Published in final edited form as:
Free Radic Biol Med. 2007 July 1; 43(1): 4–15.

Vitamin E, Antioxidant and Nothing More

Maret G. Traber[1] and **Jeffrey Atkinson**[2]

1*Linus Pauling Institute, Oregon State University, Corvallis, OR 97331*

2*Department of Chemistry, Brock University, St. Catharines, Ontario, Canada, L2S 3A1*

Abstract

All of the naturally occurring vitamin E forms, as well as those of synthetic *all rac*-α-tocopherol, have relatively similar antioxidant properties, so why does the body prefer α-tocopherol as its unique form of vitamin E? We propose the hypothesis that all of the observations concerning the in vivo mechanism of action of α-tocopherol result from its role as a potent lipid soluble antioxidant. The purpose of this review then is to describe the evidence for α-tocopherol's in vivo function and to make the claim that α-tocopherol's major vitamin function, if not only function, is that of a peroxyl radical scavenger. The importance of this function is to maintain the integrity of long-chain polyunsaturated fatty acids in the membranes of cells and thus maintain their bioactivity. That is to say that these bioactive lipids are important signaling molecules and that changes in their amounts, or in their loss due to oxidation, are the key cellular events that are responded to by cells. The various signaling pathways that have been described by others to be under α-tocopherol-regulation appear rather to be dependent upon the oxidative stress of the cell or tissue under question. Moreover, it seems unlikely that these pathways are specifically under the control of α-tocopherol given that various antioxidants other than α-tocopherol and various oxidative stressors can manipulate their responses. Thus, virtually all of the variation and scope of vitamin E's biological activity can be seen and understood in the light of protection of polyunsaturated fatty acids and the membrane qualities (fluidity, phase separation and lipid domains) that polyunsaturated fatty acids bring about.

Introduction

Vitamin E was discovered in 1922 by Evans and Bishop as a necessary dietary factor for reproduction in rats [1]. Subsequent studies showed that the presence of rancid fat in the experimental diets fed to rats and chickens was the causative agent of various pathologies in these animals and that these abnormalities could be "cured" by wheat germ oil concentrates that later were demonstrated to contain tocopherols [2-8], work that has been reviewed by Wolf [9].

Given that most other vitamins have a co-factor function, are ligands for nuclear receptors, or have some unique molecular role; the search for a more specific vitamin E function has continued since its discovery. We propose the hypothesis that all of the observations concerning the in vivo mechanism of action of α-tocopherol result from its role as a lipid soluble antioxidant. Why are the proofs of this hypothesis so scarce after more than 80 years research on vitamin E? If oxidized α-tocopherol accumulated, or even was excreted in detectable amounts, its role as an antioxidant could be readily demonstrated. Instead, the tocopheroxyl

Address for Correspondence: Maret G. Traber, Ph.D., Professor, Linus Pauling Institute, Oregon State University, Corvallis, OR 97331-6512, 541-737-7977, fax: 541-7375077 email: maret.traber@oregonstate.edu

radical is formed during the antioxidant action, but then is reduced by other antioxidants and thus the ephemeral oxidation product of α-tocopherol does not remain to be measured. Although this concept of antioxidant interactions has been discussed and demonstrated in vitro [10], it is only recently that evidence has been obtained in humans for its existence [11]. Moreover, a combination deficiency of vitamin E and either selenium [12] or vitamin C [13] results in death to guinea pigs within days of the initiation of the second deficiency. These results highlight the importance of the multiple low molecular weight antioxidant systems necessary for vitamin E's function.

The purpose of this review then is to describe the evidence for α-tocopherol's in vivo function and to make the claim that α-tocopherol's vitamin function, if not only function, is that of a peroxyl radical scavenger. The importance of this function is to maintain the integrity of long-chain polyunsaturated fatty acids in the membranes of cells and thus maintain their bioactivity. That is to say that these bioactive lipids are important signaling molecules and that changes in their amounts, or in their loss due to oxidation, or their oxidation products are the key cellular events that are responded to by cells. This hypothesis is supported by studies in plants where the genes for vitamin E synthesis have been knocked out. Sattler et al. [14] show that nonenzymatic lipid peroxidation products in the seedlings from these plants up-regulated genes, and they proposed that increased tocopherol levels in response to environmental stresses may limit nonenzymatic lipid peroxidation and thereby prevent the inappropriate induction of stress responses.

Regulation of α-tocopherol concentrations

Of the four tocopherols and four tocotrienols (designated as α-, β-, γ-, and δ-) found in food, only α-tocopherol meets human vitamin E requirements [15]. Despite the fact that all of these vitamin Es have similar antioxidant functions (rate constants for H-atom donation within an order of magnitude (Table)), non-α-tocopherols are poorly recognized by the hepatic α-tocopherol transfer protein (α-TTP) [16]. Moreover, defects in the human α-TTP [17] lead to severe vitamin E deficiency [18]. The vitamin E deficiency has been recapitulated in mice by deleting the α-TTP gene [19,20].

α-TTP is responsible for maintaining plasma α-tocopherol concentrations [21]. The crystal structure of α-TTP has been reported [22,23]. The important structural features of the ligand for recognition by α-TTP include: 1) a fully methylated chroman ring, 2) a phytyl pyrophosphate-derived tail [24] (trimethyltridecyl-residue (IUPAC) [25]), and 3) the *R*-configuration at C-2 where the tail attaches to the chromanol ring [26]. This third requirement makes α-TTP selective for the *2R*-isomers of synthetic α-tocopherol, as has also been demonstrated in α-TTP null mice [27]. The preferential binding of *RRR*-α-tocopherol by α-TTP is reflected in the 3-fold greater half-life of *RRR*-α-tocopherol (57 ± 19 hours) compared to the half-lives of *SRR*-α-tocopherol [28] or γ-tocopherol [29]. Thus, only natural α-tocopherol *(RRR*- α -tocopherol) and 2*R*-α-tocopherols in synthetic, *all rac*-α-tocopherol, not the other vitamin E forms, are maintained in human plasma and tissues by α-TTP [30]. The mechanism by which α-TTP maintains plasma α-tocopherol remains under intense investigation [31-34], but it is clear that in the absence of α-TTP the default pathway results in lysosomal accumulation of α-tocopherol and its ultimate excretion rather than the secretion of α-tocopherol into the plasma, as shown in patients with defective α-TTP [35]. Moreover, feeding a diet with 550 mg γ-tocopherol/kg diet to α-TTP null mice did not replete their tissues with γ-tocopherol, demonstrating that even in the absence of α-tocopherol, γ-tocopherol is not utilized, but rather was metabolized.

Metabolism of vitamin Es appears to also play a major role in hepatic regulation of vitamer concentrations. The various non-α-tocopherol forms are metabolized in preference to α-

tocopherol [36,37]. The metabolites of vitamin E are the CEHC (2'-carboxyethyl-6-hydroxychroman) products of the respective forms of vitamin E, i.e., α-, β-, γ-, and δ-CEHC. The various vitamin E forms are ω-oxidized by cytochrome P450s (CYPs), then following ß-oxidation are conjugated and excreted in urine [38] or bile [39]. Similarly to other xenobiotics, CEHCs are sulfated [40] or glucuronidated [36,41,42]. CEHCs are excreted urine and bile. Again metabolism is geared to eliminate non-α-tocopherol forms of vitamin E. Surprisingly, even Drosophila appear to have the mechanism to metabolize non-α-tocopherols, although they do not seem to express α-TTP; nonetheless they efficiently prefer α-tocopherol [43].

