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REVIEW

BIOCHEMICAL AND THERAPEUTICAL ASPECTS OF VITAMIN E

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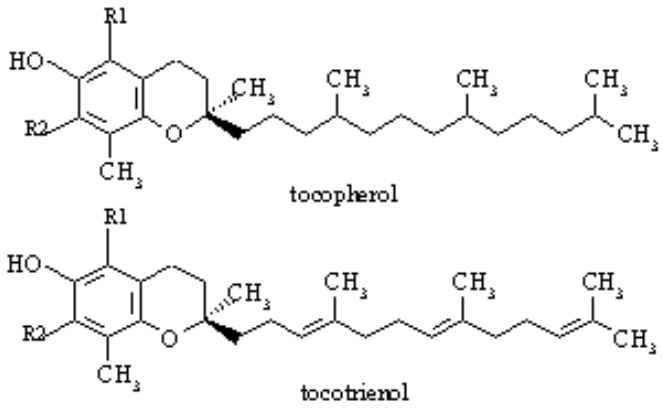
Although vitamin E has been known as an essential nutrient for reproduction since 1922, we are far from understanding the mechanisms of its physiological functions. Because of its antioxidative properties, vitamin E is believed to help prevent diseases associated with oxidative stress, such as cardiovascular disease, cancer, chronic inflammation, and neurological disorders. In the past decades scientists have discovered novel mechanisms for the antiatherosclerotic and anticarcinogenic properties of vitamin E, which involve modulation of cellular signaling, transcriptional regulation, and induction of apoptosis. Future research on this essential vitamin should focus on what makes it essential for humans, why the body apparently utilizes α-tocopherol preferentially, and what functions other forms of vitamin E have.

1. CHEMISTRY OF VITAMIN E

The term vitamin E, introduced in 1922 by Evans and Bishop from Berkeley, designates a group of chemical-related, lipid-soluble compounds with high antioxidant activity. Vitamin E occurs in nature in 8 different forms (vitamers): 4 tocopherols (α, β, γ, δ) and 4 tocotrienols (α, β, γ, δ) (Fig. 1). The tocopherols have a chromanol ring and a phytyl tail, and differ in the number and position of the methyl groups on the ring. The tocotrienols have 3 double bonds of which 2 have *trans* configuration.



R_1	R_2	Tocopherol
CH_3	CH_3	α
CH_3	H	β
H	CH_3	γ
H	H	δ

Fig. 1 – Chemical structure of tocopherols and tocotrienols.

Each tocopherol has three chiral centers (C_2, $C_{4'}$, $C_{8'}$) and 8 stereoisomers are possible. Natural tocopherols have the R configuration at all chiral centers and stereoisomers with R configuration at C_2 are more biologically active than those with S configuration at C_2.[100,101] Chemically synthesized α-tocopherol

is a mixture of 8 stereoisomers and is designated as *allrac*-α-tocoferol (*all rac*emic). Vitamin E supplements are marketed as mixed tocopherols, α-tocopherol, or α-tocopheryl esters (acetate, nicotinate or succinate).

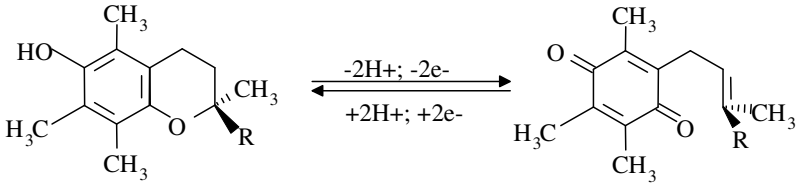
Table 1

Biological activities of α-tocopherol stereoisomers [100]

α-tocopheryl derivate	Biological activities
RRR-α-tocopheryl acetate	100%
RRS-α-tocopherol	90%
RSS-α-tocopherol	73%
SSS-α-tocopherol	60%
RSR-α-tocopherol	57%
SRS-α-tocopherol	37%
SRR-α-tocopherol	31%
SSR-α-tocopherol	21%

The 8 forms of vitamin E have different biological and antioxidant activities: γ-tocopherol has about 50% the antioxidant activity and 1/10 the biologic activity of α-tocopherol.[42] Although the amount of γ-tocoferol in the diet is 10 times higher than that of α-tocopherol, the plasma γ-tocopherol concentration is only 10-20% of that of α-tocopherol.

The most important chemical property of vitamin E is the ability to participate in redox processes according to the following reaction (R= $C_{16}H_{33}$):



alpha-tocopherol alpha-tocopherylquinone

Tocopherols act as free radical scavengers (chain-breaking antioxidants) when the phenoxylic head group encounters a free radical:[29]

$$\text{Toc-OH} + \text{LOO}\bullet \rightarrow \text{Toc-O}\bullet + \text{LOOH}$$

Toc-OH = tocopherol LOO• = lipid radical

The phenoxyl radical Toc-O• is much more stable, and less reactive, than LOO•. The aromatic nature of the chromanol ring, steric and electronic effects from methyl substituents stabilizes the tocopheroxyl radical. Toc-O• can be reduced back to Toc-OH by ascorbat or ubiquinol-10 ($CoQ_{10}H_2$) acting in conjunction with NADPH reductase.[29] In a minor pathway, tocopheryl radicals can undergo a ring scission reaction to form tocopheryl quinones as a terminal oxidation product.

