol	25-40	15-27
Y-tocopherol	8-19	3-20
8-tocopherol	1	0.3-2
5,7-dimethyl tocol (synthetic)	60*	n.e
a-tocotrienol	29	25
p-tocotrienol	5	1-5

*Bunyan et al1960 (52)

n.e. not examined

Vitamin E in membranes

Tocopherols have been shown to be very important components of biological membranes where they may act as antioxidants (56, 57) and play a role in membrane stabilization (56-59).

Vitamin E is detected in most tissues and mainly in those membranes (60-65) with the highest PUFA concentrations (66-67). This is not only because a-tocopherol is lypophylic but also its 16-carbon phytol side chain provides the ability to form an integral part of the membrane. According to Diplock and Lucy's hypothesis (68), which was based on studies of molecular models, vitamin E may stabilize biological membranes by virtue of lipid- lipid interactions between its phytyl side chain and the fatty acyl chains of PUFA, particularly those derived from arachidonic acid (figure 1). It may thus exert its free-radical scavenging function towards peroxidizing PUFA. This was confirmed by Burton and Ingold (69) who demonstrated that removal of phytyl side chain did not diminish the *in vitro* antioxidant potential of oc-tocopherol but completely destroyed its *in vivo* function, a-tocopherol becomes an integral part of the membrane exerting its free-radical quenching role. This concept unites the theory of Tappel (70) with that of Diplock and Lucy (68) who suggested that a-tocopherol acted by conferring stability on the membrane. Diplock and Lucy (68) suggested that antioxidant action is near the surface of the membrane. The methyl groups of the phytyl side chain fit into the the

pockets created by *cis* double bonds and may facilitate the molecular packing within membranes, while the hydroxyl group of the chromanol ring and polar groups of phospholipid lie together at the membrane surface (55) (fig. 1). By this localisation vitamin E helps to maintain membrane integrity and permeability (71), thus controlling unspecifically the cellular metabolism and functioning of cells (72). The consequences of forming such a complex *ie* between vitamin E and PUPA are several. Firstly oxidative destruction of PUFA either *in vivo* (73, 74) or *in vitro* (58, 75, 76) is inhibited. Secondly, the permeability of biological membranes containing high levels of PUFA, particularly arachidonic acid is reduced by the presence of Vitamin E (49, 77) that is the restriction of molecular mobility of the membrane lipid bilayer with vitamin E (68, 78). Thirdly, the complex stabilizes membrane-bound phospholipases (79-81) and stabilizes the polypeptide chains of intrinsic proteins against the modifying effects of free fatty acids. Studies have demonstrated a stabilizing effect of cc-tocopherol against the damages caused by free acids (57, 79, 81). This effect can either be due to the direct interaction of oc-tocopherol with ca^{2+}-dependent ATPase or to its interaction with free fatty acid. It has been demonstrated that vitamin E can protect biological membranes against the damaging action of phospholipases as well as the phospholipid hydrolysis products by phospholipase A namely free fatty acids and lysophospholipids (57,77, 81, 82,).

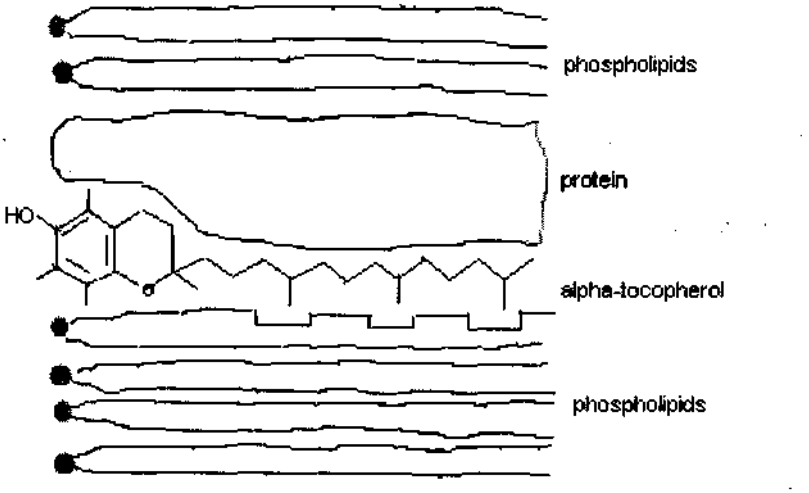


Figure 1 Diagrammatic Representation of the Proposed Interaction Between cc-Tocopherol and Polyunsaturated Phospholipids (68)