Vitamin E antioxidant activities

The curiosity is that all of the naturally occurring vitamin E forms, as well as those of synthetic *all rac*-α-tocopherol, have relatively similar antioxidant activities, so why does the body prefer α-tocopherol as its form of vitamin E? Vitamin E's antioxidant function, as a peroxyl radical scavenger that terminates chain reactions, is well-known and well-described by various chemists [44-54].

There are important differences between the various vitamin E forms with respect to their antioxidant activities when measured in vitro (Table). Whether they are measured in organic solution such as chlorobenzene or in detergent supported lipids (to mimic biological membranes), the rank order of the tocols remains the same and the greater potency of α-tocopherol versus the other vitamers is approximately constant. Surprisingly, this is true across the different chemical systems, as well as different investigators. These results are due to the relative H-atom donating ability of the different tocols, which increases in efficiency with greater ring methyl-substitution. However, relative to their efficacies in the rat fetal resorption test these differences in *in vitro* antioxidant activities are rather minor. The differences in potency of α-tocopherol versus other tocols in vivo is due to hepatic discrimination favoring α-tocopherol, as well as the preferential metabolism of non-α-tocopherol forms, as discussed above.

What has not been established is whether non-α-tocopherols follow the same rank order of antioxidant efficiency when in biological membranes. In actual membranes, the antioxidant power would include the H-atom donating ability, location (penetration) and movement within the membrane, plus the efficiency of tocopheroxyl radical recycling by cytosolic reductants, such as ascorbate. Attempts have been made to compare α-tocopherol and α-tocotrienol; in microsomal membrane systems the conclusion was that tocotrienols are better antioxidants [57], but in more chemical systems they are said to be near equivalent [58,59]. In the most recent contribution to this literature, Mukai et al. [55] describe α-tocopherol as being more efficient than other vitamers as measured by H-atom donation to aryloxy radicals (ArO•) in Triton X-100 supported micelles. Importantly, in these micelles, non-α-tocopherols behaved much worse than their relative antioxidant efficiency in organic solvents such as ethanol, ether, or benzene (TABLE 1, footnote [{b}]). It should be emphasized, however, that the Triton micelles contain no oxidizable lipid and thus, might be poor membrane mimics. However, it would appear from this study that fundamental physical organic behavior would favor the use of α-tocopherol as a protector of lipids without even considering the efficiency of in vivo recycling.

Additionally, it should be noted that the fully methylated chromanol ring of α-tocopherol prevents adduct formation at the positions that are not methylated in the non-α-tocopherols. For example, the formation of 5-nitro-γ-tocopherol has been touted as a marker of the benefit of γ-tocopherol scavenging reactive nitrogen species [60]. Provocatively, Tafazoli et al. [61] reported that the order of vitamin E analogues in catalyzing hepatocyte cytotoxicity, lipid peroxidation, and GSH oxidation was δ-tocopherol > γ-tocopherol > α-tocopherol > PMC. The toxic prooxidant activity of vitamin E analogues was therefore inversely proportional to their

antioxidant activity. The in vivo preference for α-tocopherol may then also rest on the potential "toxic" activity of the vitamin E analogues. Indeed, Cornwell's laboratory has made a case for non-α-tocopherols generating cytotoxic adducts [62,63].

The relevance of tocols other than α-tocopherol has only recently been considered, and largely has focused on γ-tocopherol. Consequently, using non-α-tocopherols as controls of apparent equal antioxidant power in cell culture experiments may be misleading since we have no expectation that they should even be as good as they are in *in vitro* experiments. For instance, if β-tocopherol is inefficiently recycled in membranes and so it is lost relatively quickly compared with α-tocopherol and thus never exerts a persistent antioxidant effect, then β-tocopherol would not be expected to be as effective as α-tocopherol towards inhibiting lipid peroxidation, nor in any possible associated activity as a regulator of cell signaling. A better test compound than β-tocopherol, Trolox or PMC (as has been used by several groups to "prove" non-antioxidant behavior of α-tocopherol) would be something that looks more like α-tocopherol but that is not capable of H-atom donation or one electron donation [64,65]. The best candidate is a 6-chloro-substituted chroman, such as 2,5,7,8-tetramethyl-2-(4',8',12'-trimethyltridecyl)-6-chlorochromane, as described by Donchenko et al. [66]. Thus, the chloro-derivative could demonstrate whether a non-antioxidant function in signaling is possible.

To further complicate the in vitro investigation of non-antioxidant α-tocopherol functions, cellular concentrations are not often measured. When the various tocols are added to cells in culture, the concentrations found in the cells can be quite variable and not necessarily the same as what is added in culture [67,68] and can vary depending on the ability of the cell to metabolize the various tocols [68]. The amount of the various tocols present in cells in vitro, therefore, should be a necessary measurement for all signaling experiments. Ideally, the cell tocol concentrations should mimic those found in vivo.

Human Vitamin E Deficiency

Human vitamin E deficiency symptoms began to be reported in the 1960s [69] in various case studies of patients with lipoprotein abnormalities and subsequently in fat malabsorption syndromes, but because these patients had malabsorption of other nutrients, especially long chain fatty acids, it was not clear the extent to which various symptoms could be attributed to lack of vitamin E. In the early 1980s [70] case studies and various reports of unique humans with vitamin E deficiency symptoms without fat malabsorption began to appear in the literature. Subsequently, the cause of this form of human vitamin E deficiency was shown to be a defect in the gene for the α-TTP [18,71,72].

The characteristic vitamin E deficiency syndrome in these humans with ataxia with vitamin E deficiency (AVED) was not anemia, as seen in many animal models of vitamin E deficiency [73], rather AVED patients had a peripheral neuropathy characterized as a dying back of the large caliber axons in the sensory nerves [74]. Remarkably, AVED patients had decreased vitamin E concentrations in their nerves prior to the appearance of abnormal nerve function [75], a phenomenon that was recapitulated in dogs fed vitamin E deficient diets [76,77].

The peripheral neuropathy of vitamin E deficiency in humans is described as a progressive dying back of the nerves [74]. Interestingly, the symptoms observed in these patients is similar to the abnormalities observed in patients with Friedreich ataxia [78-81], a genetic disorder caused by a mutation in the gene for frataxin, a mitochondrial iron-binding protein [82]. Thus, free iron in mitochondria could cause lipid peroxidation, an increased need for a lipid soluble antioxidant and therefore a decrease in α-tocopherol. The dying back of the sensory neurons in both ataxias is likely caused by insufficient nerve vitamin E resulting in apoptosis. So is this a sign of vitamin E signaling that cells should undergo apoptosis, or an oxidative stress due to

lack of vitamin E antioxidant function? We are currently studying the question, but do not have an answer.