International unit (IU) of vitamin E it was defined as 1 mg of *allrac*-α-tocopheryl acetate, and 1 mg of RRR-α-tocopheryl acetate is equivalent to 1.36 IU. The daily recommended intake is about 15-30 mg of α-tocoferol to obtain an optimal plasma concentration (30 μM).

In Tables 2 and 3 are presented some of the physico-chemical parameters of tocopherols and derivates and natural sources.

Table 2

Physico-chemical parameters of tocopherols [105]

Compound	MW	Boiling point (°C)	Melting point (°C)	λ_{max}	$[\alpha]_D^{25}$	Observations
Tocol	388	190°-195°		286 mμ		Colourless oil
α-tocopherol	430	140°	2,5-3,5	292/298 mμ	0.65°	Thermostable, resistant to light, acids, alkalis
β-tocopherol	416			297 mμ		Viscous oil; thermostable; resistant to acids and alkalis
γ-tocopherol	416		-3° - -2°	298 mμ		Viscous oil; thermostable; resistant to acids and alkalis
δ-tocopherol	402	150°				Pale yellow oil
α-tocopheril acetate	473		26,5°-27,5°		3,2° (ethanol)	

Table 3

Natural sources of tocopherols [76,90,105]

Compound	Natural sources
α-tocopherol	Wheat-germ oil; hempseed oil; cotton-seed oil; crude olive oil; green leafy vegetables; muscle tissue.
β-tocopherol	Wheat-germ oil
γ-tocopherol	Cotton-seed oil
δ-tocopherol	Soybean oil

2. BIOCHEMISTRY OF VITAMIN E

2.1. Absorption, metabolism and catabolism of vitamin E

Vitamin E is absorbed by the entherocites from the lumen of the small intestine, along with other hydrophobic dietary compounds. The absorption requires the presence of bile acids and salts. Thus, vitamin E deficiency occurs in patients with biliary obstruction, pancreatitis or cystic fibrosis.[90] In normal subjects only 15-45% of the amount of ingested vitamin E will be absorbed.[9]

Inside entherocytes tocopherols are assembled together with triacylglicerols (TAG), cholesterol, phospholipids, carotenoids and apolipoproteins (apoB 48) into chylomicrons.[67] Patients with abetalipoproteinemia and apoB knock-out mice develop vitamin E deficiency.[46]

Administration of puromycin to rats prevents the synthesis of chylomicrons and vitamin E is not secreted into the lymph.[41]

Chylomicrons are catabolized in the circulation by the endothelial-bound enzyme, lipoprotein lipase, which hydrolyzes TAG, releasing free fatty acids which are transferred to the cells. During chylomicron lipolysis, a part of vitamin E is distributed to tissues. Skeletal muscle and adipose tissue receive a significant supply of vitamin E chylomicrons during LPL-mediated metabolism.[46] Patients with LPL deficiency accumulate considerable amounts of circulating α-tocopherol in chylomicrons and VLDL.[88]

More recently, a novel role of LPL has been reported in the delivery of α-tocopherol across the blood-brain barrier into the central nervous system.[27] The nervous tissue from knock-out mice for the LPL gene, has a significantly decreased concentration in α-tocopherol than in normal mice.[99]

After partial delipidation by LPL and acquisition of apoE, chylomicron remnants are taken up by the liver parenchymal cells.[90] During the formation of chylomicron remnants α-tocopherol is transferred to HDL.

In the liver, dietary lipids are incorporated into nascent VLDL. A number of studies have demonstrated that the liver is responsible for the control and selective release of RRR-α-tocopherol into the plasma.[87, 88, 89, 90, 92, 93]

When RRR-α-tocopherol, γ-tocopherol and SRR-α-tocopherol, labeled with different amounts of deuterium, were fed in a single dose to normal humans, the plasma initially contained equal concentrations of isomers, but after 24 h it was enriched with RRR-α-tocopherol.[88] Discrimination between different isomers is made during VLDL secretion by the liver. It has been suggested that the liver contains a mechanism for the preferential secretion of RRR-α-tocopherol in nascent VLDL [89], and this could be the function of a protein named α-TTP. It has been demonstrated that α-TTP selectively recognizes α-tocopherol, the most biologically active form of vitamin E, but also has a preference for C_2-R-stereoisomers. Using a chiral HPLC technique it has been demonstrated that plasma and tissues after supplementation with allrac-α-tocopherol contain the C_2-R-epimers.[100]

α-TTP-catalyzed α-tocopherol secretion is not coupled with VLDL secretion, because brefeldin A, which inhibits VLDL secretion, has no effect on α-tocopherol secretion. This suggests that α-tocopherol is not assembled into VLDL in the liver cells, but rather becomes associated with VLDL after their secretion, possibly in the liver sinusoidal spaces.[86]

α-Tocopherol circulates in the amphipatic outer layer of lipoproteins, which should permit rapid exchange or transfer of α-tocopherol between different lipoprotein classes and lipoproteins and cells.

Upon secretion into the plasma, LPL and hepatic triglyceride lipase catabolize nascent VLDL. About half of the VLDL are partially delipidated in the circulation and returned to the liver, while the remainders are converted in the circulation to LDL.