Lucy (71) studied the molecular interactions of synthetic phospholipid molecules with a-tocopherol and its derivatives in monolayers of phospholipids at the air/water interface, a-Tocopherol was found to exhibit the maximum interaction with polyunsaturated phospholipids

and tocopherol penetration into the membrane was increased as unsaturation in the phospholipids was increased. It also appeared that a-tocopherol reduced the permeability to glucose and chromate ions when the liposomes were prepared from phospholipids containing arachidonic acid residues. Giasuddin and Diplock (83) studied the effect of adding specific unsaturated lipids on the growth and permeability of 2-deoxyglucose of isolated mouse fibroblasts. It was found that deoxyglucose transport and optimal growth depended on the presence of linoleic acid, cholesterol and vitamin E. Butylated hydroxytoluene an antioxidant, was unable to replace vitamin E which indicates that the activity of vitamin E does not depend upon its antioxidant properties alone. It depends on its lipid nature as well.

Proteins as well as lipids form an important constituent of cellular membranes. Therefore, membrane-bound proteins and enzymes may also be affected in vitamin E-deficiency. Mc Cay and King (84) proposed a scheme where NADPH-oxidase activity leads to a peroxidation attack on unsaturated membrane lipids in liver microsomes. They suggested that a-tocopherol must be localised in the membrane, close to NADPH oxidase which generates free radicals, so that the free radicals can be trapped. If vitamin E is important in maintaining the integrity of biological membranes against disruption by free radicals, other functions mediated through cell membranes such as enzymatic activities, ion transport, receptor availability etc. would be greatly affected (85, 86, 87). A loss of enzymatic activities such as cytochrome P450, glucose-6-phosphatase and UDP-glucuronyl transferase, catalase and fragmentation of lysosomal membranes with liberation of hydrolytic enzymes have been linked to lipid peroxidation (88, 89).

The ratio of PUFA to vitamin E in biological membranes was found to be compatible with the vitamin E chain-breaking antioxidant function. In mitochondrial membranes, the ratio of molecules of PUFA to molecules of a-tocopherol was 200 to 1 (90) whereas Kornbrust and Mavis (66) found a ratio of PUFA to vitamin E of 200/1 - 2000/1 in the microsomes of various rat tissues. The greatest concentrations of a-tocopherol was found in lisosomes and golgi membranes in the order of 1 : 65 phospholipid molecules (91).

The phospholipids in the red blood cell membrane contain a variety of fatty acids varying in chain length and in the number of double bonds. The physical characteristics of phospholipids are largely determined by their fatty acyl constituents. Alterations in fatty acyl groups can affect the shape and permeability (92). An increase in the degree of unsaturation of phospholipid fatty acids increases the lipid fluidity, whereas an increase in the SPH/PC ratio or in the cholesterol/phospholipid ratio decreases membrane fluidity (93).

Relatively large elevations in the dietary polyunsaturated/saturated fatty acid ratio have been shown to increase the requirement for vitamin E with respect to biological parameters (94, 95) and to influence the susceptibility of tissues to lipid peroxidation. Experiments with liposomes have shown that high lipid unsaturation results in increased lipid peroxidation (96, 97, 98). Studies performed with human erythrocytes showed a significant positive correlation between the relative concentrations of arachidonic acid ($C_{20:4}$) and lipid peroxidation (99). The

results demonstrate that in red cells obtained from healthy persons only fatty acids with four or more double bonds are involved in membrane peroxidation reactions when exposed to peroxide stress. Arachidonic acid and docosahexanoic acid are the main substrates of the red cell mehmbrane peroxidation, whereas linoleic acid is of minor importance (99).