Cell Proliferation and Apoptosis

Protein kinase C

Cell proliferation and differentiation, along with apoptosis, are important cellular regulatory mechanisms that must be closely controlled. Protein kinase C (PKC) is a key-signaling molecule involved in this regulation of growth and differentiation. The PKC family encompasses 12 different isozymes that transduce various signals as a result of receptor activation [83]. PKC is expressed in a variety of cells [84] and reportedly is inhibited by α–tocopherol [85], perhaps by activation of protein phosphatase 2A, increasing PKC-alpha dephosphorylation [85]. Moreover, α-tocopherol has been described to inhibit PKC in various cell types with consequent inhibition of platelet aggregation, endothelial cell nitric oxide production and superoxide production in neutrophils and macrophages [86]. Indeed, α-tocopherol was found to attenuate p67 phox translocation, which abrogates superoxide production by glial cells through a PKC-mediated mechanism [87]. Moreover, platelet aggregation was found to be prevented by α-tocopherol through a PKC-dependent pathway [88,89]. These studies are examples of reports showing the relationship between PKC and α-tocopherol, and there are many others. However, the relationship does not appear to be specific to an α-tocopherol regulatory function in that oxidative stress seems to have an important role in PKC regulation.

Low concentrations of peroxide added to cells in culture result in a reversible oxidation/activation of PKC [90]. Indeed, not only does oxidative stress appear to regulate PKC, micromolar concentrations of hydrogen peroxide induced the phosphorylation of mitogen-activated protein (MAP) kinases [91]. And the reverse is also true; various antioxidants modulate these kinase activities [92,93]. Gopalakrishna et al [92] point out that by having different oxidation susceptible regions, PKC can respond to both oxidants and antioxidants to elicit opposite cellular responses. Thus, it seems highly unlikely that there is some specific signaling role between PKC and α-tocopherol, but more likely this is an antioxidant phenomenon in the membrane where the activated form of PKC functions. Indeed, Numakawa et al [94] found that cell death occurred when cultured cortical neurons were stimulated with peroxide, but this was prevented by pre-treatment for 24 h with vitamin E analogs including α-tocopherol, α-tocotrienol, γ-tocopherol, or γ- tocotrienol. It should be emphasized that such manipulations are easy to perform in vitro, but achievement of similar concentrations in vivo are not possible because of the various mechanisms that result in a preference for α-tocopherol. Moreover, α-tocopherol exposure induced the activation of both the MAP kinase and PI3 kinase (PI3K) pathways [94], again suggesting that it is the oxidative stress that up-regulates kinase pathways and the antioxidant action of α-tocopherol protects the cell membrane fatty acids. Importantly, Trolox® which is not incorporated into membranes was relatively ineffective, while the various vitamin E forms that are retained in cell membranes were quite potent [94]. Thus, it is possible that the vitamin E forms changed the nature of phospholipid packing, or some measure of acyl chain fluidity, or protected critical fatty acids from oxidation. Apparently, the kind of cell, whether it is cancerous or not, and the identity of the oxidative stressor can alter the cellular response to α-tocopherol, resulting in apoptosis in some situations [95] and not in others [96,97].

It should also be noted that pharmacologic effects of tocopheryl succinate or tocopheryl ethers are not the same as the vitamin E effects discussed in this section because neither of these former two molecules have antioxidant activities [98].

Lipoxygenase and Cyclooxygenase, Prostaglandins and Arachidonic Acid

Prostaglandins I_2 (PGI_2; prostacyclin) and E_2 (PGE_2) are cyclooxygenase (COX)-derived prostanoids that counteract and/or inhibit the secretion of vasoconstrictors such as thromboxane A_2 (TXA_2) [99]. Arachidonic acid, a long chain polyunsaturated fatty acid, is a precursor of all of these molecules and vitamin E plays a key role in arachidonic acid availability by apparently regulating cytosolic phospholipase A2 ($cPLA_2$) activity. In fact, Wu et al [99] demonstrated that vitamin E increased PGE_2 and PGI_2 production in an in vitro system by increasing arachidonic acid availability. The vitamin E–induced arachidonic acid release was due to increased $cPLA_2$ activity and a higher level of $cPLA_2$ expression. Wu et al [99] also showed that the inhibitory effect of vitamin E on COX activity was not mediated through inhibition of COX-1 or COX-2 expression.

Although $cPLA_2$ has been suggested as being regulated by vitamin E, oxidative stress appears to again play a key role in $cPLA_2$ regulation. For example, when bovine pulmonary artery endothelial cells were stimulated with peroxynitrite (ONOO-) increases in the cell membrane associated protease, PKC and PLA_2 activities, as well as arachidonic acid release from the cells were observed [100]. Again, if oxidative stress increases, then an antioxidant, such as α-tocopherol should decrease their activities. If PLA_2 activation in a given model depends on PKC, PKA, cAMP, or MAPK then inhibition of these phosphorylating enzymes may alter activities of PLA_2 isoforms during cellular injury [101], thus explaining the antioxidant role of α-tocopherol in signaling pathways.

Alternatively, it appears that pancreatic PLA_2 may be regulated through modification of membrane properties that limit the imperfections in lipid packing that PLA-2 needs to get access to substrate phospholipids to release arachidonic acid. Grau and Ortiz [102] demonstrated in vitro that α-tocopherol decreases both the initial rate and the extent of hydrolysis. They suggest that α-tocopherol has an effect on the substrate, i.e. the membrane, and not on the enzyme itself. Other tocopherols, such as the isomers β-, γ- and δ-tocopherol also display PLA_2 inhibition but to a lesser extent. Importantly, the degree of inhibition of PLA_2 activity by these various vitamin E forms correlates with their penetration in the bilayer as shown by fluorescence quenching [102]. The chromanol of α-tocopherol resides higher in the membrane (towards the surface) and inhibits PLA_2 the most. Other vitamers sit deeper in the membrane and inhibit PLA_2 to a lesser degree. Thus, the mechanism of α-tocopherol's PLA_2 inhibition could result by at least two possible mechanisms. 1) The tocopherols' antioxidant action lowered the amounts of peroxylipids. These oxidized lipids increase the fluidity and packing errors of membranes, and thus would allow PLA_2 greater access to substrate phospholipids. Potentially, the different vitamers inhibit differently because they have different antioxidant efficiencies in membranes. 2) The various vitamers "repair" membrane packing errors to different degrees and thus inhibit PLA_2 differently, but in this case without recourse to antioxidant activity. Certainly, the relatively low concentrations of non-α-tocopherols in vivo would limit their effectiveness in such an activity.

The above observations then bring up the question as to why α-tocopherol, but not the other vitamin E forms, is the preferred form of the vitamin. The answer, perhaps, is the somewhat more effective membrane location, allowing optimal delivery of the antioxidant activity of α-tocopherol. Certainly, the food chemists have long appreciated that α-tocopherol in the presence of a water soluble antioxidant such as ascorbic acid is the most effective antioxidant in food, as reviewed [103]. It is worth pointing out that while the biophysical description of membrane structure and biological function grows ever more sophisticated - admitting to the role of a myriad of different phospholipids, enzymes that modify such lipids, and the domains that protein-lipid complexes might occupy - few studies that investigate the role of antioxidants under conditions of oxidative stress ever measure the specific lipids that are sacrificed by these

very conditions. The specific loss of long chain PUFAs, particularly those within a subclass of lipid head group, could have profound effects on the properties of the membrane system under study. Indeed, this topic is just beginning to be studied. Tocopheryl succinate has been shown to change the coordinated activity of kinases and phosphatases in membrane rafts [104]. Moreover, changes in membrane n-3 fatty acids were sufficient to alter signaling; a marked decrease in epidermal growth factor receptor (EGFR) levels in lipid rafts, accompanied by increases in the phosphorylation of both EGFR and p38 mitogen-activated protein kinase (MAPK), in eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA)-treated cells [105]. It is clear that membrane lipid composition can cause major alterations in cellular responses to signaling molecules.