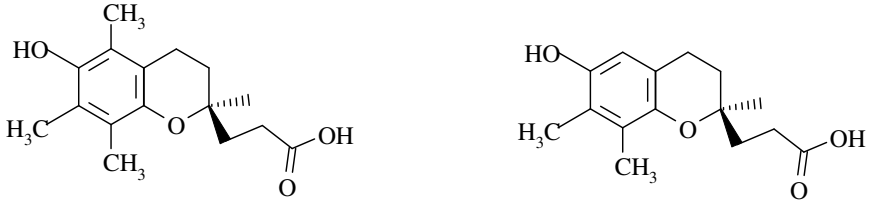
No organ functions as a storage organ for α-tocopherol, releasing it on demand. The bulk of vitamin E in the body is localized in the adipose tissue. HDL removes excess tissue tocopherol and returns it to the liver. Tocopherol efflux from adipose tissue may be important to maintain tissue levels during vitamin E deficiency.[42]

In the 1950s two major urinary metabolites of α-tocopherol, tocopheronic acid and tocopheronolactone were described (Simon's metabolites). Both metabolites are excreted in the urine as glucuronides or sulfates and have a shortened side chain and an opened chroman structure, indicating that they were formed from the α-tocopherol that had reacted as an antioxidant.[11] Today, some authors consider Simon's metabolites as artifacts produced during sample preparation.[74]

The catabolism of tocopherols is made by cytochromes P_{450}, isoforms CYP3A4, CYP3A5 and $CYP4F_2$.[8, 78] Degradation of tocopherols (α, γ, δ) leads to the carboxyethyl-hydroxychromans (α, γ, δ), which have an intact chroman ring.

The first step is a ω-hydroxylation of the side chain by the action of some CYP-dependent hydroxylases. This mechanism was indirectly proven by the inhibition of γ-CEHC formation by sesamin and ketokonazole, both which are inhibitors of the CYP3A family [60] and by the increase in α-tocopherol metabolites after induction of CYP3A by rifampicin in HepG2 cells.[8]

The initial ω-hydroxylation is followed by oxidation of the hydroxyl group to carboxyl group and β-oxidation to final carboxyethil hydroxychromans (CEHCs) (Fig. 2).



alpha-CEHC gamma-CEHC

Fig. 2 – Structures of α-CEHC (2,5,7,8-tetramethyl-2(2'-carboxyethyl)-6-hydroxychroman) and γ-CEHC (2,7,8-tetramethyl-2(2'-carboxyethyl)-6-hydroxychroman).

ω-Hydroxylation is the rate-limiting step in tocopherol metabolism. The competition of microsomal ω-hydroxylation with specific binding of α-tocopherol by α-TTP determines the metabolic fate of the individual tocopherols.[8] The excess of α-tocopherol seems to be converted to α-CEHC, whereas ingested γ- and δ-tocopherol may be almost quantitatively converted to their CEHCs and excreted in the urine.[12]

2.2. Proteins implicated in the vitamin E metabolism

In 1975, Catignani and collab., identified a cytosolic protein with a molecular weight of 32 kDa, that binds α-tocopherol, called **α-TTP** (α-tocopherol binding protein). Both Southern-blot hybridization and fluorescence *in situ* hybridization revealed a single α-TTP gene corresponding to the 8q13.1-13.3 region of chromosome 8.[86]

The specificity of α-TTP for α-tocopherol is due to: (1) all three methyl groups on the chromanol ring are important for recognition, but the methyl group from C_5 is critical for discrimination between α-,β- and γ-tocopherols; (2) hydroxyl group on the chromanol ring; (3) the structure and spatial configuration of the phytyl chain.

α-TTP is expressed at high levels in the liver and low levels in other tissues like brain,[32] spleen, lung, kidney and placenta. The levels of α-tocopherol in the diet modulate the expression of the α-TTP gene. Refeeding tocopherol to vitamin E-depleted rats increased the expression of α-TTP mRNA about 7-fold.[24] Also, an increase in the α/δ ratio in individual lipoprotein fractions is evidence of an increased α-TTP action concomitantly with the increased α-TTP mRNA expression.

Genetic defects in α-TTP are associated with a characteristic syndrome, ataxia with vitamin E deficiency **(AVED)**. AVED patients have neurological abnormalities, very similar to those of Friedreich's ataxia.[86] When these patients were given a high dose of α-tocopherol, the progression of the disease was slowed down.[96]

Recently, a family of cellular tocopherol associated proteins **(TAPs)**, that show GTP-ase activity, has been identified. TAP1 binds α-tocopherol better than the other tocopherols.[96] The highest amounts of TAP are found in the liver, brain and prostate. Nuclear translocation of TAP1 and transcriptional activation of some genes have been suggested to occur under the influence of α-tocopherol.[95]

A 15 kDa tocopherol-binding protein **(TBP)** that preferentially binds α-tocopherol may be responsible for intracellular distribution of α-tocopherol. The presence of a membrane TBP in the tissues may regulate their α-tocopherol levels.[22]

Phospholipid transfer protein **(PLTP)** is a plasma protein that is capable of mediating the rapid exchange of α-tocopherol between different lipoproteins classes and cells[43] and also facilitates the net transfer of α-tocopherol to endothelial cells.[12] PLTP deficiency results in the enhanced accumulation of vitamin E in the atherogenic apoB lipoproteins (VLDL and HDL) that leads to a decrease in their susceptibility to oxidative modification. PLTP knock-out mice were recently shown to be resistant to atherosclerosis.[37] If PLTP plays a similar role in humans, then PLTP inhibition may be a novel strategy to decrease atherosclerosis.[38]

There are proteins implicated in cellular selective α-tocopherol uptake. **SR-BI** (**s**cavenger **r**eceptor class **B** type **I**) is a cell surface glycoprotein that binds plasma HDL with high affinity and mediates selective uptake of HDL-associated lipids, including α-tocopherol. In rodents, tissues with the highest levels of SR-BI expression (adrenal gland) are those with the highest amount of α-tocopherol per gram of tissue.