Diplock and Lucy (68) calculated the ratio of arachidonic acid residues to a-tocopherol to be about 500 : 1 in red blood cell membranes. This ratio is lower in mitochondrial membranes (90) when compared to microsomes of tissues (66) and red blood cell membranes (68). a-Tocopherol is more concentrated in the mitochondria than these two tissues (90).

cc-Tocopherol as an antioxidant

Our health is under constant attack from free radicals. Free radicals are highly reactive molecules produced by cellular (100-102) and environmental sources (103, 104). These unstable molecules attack healthy cells, weakening cell membranes and leading to cell damage and diseases (105, 106). Polyunsaturated fatty acids are very susceptible to attack by free radicals. Vitamin E is thought to prevent the spread of peroxidation initiated by free radicals through the fatty acids of phospholipids as a chain-breaking antioxidant (107-114).

Initiation of peroxidation of an unsaturated fatty acid (LH) is caused following attack by any chemical species (free radical e. g., a hydroxyl radical) that has sufficient reactivity to abstract a hydrogen atom from a methylene carbon in the side chain to form a fatty acyl radical (L) (1). The resulting carbon centered radical (L) can have several fates the most likely one is to undergo molecular rearrangement. This is followed by acceptance of oxygen (2) to give a fatty acid peroxy radical (LOO) (2).

$$HO^{\cdot} + H \longrightarrow H_2O + L^{\cdot} \quad (1)$$

$$L^{\cdot} + O_2 \longrightarrow LOO \quad (2)$$

These peroxy radicals are very highly reactive themselves and easily set up a chain reaction. They require an additional H to become stable. This hydrogen may be donated from an adjacent fatty acid molecule (LH'), forming another free radical (L') thereby setting up a chain reaction (3).

$$LOO^{\cdot} + LH' \longrightarrow LOOH + L'^{\cdot} \quad (3)$$

The carbon atom most vulnerable to radical formation is the one between two *cis* double bonds *ie* -CH=CH-CH2-CH=CH-. Therefore oleic acid with only one *cis* double bond has no such carbon but any carbon atom adjacent to the double bond has vulnerability to some extent. Linoleic acid (Ci8:2) has one carbon atom between two double bonds and is much more vulnerable to radical formation, linolenic acid (Ci8.3) has two such carbon atoms and arachidonic acid (C20:4) has three. The chemistry of these compounds is such that linolenic acid is twice as vulnerable as linoleic acid and arachidonic acid is three times as vulnerable.

Vitamin E and other phenolic antioxidants inhibit reaction 3 and are thus chain breaking antioxidants (28, 115-117). The effect of a-tocopherol stops this peroxidation spiral. The peroxy radical can be quenched by the H of phenolic OH group of oc-tocopherol (a-T) (3). The principle role of a-tocopherol is to scavenge the lipid peroxyl radical before it attacks the target lipid.

Two peroxyl radicals are trapped for every a-tocopherol molecule oxidized (118). Tocopherol itself becomes a relatively stable free radical (tocopheroxyl radical a-T) (50) that does not propagate the radical chain (fig .2).

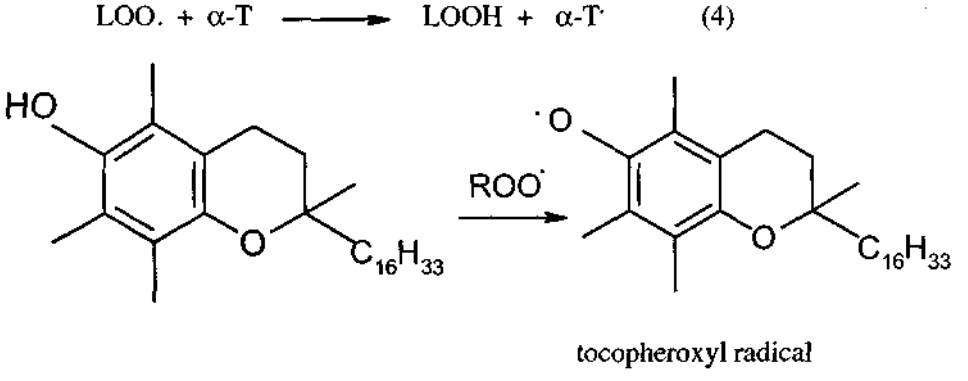


Figure 2. Peroxyl trapping by a-tocopherol

Reaction of the tocopheroxyl radical with a peroxyl radical yields two groups of products (119, 120). The first group consists of 8 a-substituted tocopherons which either have a peroxyl-derived adduct or a hydroxyl group at C-8 a of the chromanol system. The 8 a-alkyldioxytocopherons hydrolyse to 8 a-hydroxy tocopherons and then arranges to a-tocopherylquinone (fig. 3) (121). The second group consists of epoxy 8 a-hydroperoxytocopherons which have an epoxide at either C-4a/C-5 or C-7/C-8 of the chromanol system and a hydroperoxy substutient at C-8a which hydrolyse and rearrange to epoxyquinones (fig .3). a-Tocopherol is vulnerable to oxidation to minor products as dimers and trimers during peroxyl scavenging as well (120).