Cytokines and Inflammation

The release of arachidonic acid from membranes and its cellular availability appear to be critical for the inflammatory responses of macrophages. Moller and Lauridsen [106] showed that dietary fish oil supplemented at a level of 5% of the diet, but not supplemental vitamin E, decreased the inflammatory responses of alveolar macrophages isolated from weaned pigs. Production of TNF-alpha, IL-8, LTB-4, and PGE-2 were decreased with fish oil feeding, a difference likely caused by the decreased synthesis of arachidonic acid [107].

The Jialal laboratory has been a major proponent of the anti-inflammatory action of α-tocopherol, as reviewed [108]. With regard to cytokine release, they demonstrated that in activated monocytes isolated from subjects supplemented with vitamin E, that α-tocopherol inhibits interleukin-1 (IL-1) release from the cells by inhibiting 5-lipoxygenase. Importantly α-tocopherol's effect was at post-transcriptional levels [109], again suggesting that α-tocopherol is not functioning as a signaling molecule, but rather is altering the environment where signaling is taking place. Furthermore, they showed that the increased IL-6 release from monocytes under hyperglycemia (an oxidative stressor) is mediated via up-regulation of PKC, through p38 MAPK and NFκB, resulting in increased IL-6 mRNA and protein [110]. Not surprisingly, when cells were α-tocopherol-enriched they released significantly less IL-6 because, as we believe, the cells were protected by α-tocopherol's antioxidant function.

An alternative explanation for the decrease in IL-6 with increased α-tocopherol is that there is a decrease in PKC activity. But, as discussed above, PKC activity, as well as that of other kinases, appears to be regulated through oxidative stress-related mechanisms. Thus, addition of α-tocopherol prevents the generation of the signal that increases PKC and results in the ultimate up-regulation in the amounts of IL-6. Although α-tocopherol may alter PKC activity, the question to be addressed here, is whether this is a specific vitamin function for α-tocopherol.

γ-Tocopherol, as well as its metabolite (γ-CEHC), possess anti-inflammatory properties because stimulated macrophages and epithelial cells treated with γ-tocopherol in vitro have decreased cyclooxygenase-2 activity and lower levels of PGE_2 synthesis [111]. Moreover, in rats subjected to carrageenan-induced inflammation, PGE_2 and leukotriene B_4 synthesis were decreased by 46% and 70%, respectively, by γ-tocopherol gavage, while α-tocopherol had no effect [112]. Similarly, in three human lung cell lines, α-tocopheryl succinate, but not α-tocopherol or α-tocopheryl acetate inhibited PMA-stimulated PGE_2 production. Thus, it appears that the regulation of PGE_2 production is not a specific α-tocopherol function.

Nuclear Receptors

The nuclear receptor superfamily of ligand-dependent transcription factors functions to regulate a diverse number of genes involved in reproduction, development, metabolism and immune responses [113]. The ligands for about half of these nuclear receptors have not been identified, so are called "orphan" nuclear receptors. Specific members of the nuclear receptor

superfamily bind fat-soluble vitamins A and D; thus, it is not unreasonable to expect that α-tocopherol might also function to control pathways based on hypothetical nuclear receptor binding. There are two nuclear receptor classes that respond to modulation by vitamin E; these are the Pregnane X Receptor (PXR) and the Peroxisome Proliferator-Activated Receptors (PPARs).

PXR regulates a variety of xenobiotic pathways and responds to a wide range of potentially toxic foreign compounds [114]. With regard to PXR, tocotrienols were more effective ligands than was α-tocopherol in stimulating downstream responses [115]. Indeed, PXR has been called promiscuous in its ability to bind a variety of ligands [116]. This characteristic makes PXR ideal for recognizing foreign compounds, but unlikely that α-tocopherol's specific vitamin function relates to binding to PXR. This α-tocopherol phenomenon is more likely a mechanism involved in the prevention of accumulation of excess vitamin E.

With regard to PPARs, these are three different, but closely related nuclear receptors: PPARα, PPARβ/δ and PPARγ, that are encoded by separate genes [117]. PPARα is found largely in the liver, where it regulates energy homeostasis, as well as a variety of other functions including heme synthesis, lipoprotein assembly, and cholesterol catabolism. PPARβ/δ is found largely in the gut and placenta. It too is involved in fatty acid catabolism, as well as control of cell proliferation, differentiation and survival. PPARγ is found mainly in the adipose tissue, but also the liver. PPARγ regulates lipid storage and glucose metabolism. In general, PPARs act as lipid sensors that translate changes in fatty acid concentrations into metabolic activity [117].

Troglitazone, a member of the thiazolidinedione family of drugs used in treatment of Type 2 diabetes, was one of the first pharmaceutical agents that acted as a PPARγ agonist. Troglitazone is unique in that the chromanol portion of α-tocopherol forms part of its structure [118]. Similarly to troglitazone, α-tocopherol itself increases PPARγ expression in hepatocytes in vitro, but effective α-tocopherol concentrations in the medium were 50 times higher than usual plasma α-tocopherol concentrations [118]. Unfortunately, troglitazone, unlike other thiazolidinediones that do not contain an α-tocopherol-like chomanol structure, caused an idiosyncratic liver dysfunction, the cause of which remains unknown, but was sufficiently serious to cause the drug to be withdrawn from the market [119,120]. Nonetheless, the reports of PPAR modulation by α-tocopherol have stimulated interest in its ability to function as a PPAR ligand.

When α-, β-, γ-, or δ-tocopherol were tested individually as to whether they could influence transcriptional activity by modulating the activity of various nuclear receptors, the tocopherols positively modulated only the reporter construct containing a consensus element for PPARγ [121]. Unfortunately, this was not an α-tocopherol specific effect, in that δ-tocopherol caused the greatest response [121].

Once again oxidative stress is an important regulator of PPARs [122]. Oxidative metabolites of linoleic and arachidonic acids are PPAR-ligands, and perhaps more importantly, long chain polyunsaturated fatty acids are potent regulators of PPARs [123-125]. Given that it has been pointed out that all cells in culture are under oxidative stress [126], it is likely that regulation of PPARs by tocopherols is not a specific α-tocopherol-regulatory function, but rather is likely due to modulation of the concentrations or preventing the oxidation of some yet to be identified fatty acid(s).

Molecular effects of α-tocopherol have been studied by analyzing gene expression. Sulzle et al. [122] fed rats diets for 63 d with 25 or 250 mg α-tocopherol/kg diet containing fresh fat or oxidized fat, then differences in gene expression were evaluated. Irrespective of the dietary vitamin E concentrations, the dietary oxidized fats caused an activation of a series of target

PPAR-α genes, as well as, increasing peroxisome proliferation [122]. In contrast, pigs were studied in an ischemia reperfusion model to test the role of PPARγ in protecting the myocardium from injury. Thiazolidinediones (rosaglitazone and troglitazone) were compared with an α-tocopherol treatment prior to ischemia-reperfusion injury. Remarkably, both troglitazone and α-tocopherol had benefit, causing the authors to conclude that the benefit was due to the *antioxidant activity*, not a PPARγ-dependent process. Importantly, α-tocopherol did not materially alter PPARγ expression. Both troglitazone and α-tocopherol preserved myocardial contractile function and stimulated greater lactate uptake. The authors concluded that the *antioxidant*, α-tocopherol, prevented the increase in the pro-inflammatory cytokines IL-1, IL-6, and IFN-γ mRNA and protein compared with the ischemic-reperfused myocardium from untreated pigs and compared to the non-injured area.