The level of vitamin E from the diet modulates the expression of SR-BI. Feeding rats with a vitamin E-depleted diet resulted in an 11-fold increase in the SR-BI protein level in the liver tissue.[103] The same effect was observed in the case of human liver-derived tumor cell line HepG2. The cellular level of vitamin E exerts a tight control over the expression of SR-BI, by a signaling pathway which involves PKC.[103]

ABCA1 (**A**TP-**b**inding **c**assette transporter **A1**) is a membrane protein mainly known for its function in transporting cellular cholesterol and phospholipids to lipid-poor HDL apolipoproteins such as apolipoprotein AI. Mutations in the ABCA1 gene cause Tangier disease, a severe HDL deficiency

syndrome characterized by accumulation of sterols in tissue macrophages and prevalent atherosclerosis.[55] Previous studies suggested that HDL and/or its major apolipoprotein (apoA-I), play(s) a role in promoting secretion of α-tocopherol from cells. Lipid-free apoA-I promotes α-tocopherol efflux from macrophages in parallel to and just as effectively as cholesterol and phospholipid efflux.[58] Knock-out mice for the ABCA1 gene have a severe deficiency in fat-soluble vitamins, including vitamin E.[57]

3. FUNCTIONS OF VITAMIN E

Biochemistry textbooks describe vitamin E as a lipid soluble, chain breaking radical scavenger (anti-oxidant function). But in the past decades many studies have suggested that vitamin E has also many non-antioxidat functions. As the Roman god Janus that had two faces in one body, vitamin E, like 17-β-estradiol and all-*trans*-retinol, is named a **"Janus molecule".**[67]

3.1. Antioxidant functions

Vitamin E has the capacity to function as an anti-oxidant agent protecting from the oxidative stress caused by reactive oxygen species (ROS) and reactive nitrogen species (RNS).

The lung is one of the body organs directly exposed to pollutants from air, and so the alveolar surfactant is a target of air oxidants. The alveolar surfactant is supplemented with vitamin E during its formation by type II pneumocytes and vitamin E turnover increased in response to hyperoxia in type II pneumocytes, lamellar bodies and lung lavage. Protection of lung tissue by vitamin E is suggested by the finding that the vitamin E content of lung tissue is about twice that of other tissues.[24]

Atherosclerosis is a common form of cardiovascular disease and the most frequent cause of death in western countries. A hallmark of atherosclerosis is the accumulation of cholesterol beneath the intact endothelium-lining vessels. The accumulating cholesterol is derived from lipoproteins, primarily LDL.

According to the oxidative modification hypothesis, the most plausible and biologically relevant modification of LDL is oxidation. LDL can be oxidatively modified by all major cells of the arterial wall.[6, 70] Native LDL enters the sub-endothelial space, where it is retained by components of the extracellular matrix (proteoglycans).[79] As a result of this residence in the early phase occurs a mild oxidation of LDL with formation of minimally modified LDL (MM-LDL) in the subendothelial space. MM-LDL stimulates production of monocyte chemotactic protein-1 (MCP-1) that promotes monocytes chemotaxis and also stimulates expression of adhesion molecules on endothelial cells. In the subendothelial space MM-LDL stimulates the production of monocyte colony stimulating factor (M-CSF) which promotes the differentiation of monocytes into macrophages.[40] Macrophages can further oxidize MM-LDL to Ox-LDL, which is not recognized by the LDL receptor but is taken avidly by scavenger receptors of macrophage. The expression of this scavenger receptors is not feed-back regulated so macrophage become overloaded with cholesteryl esters leading to the "foam-cell" formation.[40]

Ox-LDL has several biological consequences: promotes vasoconstriction, promotes adhesion, stimulates cytokine (IL-1), increases platelet aggregation, inhibits nitric oxide tissue factor secretion and stimulates plasminogen activator inhibitor-1 synthesis.[40]

Monocytes and macrophages secretes several proinflammatory, pro-atherogenic cytokines, such as IL-1β and TNF-α, which augment monocyte-endothelial adhesion.[64] IL-1β stimulates smooth muscle proliferation *via* PDGF (**p**latelet **d**erived **g**rowth **f**actor) and TNF-α promotes apoptosis of macrophages and smooth muscle cells (SMC). Many stimuli induce SMC migration and subsequent proliferation, resulting in narrowing of the lumen.[70] SMC proliferation represents a significant central event in the fibrous plaque formation.

Vitamin E could act as an antioxidant or pro-oxidant under different circumstances.

LDL ensures the distribution of vitamin E to peripheral tissues. Each LDL particle is a mixture of cholesterol, phospholipids, cholesteryl esters, TAGs, carotenoids and 6-10 molecules of α-tocopherol.[23]

LDL's content of polyunsaturated lipids is around 50%, predisposing LDL particles to oxidative modifications.[101]

The pro-oxidant activity of α-tocopherol can be explained by a mechanism called **tocopherol-mediated peroxidation (TMP)** (Fig. 3).