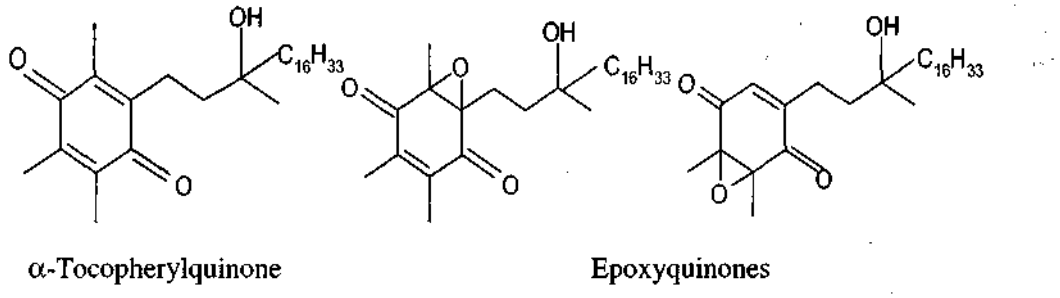


Figure 3. Oxidation products of a-Tocopherol by peroxyl radicals

Vitamin E reacts primarily with peroxyl radicals because of the following reasons:

1) Propagation is the slowest step (reaction 3) in lipid peroxidation and peroxyl radicals are much higher concentrations than other radicals involved (122).

2) Peroxyl radicals react with vitamin E more faster than other molecules (f.<?.adjacent polyunsaturated fatty acids) (50).

3) Vitamin E is poor inhibitor of reactions 1 and 2 (123).

Tocopherols react with other reactive oxidants, including alkoxyl radicals (124, 125), singlet oxygen (126, 127), nitrogen dioxide (128), peroxinitrite (129), ozone (130) and superoxide (131). Reaction of a-tocopherol with other oxidant has been studied in several model systems. Although some of the products are identical to those produced by peroxyl radicals, some characteristic products are produced by individual oxidants (131, 125) Except for singlet oxygen a-tocopherol has relatively little kinetic advantage in scavenging these other oxidants. These oxidants may probably react directly with a-Tocopherol to a much smaller extent than do peroxyl radicals.

The antioxidant theory (69) was supported by the fact that several vitamin E-deficiency diseases of animals are prevented by antioxidants (132-135) and furthermore there is evidence of increased lipid peroxidation in tissues from deficient animals (136-138).

According to the antioxidant theory it should be possible to detect tocopherol metabolites, a-tocopherol can be converted to a-tocopheryl quinone *in vivo* and oxidation products of a-tocopherol were reported in some animals (139-141). However the quantity of cc-tocopheryl quinone detected in tissues is very small. Tappel has suggested that the tocopheroxy radicals formed from tocopherol by removal of the phenolic hydrogen can be reduced back to tocopherol by vitamin C (142). The antioxidant efficiency of vitamin E appears to be greatly enhanced by regeneration of the vitamin from its oxidised products (143). Appreciation of other antioxidants (144-146) has developed the notion that while vitamin E protects lipids other antioxidants must recycle vitamin E. The other major antioxidants are coenzyme Q and vitamin C (147, 148) Regeneration of a-tocopherol from its tocoperoxyl radical have been demonstrated by vitamin

A (149) , C (150) and coenzyme Q (152) *in vitro.* However there is some doubt about the significance of regeneration by vitamins A and C *in vivo.*

Another explanation for the low levels of cc-TQ in tissues is that the tocopherol may be one of the last antioxidant substances to be consumed during oxidative stress. Burton and Ingold, found that other antioxidants such as sulphydryl groups in proteins were found to be used preferentially to tocopherols during oxidations initiated by free radicals in human plasma (28, 153). Indeed, a radical (tocopheroxy radical) reductase activity has been found by Packer *et al,* (154). Therefore the. tocopheroxy radical may be reduced to the native form of vitamin E by this enzyme.