Taken together, despite the fact that troglitazone was one of the first pharmaceutical agents to modify PPAR function and it contained a chromanol similar to α-tocopherol as part of this molecular structure, it is unlikely that the vitamin function of α-tocopherol is that of a PPAR regulator.

Oxidative Stress in Humans

Does vitamin E act as an antioxidant in humans? In general, there are numerous studies showing that vitamin E supplements can decrease measures of lipid peroxidation in subjects with oxidative stress, examples include: decreasing urinary F2-isoprostanes in hypercholesterolemic subjects [127] and in diabetics [128]. However, normal subjects not under oxidative stress showed no effect of vitamin E supplements on markers of oxidative damage [129]. We believe that the level of antioxidant protection by the interaction of various antioxidants is so efficient that under normal circumstances, the levels of oxidized lipids are kept in check. This contention is supported by studies in selenium deficient guinea pigs that are subsequently fed a vitamin E deficient diet [12], or vitamin C deficent guinea pigs that are subsequently fed a vitamin E deficient diet [13]. In both cases, the depletion of two antioxidant systems leads to a rapid system failure and death within days.

To evaluate whether vitamin E acts as an antioxidant in humans, first it was necessary to establish model systems where humans are under ethically accepted modes of oxidative stress. We have used two different model systems, both extreme exercise and cigarette smoking.

Studies in Extreme Exercisers

An ultramarathon race (50 k or 32 miles) is run annually in McDonald forest, a research forest belonging to OSU, Corvallis, OR. The racecourse is up and down hills, usually takes the runners 5 to 7 h, and the runners expend around 7,000 calories during the race. In our first study, we found that both vitamin E disappearance rates and F2-isoprostane concentrations, increased in the runners during the race as compared with a rest period [130]. To evaluate, whether prior supplementation with antioxidants (vitamins E and C) would prevent the increased oxidative stress, a more extensive follow-up study was carried out in the ultramarathon racers.

In this second study, the 50 km ultramarathon run again caused oxidative damage measured as increased F_2-isoprostane concentrations [131] and DNA damage [132]. Muscle damage was characterized by measures of fatigue, as well as increased circulating muscle damage markers [133]. Circulating inflammatory markers elicited by the run increased in the characteristic progression of cytokine responses to tissue damage and inflammation [131]. Supplementation with both vitamins E and C completely inhibited exercise-induced lipid peroxidation, but had no effect on other parameters, such as inflammation [131], DNA damage [132], muscle damage markers, fatigue, or recovery [133]. Importantly, at post-race, when oxidative stress was maximal, F_2-Isoprostane concentrations were inversely correlated both with α-tocopherol/

lipids (R= -0.61, p<0.003) and ascorbic acid (R= -0.41, p= 0.05); further substantiating that antioxidants were responsible for preventing lipid peroxidation, but appeared to have little other protective effects.

Cigarette Smokers as Examples of Oxidative Stress in Humans

Cigarette smoke is an exogenous source of an enormous number of reactive oxygen and nitrogen species, as well as an inflammatory stimulant. The hypothesis of this study was to determine if the chronic oxidative stress of cigarette smoking would result in a more rapid *in vivo* disappearance of α-tocopherol [134]. The investigation (10 subjects/group) was conducted among nonsmokers and smokers (>10 cigarettes/day). The subjects were healthy, collegeaged, non-dietary supplement users with normal cholesterol status. The α-tocopherol fractional disappearance rates in cigarette smokers (0.215 ± 0.001) were ~13% greater than in nonsmokers (0.191 ± 0.001 pools/day; $p<0.05$). Moreover, the smokers with the lowest plasma ascorbic acid concentrations had the fastest α-tocopherol disappearance rates, presumably because vitamin C regenerates vitamin E [10]. To test this hypothesis, smokers and nonsmokers were supplemented for 2 wk with placebo or vitamin C (1000 mg/d), then their plasma vitamin E disappearance rates were measured [11]. Using a crossover design, the same subjects were tested again on the opposite supplement. Marginal vitamin C status in smokers was associated with increased rates of vitamin E disappearance from plasma (as previously observed [134]), and these rates were normalized by prior vitamin C supplementation [11]. Importantly, both α- and γ-tocopherols were similarly affected by vitamin C status, suggesting that oxidation of the tocopherols is the mechanism for the faster vitamin E disappearance [11].

Conclusion

The thrust of this review is to propose the idea that the various signaling pathways that have been described by others to be under α-tocopherol regulation, appear rather to be dependent upon the oxidative stress of the cell or tissue under question. Moreover, it seems unlikely these pathways are specifically under the control of α-tocopherol given that various antioxidants and oxidative stressors can manipulate their responses. For example, regulation of the expression of the lipoprotein receptors, scavenger receptor BI [135] and its homolog, CD36 [136,137], by α-tocopherol have been reported. Specifically, high cellular α-tocopherol concentrations decrease and low concentrations increase their expression; however, both are also modulated by oxidatively-modified lipids [138], by membrane signaling pathways such as kinases [139] and specifically by eicosapentaenoic acid concentrations [140]. Thus, regulation by alterations in membrane fluidity or changes in the specific lipids could also explain the observed regulation by α-tocopherol concentrations. Indeed, Klein et al. [141] have demonstrated that α-tocopherol reduces phosphatidyl-serine externalization in circulating erythrocytes, thereby modulating their procoagulant properties in vivo. It seems likely that tocopherols can alter membrane properties by protecting oxidizable lipids and thereby modulate receptors and signaling pathways that are dependent upon insertion in specific membrane regions.

Thus, we are left with proposing that the specific vitamin function of α-tocopherol is to protect long chain polyunsaturated fatty acids and thus maintain their concentrations for important signaling events. An example of such a fatty acid is DHA with 22-carbons and 6 double bonds, an omega-3 polyunsaturated fatty acid that is found in membrane phospholipids, especially of the nervous system, a critical site harmed during vitamin E deficiency. Tonito et al. [142] report in α-TTP null mice fed a vitamin E deficient diet that the retina was depleted in DHA and its precursor linolenic acid, while arachidonic acid concentrations were elevated, suggesting that synthesis of arachidonic acid to replace DHA was occurring and further suggesting that vitamin E deficiency allowed DHA depletion. Stillwell and Wassall [143] note that “Once esterified into phospholipids, DHA has been demonstrated to significantly alter many basic properties

of membranes including acyl chain order and “fluidity”, phase behavior, elastic compressibility, permeability, fusion, flip-flop and protein activity.” Thus, virtually all of the variation and scope of vitamin E’s biological activity can be seen and understood in the light of protection of polyunsaturated fatty acids and the membrane qualities (fluidity, phase separation and lipid domains) that polyunsaturated fatty acids bring about.

Acknowledgements

MGT has received support from NIH DK 067930. JKA has received support from Discovery and Equipment Grants from the Natural Sciences and Engineering Research Council (NSERC) of Canada; NIH ES012249; Research Corporation, Cottrell College Science Award; and a Premier’s Research Excellence Award from the Province of Ontario.