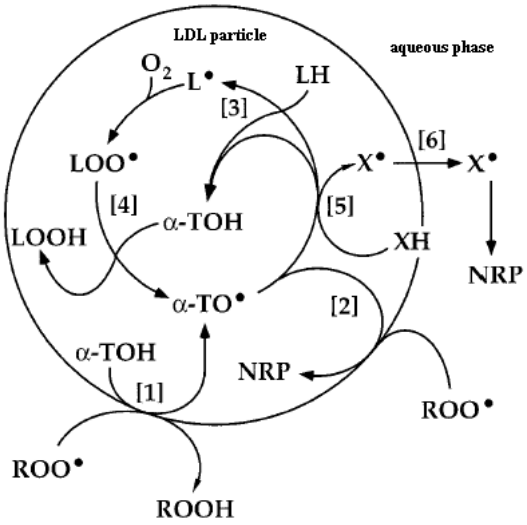


Fig. 3 – Anti-oxidant and pro-oxidant effects of tocopherol [after Upston, 1999].

The oxidant, the peroxyl radical (ROO•) can interact with either the surface lipid of the LDL particle or α-tocopherol. Due to the redox-active hydroxyl group of the chromanol ring, located very close to the surface of the LDL particle, ROO• tends to react preferentially with α-tocopherol (reaction 1, Fig. 3), generating the α-tocopheroxyl radical (α-TO•).

The fate of the resulting α-TO• determines whether the overall result of this initial reaction is pro- or antioxidant.

In the presence of a high concentration of ROO•, termination reactions between α-TO• and ROO• are frequent (reaction 2, Fig. 3). So the lipid peroxidation is diminished and α-tocopherol is consumed (antioxidant activity). In the presence of a low concentration of ROO• termination reactions are infrequent. In this situation α-TO• moves within the LDL particle (pro-oxidant activity), mechanism called phase transfer activity of α-tocopherol (reaction 1, Fig. 3).

Inside the LDL particle, in the absence of other radicals, α-TO• triggers the formation of LOO• (reaction 3, Fig. 3). α-tocopherol from the inside of the particle rapidly scavenges LOO• to generate LOOH and α-TO•, which will continue the cycle (reaction 4, Fig. 3). If this cycle is not interrupted, a great percentage of LDL lipids will become oxidized without substantial loss of the vitamin. The ability of α-TO• to maintain this cycle is called chain transfer activity of α-tocopherol (reactions 3 and 4, Fig. 3).

A strategy to inhibit radical-induced lipid peroxidation of LDL and other lipoproteins is the elimination of α-TO•. This may take place by a bimolecular termination reaction (reaction 2, Fig. 3) or by reaction of α-TO• with an co-antioxidant (XH) (reaction 5, Fig. 3). The resulting X• must be relatively unreactive toward LH and it must be able to rapidly exit the particle or transfer its radical character to another species that leaves the LDL particle. This mechanism could explain why the balance α-TOH/co-antioxidants, rather than α-TOH alone, determines if the LDL particle will undergo lipid peroxidation.[93] The most important co-antioxidants are ubiquinol-10 ($CoQ_{10}H_2$), α-tocopherylhydroquinone, ascorbate, bilirubin and 3-hydroxyanthranillic acid (a metabolite of tryptophan).[80]

In the LDL particle $CoQ_{10}H_2$ acts predominantly as a co-antioxidant for α-TOH.[28] Co-enrichment of LDL with $CoQ_{10}H_2$ and α-TOH demonstrates that the co-antioxidant also attenuates efficiently the pro-oxidant activity of vitamin E.[26, 84]

The regeneration of α-TOH from α-TO• by ascorbate (Fig. 4), with concomitant generation of the ascorbyl radical, is well known.[75] In an aqueous environment the oxidation of LDL by ROO• is rapidly inhibited by vitamin C, causing cessation of α-TOH consumption and lipid oxidation. After removal of ascorbate, α-TOH consumption and LDL's lipids oxidation will have the same rates as those prior to the addition of ascorbate.[10, 12]

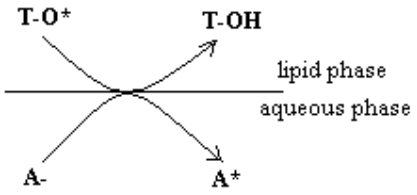


Fig. 4 – Interaction of ascorbate with α-tocopheroxyl radical (T-O*=α-tocopheroxyl radical; T-OH=α-tocopherol; A^-=ascorbate; A*=ascorbyl radical.

The question of whether vitamin E promotes or inhibits atherosclerosis has no conclusive answer, because of the discrepancy between *in vitro* and *in vivo* studies. Many trials suggest that vitamin E, together with co-antioxidants might be superior in protecting LDL from oxidation than α-tocopherol alone. These results could be correlated with the observation that foods containing lesser amounts of vitamin E generally provide greater benefit than de pharmacologically large doses of vitamin E alone.[79]