Requirements of vitamin E

At present estimations of ideal vitamin E intake and requirements are mainly based on its interaction with polyunsaturated fatty acids (PUFA). High intake of PUFA increases the requirement for vitamin E. This is based on the data from animal and a few human studies (155). Food with high PUFA often contains high vitamin E but this is not always the case. Margarines and vegetable oils are relatively rich in y-tocopherol with 10% the biological activity of a-tocopherol (155). Therefore increasing PUFA in the diet will not always increase total vitamin E intake to maintain an ideal ratio of above 0.4 mg d-cc-tocopherol/g PUFA.

In order to set the ideal intake of vitamin E several other factors should be considered. These include the physical activity, exposure to environmental pollutants, intake of total calories, percent energy from total fat and from PUFA, intake of vitamin C, |3-carotene and other antioxidants. High levels of vitamin C has been shown to decrease vitamin E requirements in the animal experiments and remains to be shown in human. Furthermore environmental pollutants, lifestyle, habits like smoking, exercise and alcohol may induce oxidative stress and increase antioxidant requirement.

The dietary reference intakes (DRIs) that is the latest recommended vitamin E requirements, were published in 2000 by the Food and Nutrition Board of the Institute of Medicine, National Academy of Sciences (156). These new DRIs were largely based on the studies by Horwitt at the Elgin State Hospital in the 1950s and 1960s (157-160). The recommended dietary allowance (RDA) for vitamin E, based on a long term study was 30 IU in the 1968 edition but was decreased to 15 IU (155). The 1989 RDA was expressed in a-tocopherol equivalents, for which y-tocopherol was estimated to be equivalent to 1/10* of the activity of cc-tocopherol (161). However 2000 DRI in contrast with 1989 RDA, indicates that only a-tocopherol can meet vitamin E requirements. The 2000 DRI was based on the observation that only a-tocopherol has a protein, a-tocopherol transfer protein (oc-TTP) that regulates plasma a-tocopherol concentrations. The studies with a-TTP indicates that oc-TTP binds a-tocopherol but has little affinity for y-tocopherol (162). Humans having a-TTP gene defect become deficient in vitamin E (162) as do mice (163). Moreover administration of a-

tocopherol reverses human vitamin E deficiency sendroms providing evidence that humans require a-tocopherol. y-Tocopherol may have health benefits but these have not been shown in humans yet.

The basis for the 2000 dietary reference intake (DRI) for vitamin E is the health benefit of the vitamin for humans (156). The new DRI take into account not only the deficiency sendromes but also the amount of minerals and vitamins that provide an optimum benefit across the life span. The *in vitro* haemolysis test was used as a marker of vitamin E status due to the fact that erythrocytes become sensitive to peroxide-induced haemolysis *in vitro* and have a faster turnover rate *in vivo* in vitamin E deficiency with neurologic abnormalities (164). All of these were reversed by a-tocopherol supplements (156). Horwitt *et al* (157) also used haemolysis test to evaluate vitamin E status in humans who had vitamin E deficient diets for longer than 5 years and then were given vitamin E supplements. These data were used to set both the 1968 RDA (30 IU vitamin E) and the 2000 DRI (15 mg 2fl-a-tocopherol or 22 IU /?/?i?-a-tocopherol) (natural) or 33 IU a//-rac-oc-tocopherol (synthetic).

CONCLUSION

Vitamin E is lipid-soluble vitamin comprised of a family of 8 stereoisomers characterised by a chromanol ring with a phytyl side chain referred to as tocopherols and tocotrienols. oc-tocopherol is a predominant isomer found in the body especially in membranes and the most potent.

The molecular mechanism of vitamin E which plays a role in a variety of physiological and biochemical functions is probably mediated either by the antioxidant function or its membrane stabilizing effect. It breaks peroxyl chain propagation reactions and is an efficient lipid peroxyl radical scavenger. Vitamin E is may be regenerated form its radical form by redox reactions involving coenzyme Q, enhancing its role as a lipid antioxidant.

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