References

1. Evans HM, Bishop KS. On the existence of a hitherto unrecognized dietary factor essential for reproduction. Science 1922;56:650–651. [PubMed: 17838496]
2. Sondergaard E, Dam H. Influence of the level of dietary linoleic acid on the amount of d-alpha-tocopherol acetate required for protection against encephalomalacia. Z Ernahrungswiss 1966;6:253–258. [PubMed: 5987069]
3. Dam H. Influence of antioxidants and redox substances on signs of vitamin E deficiency. Pharmacol Rev 1957;9:1–16. [PubMed: 13431414]
4. Dam H. Fat-soluble vitamins. Annu Rev Biochem 1951;20:265–304. [PubMed: 14847531]
5. Dam H, Granados H. The influence of certain substances on massive hepatic necrosis and lung hemorrhage in rats fed low-protein, vitamin E deficient diets. Acta Pharmacol Toxicol (Copenh) 1951;7:181–188.
6. Granados H, Dam H. On the histochemical relationship between per-oxidation and the yellow-brown pigment in the adipose tissue of vitamin E-deficient rats. Acta Pathol Microbiol Scand 1950;27:591–598. [PubMed: 14770853]
7. Harris PL, Embree ND. Quantitative consideration of the effect of polyunsaturated fatty acid content of the diet upon the requirements for vitamin E. Am J Clin Nutr 1963;13:385–392. [PubMed: 14101400]
8. Herting DC. Perspective on vitamin E. Am J Clin Nutr 1966;19:210–218. [PubMed: 5331044]
9. Wolf G. The discovery of the antioxidant function of vitamin E: the contribution of Henry A. Mattill. J Nutr 2005;135:363–366. [PubMed: 15735064]
10. Buettner GR. The pecking order of free radicals and antioxidants: lipid peroxidation, alpha-tocopherol, and ascorbate. Arch Biochem Biophys 1993;300:535–543. [PubMed: 8434935]
11. Bruno RS, Leonard SW, Atkinson J, Montine TJ, Ramakrishnan R, Bray TM, Traber MG. Faster plasma vitamin E disappearance in smokers is normalized by vitamin C supplementation. Free Radic Biol Med 2006;40:689–697. [PubMed: 16458200]
12. Hill KE, Motley AK, Li X, May JM, Burk RF. Combined selenium and vitamin E deficiency causes fatal myopathy in guinea pigs. J Nutr 2001;131:1798–1802. [PubMed: 11385070]
13. Hill KE, Montine TJ, Motley AK, Li X, May JM, Burk RF. Combined deficiency of vitamins E and C causes paralysis and death in guinea pigs. Am J Clin Nutr 2003;77:1484–1488. [PubMed: 12791628]
14. Sattler SE, Mene-Saffrane L, Farmer EE, Krischke M, Mueller MJ, Dellapenna D. Nonenzymatic lipid peroxidation reprograms gene expression and activates defense markers in Arabidopsis tocopherol-deficient mutants. Plant Cell 2006;18:3706–3720. [PubMed: 17194769]
15. Food and Nutrition Board; Institute of Medicine. Dietary Reference Intakes for Vitamin C, Vitamin E Selenium, and Carotenoids. 529. National Academy Press; Washington: 2000.
16. Hosomi A, Arita M, Sato Y, Kiyose C, Ueda T, Igarashi O, Arai H, Inoue K. Affinity for alpha-tocopherol transfer protein as a determinant of the biological activities of vitamin E analogs. FEBS Lett 1997;409:105–108. [PubMed: 9199513]

17. Arita M, Sato Y, Miyata A, Tanabe T, Takahashi E, Kayden H, Arai H, Inoue K. Human alpha-tocopherol transfer protein: cDNA cloning, expression and chromosomal localization. Biochem J 1995;306:437–443. [PubMed: 7887897]

18. Ouahchi K, Arita M, Kayden H, Hentati F, Ben Hamida M, Sokol R, Arai H, Inoue K, Mandel JL, Koenig M. Ataxia with isolated vitamin E deficiency is caused by mutations in the alpha-tocopherol transfer protein. Nature Genetics 1995;9:141–145. [PubMed: 7719340]

19. Terasawa Y, Ladha Z, Leonard SW, Morrow JD, Newland D, Sanan D, Packer L, Traber MG, Farese RV Jr. Increased atherosclerosis in hyperlipidemic mice deficient in alpha-tocopherol transfer protein and vitamin E. Proc Natl Acad Sci USA 2000;97:13830–13834. [PubMed: 11095717]

20. Yokota T, Igarashi K, Uchihara T, Jishage K, Tomita H, Inaba A, Li Y, Arita M, Suzuki H, Mizusawa H, Arai H. Delayed-onset ataxia in mice lacking alpha-tocopherol transfer protein: model for neuronal degeneration caused by chronic oxidative stress. Proc Natl Acad Sci USA 2001;98:15185–15190. [PubMed: 11752462]

21. Traber MG, Sokol RJ, Burton GW, Ingold KU, Papas AM, Huffaker JE, Kayden HJ. Impaired ability of patients with familial isolated vitamin E deficiency to incorporate alpha-tocopherol into lipoproteins secreted by the liver. J Clin Invest 1990;85:397–407. [PubMed: 2298915]

22. Meier R, Tomizaki T, Schulze-Briese C, Baumann U, Stocker A. The molecular basis of vitamin E retention: structure of human alpha-tocopherol transfer protein. J Mol Biol 2003;331:725–734. [PubMed: 12899840]

23. Min KC, Kovall RA, Hendrickson WA. Crystal structure of human α-tocopherol transfer protein bound to its ligand: Implications for ataxia with vitamin E deficiency. Proc Natl Acad Sci USA 2003;100:14713–14718. [PubMed: 14657365]

24. DellaPenna D, Pogson BJ. Vitamin synthesis in plants: tocopherols and carotenoids. Annu Rev Plant Biol 2006;57:711–738. [PubMed: 16669779]

25. IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN). Nomenclature of tocopherols and related compounds. Recommendations 1981. Mol Cell Biochem 1982;49:183–185. [PubMed: 7162509]

26. Panagabko C, Morley S, Hernandez M, Cassolato P, Gordon H, Parsons R, Manor D, Atkinson J. Ligand specificity in the CRAL-TRIO protein family. Biochemistry 2003;42:6467–6474. [PubMed: 12767229]

27. Leonard SW, Terasawa Y, Farese RV Jr, Traber MG. Incorporation of deuterated *RRR*- or *all rac* α–tocopherol into plasma and tissues of α–tocopherol transfer protein null mice. Am J Clin Nutr 2002;75:555–560. [PubMed: 11864863]

28. Traber MG, Ramakrishnan R, Kayden HJ. Human plasma vitamin E kinetics demonstrate rapid recycling of plasma *RRR*-α-tocopherol. Proc Natl Acad Sci USA 1994;91:10005–10008. [PubMed: 7937827]

29. Leonard SW, Paterson E, Atkinson JK, Ramakrishnan R, Cross CE. Traber, M. G. Studies in humans using deuterium-labeled α- and γ-tocopherol demonstrate faster plasma γ-tocopherol disappearance and greater γ-metabolite production. Free Radic Biol Med 2005;38:857–866. [PubMed: 15749381]

30. Traber MG, Burton GW, Hamilton RL. Vitamin E trafficking. Ann NY Acad Sci 2005;1031:1–12. [PubMed: 15753129]

31. Horiguchi M, Arita M, Kaempf-Rotzoll DE, Tsujimoto M, Inoue K, Arai H. pH-dependent translocation of alpha-tocopherol transfer protein (alpha-TTP) between hepatic cytosol and late endosomes. Genes Cells 2003;8:789–800. [PubMed: 14531858]