Because of its lack of one of the electron-donating methyl groups on the chromanol ring, γ-tocopherol is somewhat less potent in donating electrons than α-tocopherol is and it is a slightly less powerful antioxidant.[39] However, the unsubstituted C-5 position of γ-tocopherol appears to make it better able to trap lipophilic electrophiles such as reactive nitrogen oxide species (RNOS). Excess generation of RNOS is associated with chronic inflammation-related diseases such as cancer, cardio-vascular diseases (CVD) and neurodegenerative disorders.[1] RNOS formed during inflammation include peroxynitrite, nitrogen dioxide and nitrogen dioxide like species generated from myeloperoxidase or superoxide dismutase. Cooney and collab.[18] showed that γ-tocopherol reduces nitrogen dioxide to less harmful nitric oxide or traps nitrogen dioxide to form 5-nitro-γ-tocopherol (5-Nγ-T). Also, γ-tocopherol can be nitrated by peroxynitrite. Because the chromanol ring of α-tocopherol is fully substituted, this form of vitamin E cannot form a stable adduct.[17]

5-Nγ-T was proposed as a novel marker for detecting the formation of RNOS. Hensley and collab.[31] reported an HPLC method for measuring 5-Nγ-T with a coulometric array detection system. Christen and collab. developed a highly sensitive HPLC assay with electrochemical detection in which the 5-Nγ-T tissue can be measured simultaneously with α-tocopherol, γ-tocopherol and unesterified cholesterol.

5-Nγ-T has elevated concentrations in the plasma of subjects with coronary heart disease.[50] So γ-tocopherol may be an important mechanism for detoxification of RNOS *in vivo*. Such a role is supported by the observation that patients with coronary heart disease have a higher α-tocopherol/γ-tocopherol ratio than healthy controls, but no difference in α-tocopherol, suggesting selective depletion of γ-tocopherol.

NO_2 can form in biologic systems from the reaction of NO• with O_2. NO• can act either as a pro-oxidant or an antioxidant. NO• alone acts as a potent antioxidant by rapidly scavenging lipid peroxyl radicals.[71] NO can, however, react rapidly with the superoxide radical to yield peroxynitrite ($ONOO^-$), which is a potent oxidant capable of initiating lipid peroxidation reactions[62] and leads to formation of 3-nitrotyrosine which affects the function of many proteins.[77]

NO• acts as a pro-oxidant when the rate of production of superoxid radical is greater than that of NO• production.[29]

3.2. Non-antioxidant functions

Several tocopherols have special properties that are unrelated to their anti-oxidant capacity. These effects can be explained by the modulation of protein kinase C (PKC) activity by α-tocopherol in different cell types, including monocytes, macrophages, neutrophiles, fibroblasts and mesangial cells.

In a cell there are more different isoforms of PKC but only PKCα is inhibited by α-tocopherol. α-tocopherol activates the protein phosphatase type 2A, which in turn dephosphorylates PKCα and inhibits its activity.[66]

Vascular SMC proliferation represents a significant event in a number of diseases such as arteriosclerosis and hypertension. SMC proliferation is controlled by growth factors released from blood cells, by inhibitors or stimulants produced by the vessel wall cells, by tocopherols and by ROS.

α-Tocopherol inhibits at physiological concentrations the SMC proliferation, by blocking the PKC activity, whereas β-tocopherol is ineffective.[83]

In atherogenesis foam cells are formed by the uncontrolled uptake of oxidized LDL (Ox-LDL) containing cholesterol and lipids. Inhibition of PKC by α-tocopherol could lead to changes of the gene expression patterns altered in atherosclerosis. Candidate genes for such alteration are the scavenger receptors, which take up modified LDL. These receptors include SR-A, SR-B1, CD36, CD68 and LOX-1.[95] Knock-out mice for the macrophage scavenger receptors have shown reduced incidence of atherosclerosis.[82]

The CD36 scavenger receptor is expressed in megakaryocytes/platelets, monocytes/macrophages, mammary epithelial cells, adipocytes, capillary endothelial cells of adipose, cardiac and muscle tissue and at low levels in the vascular endothelium of the brain, lung and kidneys.[28] CD36 can bind to a large variety of ligands: thrombospondin, collagens type I and IV, fatty acids, anionic phospholipids, HDL and oxLDL.[94] Monocytes/macrophages from CD36-deficient patients show a reduced capacity to bind and internalize oxLDL.[54] Both the CD36 mRNA and the corresponding protein are down-regulated by α-tocopherol, and the reduction of CD36 expression leads to a reduction of oxLDL uptake.[69]

Binding of Ox-LDL to CD36 activates transcriptional factors such as NF-kB [45] and the nuclear receptor PPARγ.[85] In human macropahges expressing CD36 activation of NF-kB by Ox-LDL induces the expression of several cytokines such as interleukin (IL)-1Ra, IL-1β, IL-6, TNF-α, TNF-β, IFN-γ and IFN-β, implicated in the development of atherosclerosis.[35]

α-Tocopherol leads to the up-regulation of α-tropomyosin expression [2], inhibits liver collagen α1(I) gene expression.[16]

In vitro enrichment of human aortic endothelial cells with α-tocopherol significantly inhibited LDL-induced adhesion of monocytes to endothelium in a dose-dependent manner with a concomitant reduction in levels of ICAM-1 (**i**ntercellular **c**ell **a**dhesion **m**olecule **1**).[47] Pretreatment of monocytes with α-tocopherol leads to a decreased expression of CD11b and VLA-4 (**v**ery **l**ate **a**ntigen 4), possibly by inhibiting the activation of NF-kB.[34]

α-Tocopherol inhibits aggregation of human platelets by a PKC-dependent mechanism, both *in vitro* and *in vivo* [25] and delays intra-arterial thrombus formation.[72]