32. Qian J, Morley S, Wilson K, Nava P, Atkinson J, Manor D. Intracellular trafficking of vitamin E in hepatocytes: Role of tocopherol transfer protein. J Lipid Res 2005;46:2072–2082. [PubMed: 16024914]

33. Morley S, Cross V, Cecchini M, Nava P, Atkinson J, Manor D. Utility of a fluorescent vitamin E analogue as a probe for tocopherol transfer protein activity. Biochemistry 2006;45:1075–1081. [PubMed: 16430203]

34. Qian J, Atkinson J, Manor D. Biochemical consequences of heritable mutations in the alpha-tocopherol transfer protein. Biochemistry 2006;45:8236–8242. [PubMed: 16819822]

35. Traber MG, Sokol RJ, Kohlschütter A, Yokota T, Muller DPR, Dufour R, Kayden HJ. Impaired discrimination between stereoisomers of α-tocopherol in patients with familial isolated vitamin E deficiency. J Lipid Res 1993;34:201–210. [PubMed: 8429255]

36. Swanson JE, Ben RN, Burton GW, Parker RS. Urinary excretion of 2,7, 8-trimethyl-2-(beta-carboxyethyl)-6-hydroxychroman is a major route of elimination of gamma-tocopherol in humans. J Lipid Res 1999;40:665–671. [PubMed: 10191290]

37. Sontag TJ, Parker RS. Cytochrome P450 omega-hydroxylase pathway of tocopherol catabolism: Novel mechanism of regulation of vitamin E status. J Biol Chem 2002;277:25290–25296. [PubMed: 11997390]

38. Brigelius-Flohé R, Traber MG. Vitamin E: function and metabolism. FASEB J 1999;13:1145–1155. [PubMed: 10385606]

39. Kiyose C, Saito H, Kaneko K, Hamamura K, Tomioka M, Ueda T, Igarashi O. Alpha-tocopherol affects the urinary and biliary excretion of 2,7,8-trimethyl-2 (2'-carboxyethyl)-6-hydroxychroman, gamma-tocopherol metabolite, in rats. Lipids 2001;36:467–472. [PubMed: 11432458]

40. Jiang Q, Freiser H, Wood KV, Yin X. Identification and quantitation of novel vitamin E metabolites, sulfated long-chain carboxychromanols, in human A549 cells and in rats. J Lipid Res. Feb 13;2007 Epub ahead of print

41. Stahl W, Graf P, Brigelius-Flohe R, Wechter W, Sies H. Quantification of the alpha- and gamma-tocopherol metabolites 2,5,7,8-tetramethyl-2-(2'-carboxyethyl)-6-hydroxychroman and 2,7,8-trimethyl-2-(2'-carboxyethyl)-6-hydroxychroman in human serum. Anal Biochem 1999;275:254–259. [PubMed: 10552913]

42. Pope SA, Burtin GE, Clayton PT, Madge DJ, Muller DP. Synthesis and analysis of conjugates of the major vitamin E metabolite, alpha-CEHC. Free Radic Biol Med 2002;33:807–817. [PubMed: 12208368]

43. Parker RS, McCormick CC. Selective accumulation of alpha-tocopherol in Drosophila is associated with cytochrome P450 tocopherol-omega-hydroxylase activity but not alpha-tocopherol transfer protein. Biochem Biophys Res Commun 2005;338:1537–1541. [PubMed: 16289043]

44. Tappel AL. Vitamin E as the biological lipid antioxidant. Vitamins and Hormones 1962;20:493–510.

45. Tappel AL. Vitamin E and selenium protection from in vivo lipid peroxidation. Ann N Y Acad Sci 1980;355:18–31. [PubMed: 6940474]

46. Dillard CJ, Sagai M, Tappel AL. Respiratory pentane: a measure of in vivo lipid peroxidation applied to rats fed diets varying in polyunsaturated fats, vitamin E, and selenium and exposed to nitrogen dioxide. Toxicol Lett 1980;6:251–256. [PubMed: 7423553]

47. Dillard CJ, Litov RE, Tappel AL. Effects of dietary vitamin E, selenium, and polyunsaturated fats on in vivo lipid peroxidation in the rat as measured by pentane production. Lipids 1978;13:396–402. [PubMed: 672480]

48. Tappel A, Zalkin H. Inhibition of lipid peroxidation in microsomes by vitamin E. Nature 1960;185:35. [PubMed: 13836891]

49. Burton GW, Joyce A, Ingold KU. First proof that vitamin E is major lipid-soluble, chain-breaking antioxidant in human blood plasma. Lancet 1982;8293:327. [PubMed: 6124736]

50. Burton GW, Doba T, Gabe E, Hughes L, Lee FL, Prasad L, Ingold KU. Autoxidation of biological molecules. 4. Maximizing the antioxidant activity of phenols. J Am Chem Soc 1985;107:7053–7065.

51. Burton GW, Ingold KU. Autoxidation of biological molecules. I. The antioxidant activity of vitamin E and related chain-breaking phenolic antioxidants in vitro. J Amer Chem Soc 1981;103:6472–6477.

52. Mukai K, Kageyama Y, Ishida T, Fukuda K, Okabe K, Hosose H. Synthesis and kinetic study of antioxidant activity of new tocopherol (vitamin E) compounds. J Org Chem 1989;54:552–556.

53. Pryor WA, Cornicelli JA, Devall LJ, Tait B, Trivedi BK, Witiak DT, Wu M. A rapid screening test to determine the antioxidant potencies of natural and synthetic antioxidants. J Org Chem 1993;58:3521–3532.

54. Pryor WA, Strickland T, Church DF. Comparison of the Efficiencies of Several Natural and Synthetic Antioxidants in Aqueous Sodium Dodecyl-Sulfate Micelle Solutions. Journal of the American Chemical Society 1988;110:2224–2229.

55. Mukai K, Tokunaga A, Itoh S, Kanesaki Y, Ohara K, Nagaoka S, Abe K. Structure-activity relationship of the free-radical-scavenging reaction by vitamin E (alpha-, beta-, gamma-, delta-

tocopherols) and ubiquinol-10: pH dependence of the reaction rates. J Phys Chem B Condens Matter Mater Surf Interfaces Biophys 2007;111:652–662. [PubMed: 17228924]