In phagocytic cells the multicomponent enzyme NADPH-oxidase makes the production of superoxide anion ($O2^-$). This complex includes membrane-bound cytochrome b_{558} and cytosolic proteins ($p47^{phox}$, $p67^{phox}$, Rac1/2 si $p40^{phox}$) [14] that translocate to the membrane during stimulation to form a catalytically active oxidase.[20] During NADPH-oxidase activation, $p47^{phox}$ is phosphorylated on several Ser residues [5] by PKC.[3]

α-Tocopherol inhibits O_2^- production by human adherent monocytes by impairing the assembly of the NADPH-oxidase. The inhibition of phosphorylation and translocation of the cytosolic factor $p47^{phox}$ results from a decrease in PKC activity.[13]

α-Tocopherol blocks at the post-transcriptional level 5-lipooxygenase inhibiting the release of IL-1 by human activated monocytes.[21]

α-Tocopheryl succinate (α-TOS) exhibits *in vivo* anti-tumor activity and, *in vitro*, induces apoptosis of cultured tumor cells.[52] This activity requires the intact succinyl ester moiety, as neither α-tocopherol nor α-tocopheryl acetate induced apoptosis in a variety of cells. α-TOS has been shown to cause deregulation of the protein phosphatase-2A (PP2A)/protein kinase C (PKC) pathway, which can be important for maintaining the anti-apoptotic function of Bcl-2.[51] The α-TOS-induced apoptosis may be a consequence of lysosomal destabilization, probably due to the detergent-like activity of the agent.[52] Because α-TOS is a weak acid with a pK_a of 5.64, it is possible that it may act as a potentially selective

and effective anti-tumour drug at the acidic pH of tumor interstitium, a potentially powerful anti-cancer strategy that must be explored.[63]

It is generally accepted that α-tocopherol exerts its inhibitory action on a number of cell reactions in a PKC-dependent manner. But the expression of several genes is regulated in a PKC-independent way.

γ-Tocopherol was mostly ignored in the past because of its relatively low animal plasma and tissue concentrations. γ-tocopherol possesses anti-inflammatory activity and at physiological concentrations it may be important in the prevention of some human diseases with an inflammatory component (cancer, vascular heart diseases).[36]

Cyclooxygenase 2 (COX-2)-catalyzed PGE_2 is one of the most important early events during inflammation. As a key inflammatory mediator, PGE_2 is known to stimulate cytokine generation, cause vasodilatation, and mediate fever and pain.[102]

At physiological concentrations, γ-tocopherol reduces PGE_2 synthesis in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages and IL-1β-treated A549 human epithelial cells.[36] It remains to be determined whether the inhibitory activity of γ-tocopherol is caused by its competing with arachidonic acid at the binding site of COX-2, or inhibition of lipid peroxidation as a nucleophile, or both.

γ-Tocopherol modulates cholesterol biosynthesis by post-traslational inhibition of HMG CoA-reductase, the rate-limiting enzyme in cholesterol synthesis.[59]

Recently, for the first time, a direct transcriptional gene activation by tocopherols and tocotrienols has been demonstrated. Gene activation is mediated by the nuclear receptor PXR (pregnan X receptor) that regulates the expression of a variety of genes coding for cytochromes P_{450}.[97] γ-tocotrienol was the most effective activator of PXR at concentrations of 1-10 μM which can be reached in human plasma.[44]

Vitamin E deficiency impairs immune function, including both T and B cell-mediated functions.[48] Lipopolysaccharide-stimulated macrophages isolated from old mice produced significantly more PGE_2 than those from young mice did. Moreover, vitamin E supplementation of old mice shifted the response of their macrophages to one typical of the macrophage of young mice. Increased PGE_2 production was correlated with an increased activity of COX.[104]

4. TOCOPHEROLS AND THERAPEUTIC IMPLICATIONS

α-Tocopherol was originally described as a fertility maintenance agent whose absence from the diet rendered rodents infertile. After this discovery, the biological activity of different tocopherols analogues was described using the rat fetal resorption assay.[7]

During **pregnancy**, there is a proportional elevation of α-tocopherol concentration with the concentration of blood lipids. It is supposed that vitamin E requirements are increased during the pregnancy. For this reason, vitamin supplements are prescribed (50 mg of vitamin E) for pregnant women.[11]

The effects of α-tocopherol supplementation on the **cardiovascular system** was evaluated in four larger prospective clinical trials: the α-Tocopherol β-Carotene **(ATBC)** study, the Cambridge Heart Antioxidant Study **(CHAOS)**, the Gruppo Italiano per lo Studio della Supervienza nell'Infarto miocardico **(GISSI)** and the Heart Outcomes Prevention Evaluation **(HOPE)** study.

In the ATBC study, supplementation with 50 mg *allrac*-α-tocopheryl acetate/day for 5-8 years generates only a lower incidence of angina pectoris in contrast with control subjects. In the case of male smokers there wasn't a significant difference in cardiovascular mortality between the subjects who received and those who didn't receive the vitamin supplement.

In the CHAOS study administration of a α-tocopherol supplement (800 IU/d and 400 IU/d) significantly decreased the frequency (77%) of nonfatal myocardial infarctions as compared to the control subjects.

In GISSI trial, subjects with a previous myocardial infarction received a daily supplement of *allrac*-α-tocopherol (300 mg) alone or in combination with 1 g of polyunsaturated fatty acids. The result was a

significant decrease of cardiovascular deaths (20%) in the α-tocopherol group as compared to the control group.

Cystic fibrosis is an autosomal inherited genetic disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator gene. Patients with cystic fibrosis exhibit frequently vitamin E deficiency due to the malabsorbtion of lipids. Also, these patients have subacute inflammatory processes almost continuously. Supplementation of vitamin E has become part of the routine therapy.[65] The recommended dose for adults is 200-400 IU/day without any specification of the form.

The ATBC study was designated to evaluate the effects of vitamin E supplementation on **prostate cancer** development. Male smokers who took vitamin E supplements had a 34% lower incidence of prostate cancer and 41% lower mortality from prostate cancer than did those who did not take the supplements.[30] But α-tocopherol had no effect on advanced prostate cancer.

In the case of **preeclampsia**, characterized by vasospasm, increased vascular resistance and reduced organ reperfusion, free radicals are incriminated. In the blood samples from women with preeclampsia there are elevated levels of markers of lipid peroxidation like malondialdehyde [33] and 8-epiprostaglandin-$F_{2\alpha}$.[4]

In a trial with 283 women who were at an increased risk of preeclampsia, those who were supplemented with vitamins E (263mg/day) and C (1000mg/day) had an 8% incidence of preeclampsia and those who received placebo had a 17% incidence of the disorder.[15]

Inadequate intake of vitamin E causes neuropathologic changes to the **nervous system**. Many studies incriminate an excessive oxidative stress that is the causative factor in the development of some diseases of the nervous system like Parkinson and Alzheimer.

Effects of vitamin E administration in the case of **Parkinson disease** was investigated in a **DATATOP** (**d**eprenyl **a**nd **t**ocopherol **a**ntioxidant **t**herapy **o**f **P**arkinson disease) study. DATATOP was a double-blind placebo-controlled study involving the use of 2000 IU vitamin E/day, 10 mg deprenyl/day, or both for the treatement of Parkinson disease.[61]

Sano and collab conducted a study similar to DATATOP in the case of **Alzheimer disease.**[71] This was a two year, double-blind, placebo-controlled, randomized, multicenter clinical trial involving 341 patients with Alzheimer disease of moderate severity. Patients were given placebo, vitamin E (2000 IU/day), selegiline (10 mg/day), or a combination of vitamin E and selegiline.

A treatment with vitamin E may be required for several years to improve the cognitive state of patients with nervous system diseases. There still remains the question if administration of antioxidants, like vitamin E, could have the ability to regenerate the neurons that were irreversibly damaged, by an oxidative stress, before the diseases became clinically manifest.

So, to determine the effects of vitamin E supplementation in the case of patients with Alzheimer and Parkinson diseases, it is necessary to design long-term studies, involving patients at as early a stage as possible.

In 1996 Wechter and collab., first identified γ-CEHC (a γ-tocopherol metabolite) as an endogenous natriuretic factor in uraemic patients that promotes Na^+ excretion and contributes to the regulation of extracellular fluid volume.[98] The natriuretic action of γ-CEHC is highly Na^+ specific, sparring K^+ from excretion. The natriuretic activity could be beneficial for the cardiovascular system by lowering blood pressure.

F_2-isoprostanes, isomers of PGF_2, has been suggested as a reliable index of *in vivo* free radical generation and oxidative lipid damage. 8-iso-$PGF_{2\alpha}$ is one of the most abundant F_2-isoprostanes produced *in vivo* by humans,[49] and its excretion is depressed by antioxidant vitamins.[68]

Enhanced formation of F_2-isoprostanes is associated with several cardiovascular risk factors, including hypercholesterolemia, diabetes mellitus and cigarette smoking, characterized by increased lipid peroxidation in response to complex metabolic abnormalities or various constituents of cigarette smoke.

In **hypercholesterolemic** and **diabetic patients**, 2-week supplementation with vitamin E (600 mg/day) was associated with normalization of enhanced F_2-isoprostane formation. Short-term administration of vitamin E (100 and 800 IU/day for 5 days) to healthy chronic smokers failed to suppress urinary 8-iso-$PGF_{2\alpha}$, and administration of vitamin C (2g/day), alone or in combination with vitamin E, only partially reduced isoprostane excretion (by 20%-30%).[68] Small sample size and short duration of vitamin E supplementation could explain this discrepancy.

5. CONCLUDING REMARKS

For long enough, vitamin E has been mislabeled simply as a lipid-soluble antioxidant. Clearly, the tocopherols can act as chain-breaking free radical scavengers, and α-tocopherol, in particular has *in vivo* potency in this respect.

Recent work has begun to focus on so-called desmethyl tocopherols such as γ-tocopherol and CEHC metabolites as candidates for new biological properties. For example γ-tocopherol might exert anti-inflammatory, natriuretic actions, being a scavenge reactive nitrogen species more efficient than α-tocopherol and thereby providing special benefits.

Even after 80 years of research on vitamin E biochemistry it would be more prudent to admit that much remains to be discovered, and to guard against unwarranted assumptions such as that α-tocopherol supplementation is always helpful and never harmful. Future investigation should pay close attention to γ-tocopherol/α-tocopherol ratio and individual vitamers.

The different vitamers of vitamin E are very good examples of the relationship between the stereochemistry and biological activity of a compound.

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