56. Leth T, Sondergaard H. Biological activity of vitamin E compounds and natural materials by the resorption-gestation test, and chemical determination of the vitamin E activity in foods and feeds. J Nutr 1977;107:2236–2243. [PubMed: 925769]
57. Suzuki YJ, Tsuchiya M, Wassall SR, Choo YM, Govil G, Kagan VE, Packer L. Structural and dynamic membrane properties of alpha-tocopherol and alpha-tocotrienol: implication to the molecular mechanism of their antioxidant potency. Biochemistry 1993;32:10692–10699. [PubMed: 8399214]
58. Yoshida Y, Niki E, Noguchi N. Comparative study on the action of tocopherols and tocotrienols as antioxidant: chemical and physical effects. Chemistry and Physics of Lipids 2003;123:63–75. [PubMed: 12637165]
59. Suarna C, Hood RL, Dean RT, Stocker R. Comparative antioxidant activity of tocotrienols and other natural lipid-soluble antioxidants in a homogeneous system, and in rat and human lipoproteins. Biochim Biophys Acta 1993;1166:163–170. [PubMed: 8443232]
60. Hensley K, Benaksas EJ, Bolli R, Comp P, Grammas P, Hamdheydari L, Mou S, Pye QN, Stoddard MF, Wallis G, Williamson KS, West M, Wechter WJ, Floyd RA. New perspectives on vitamin E: gamma-tocopherol and carboxyethylhydroxychroman metabolites in biology and medicine. Free Radic Biol Med 2004;36:1–15. [PubMed: 14732286]
61. Tafazoli S, Wright JS, O'Brien PJ. Prooxidant and antioxidant activity of vitamin E analogues and troglitazone. Chem Res Toxicol 2005;18:1567–1574. [PubMed: 16533021]
62. Cornwell DG, Williams MV, Wani AA, Wani G, Shen E, Jones KH. Mutagenicity of tocopheryl quinones: evolutionary advantage of selective accumulation of dietary alpha-tocopherol. Nutr Cancer 2002;43:111–118. [PubMed: 12467142]
63. Wang X, Thomas B, Sachdeva R, Arterburn L, Frye L, Hatcher PG, Cornwell DG, Ma J. Mechanism of arylating quinone toxicity involving Michael adduct formation and induction of endoplasmic reticulum stress. Proc Natl Acad Sci U S A 2006;103:3604–3609. [PubMed: 16505371]
64. Lee SB, Willis AC, Webster RD. Synthesis of the phenoxonium cation of an alpha-tocopherol model compound crystallized with non-nucleophilic [B(C6F5)(4)](-) and (CB11H6Br6)(-) anions. J Am Chem Soc 2006;128:9332–9333. [PubMed: 16848459]
65. Wilson GJ, Lin CY, Webster RD. Significant differences in the electrochemical behavior of the alpha-, beta-, gamma-, and delta-tocopherols (vitamin E). J Phys Chem B Condens Matter Mater Surf Interfaces Biophys 2006;110:11540–11548. [PubMed: 16771430]
66. Donchenko GV, Kuz'menko IV, Kovalenko VN, Basalkevich ED, Koliadenko EV. Ubiquinone content and the oxidative-reductive enzymatic system activity in the liver of vitamin E-deficient rats administered alpha-tocopherol and its chloro-derivative. Vopr Med Khim 1981;27:707–710. [PubMed: 6797129]Article in Russian
67. Noguchi N, Hanyu R, Nonaka A, Okimoto Y, Kodama T. Inhibition of THP-1 cell adhesion to endothelial cells by alpha-tocopherol and alpha-tocotrienol is dependent on intracellular concentration of the antioxidants. Free Radic Biol Med 2003;34:1614–1620. [PubMed: 12788481]
68. Sontag TJ, Parker RS. Comparative influence of major structural features of tocopherols and tocotrienols on kinetics of their omega -oxidation by cellular and microsomal tocopherol-omega -hydroxylase. J Lipid Res. Feb 6;2007 Epub ahead of print
69. Kayden HJ, Silber R, Kossmann CE. The role of vitamin E deficiency in the abnormal autohemolysis of acanthocytosis. Trans Assoc Am Physicians 1965;78:334–342. [PubMed: 5864979]
70. Burck U, Goebel HH, Kuhlendahl HD, Meier C, Goebel KM. Neuromyopathy and vitamin E deficiency in man. Neuropediatrics 1981;12:267–278. [PubMed: 6945489]
71. Ben Hamida C, Doerflinger N, Belal S, Linder C, Reutenauer L, Dib C, Gyapay G, Bignal A, Le Paslier D, Cohen D, Pandolfo M, Mokini V, Novelli G, Hentati F, Ben Hamida M, Mandel JL, Koenig M. Localization of Friedreich ataxia phenotype with selective vitamin E deficiency to chromosome 8q by homozygosity mapping. Nature Genetics 1993;5:195–200. [PubMed: 8252047]
72. Doerflinger N, Linder C, Ouahchi K, Gyapay G, Weissenbach J, Le Paslier D, Rigault P, Belal S, Ben Hamida C, Hentati F, Ben Hamida M, Pandolfo M, DiDonato S, Sokol R, Kayden H, Landrieu P, Durr A, Brice A, Goutières F, Kohlschütter A, Sabouraud P, Benomar A, Yahyaoui M, Mandel J-L, Koenig M. Ataxia with vitamin E deficiency: refinement of genetic localization and analysis of

linkage disequilibrium by using new markers in 14 families. Am J Hum Genet 1995;56:1116–1124. [PubMed: 7726167]

73. Machlin, LJ. Vitamin E. In: Machlin, LJ., editor. Handbook of Vitamins. New York: Marcel Dekker; 1991. p. 99-144.
74. Sokol, RJ. Vitamin E deficiency and neurological disorders. In: Packer, L.; Fuchs, J., editors. Vitamin E in Health and Disease. New York: Marcel Dekker, Inc.; 1993. p. 815-849.
75. Traber MG, Sokol RJ, Ringel SP, Neville HE, Thellman CA, Kayden HJ. Lack of tocopherol in peripheral nerves of vitamin E-deficient patients with peripheral neuropathy. N Engl J Med 1987;317:262–265. [PubMed: 3037372]
76. Pillai SR, Traber MG, Steiss JE, Kayden HJ. Depletion of adipose tissue and peripheral nerve α-tocopherol in adult dogs. Lipids 1993;28:1095–1099. [PubMed: 8121251]
77. Pillai SR, Traber MG, Steiss JE, Kayden HJ, Cox NR. α-Tocopherol concentrations of the nervous system and selected tissues of dogs fed three levels of vitamin E. Lipids 1993;28:1101–1105. [PubMed: 8121252]
78. Stumpf DA SR, Bettis D, Neville H, Ringel S, Angelini C, Bell R. Friedreich's disease: V. Variant form with vitamin E deficiency and normal fat absorption. Neurology 1987;37(1):68–74. [PubMed: 3796840]
79. Belal S, Hentati F, Ben Hamida C, Ben Hamida M. Friedreich's ataxia-vitamin E responsive type. The chromosome 8 locus. Clin Neurosci 1995;3:39–42. [PubMed: 7614093]
80. Hammans SR, Kennedy CR. Ataxia with isolated vitamin E deficiency presenting as mutation negative Friedreich's ataxia. J Neurol Neurosurg Psychiatry 1998;64:368–370. [PubMed: 9527151]
81. Yokota T, Shiojiri T, Gotoda T, Arita M, Arai H, Ohga T, Kanda T, Suzuki J, Imai T, Matsumoto H, Harino S, Kiyosawa M, Mizusawa H, Inoue K. Friedreich-like ataxia with retinitis pigmentosa caused by the His101Gln mutation of the alpha-tocopherol transfer protein gene. Ann Neurol 1997;41:826–832. [PubMed: 9189046]
82. Rotig A, de Lonlay P, Chretien D, Foury F, Koenig M, Sidi D, Munnich A, Rustin P. Aconitase and mitochondrial iron-sulphur protein deficiency in Friedreich ataxia. Nat Genet 1997;17:215–217. [PubMed: 9326946]
83. Ventura C, Maioli M. Protein kinase C control of gene expression. Crit Rev Eukaryot Gene Expr 2001;11:243–267. [PubMed: 11693964]
84. Buchner K. The role of protein kinase C in the regulation of cell growth and in signalling to the cell nucleus. J Cancer Res Clin Oncol 2000;126:1–11. [PubMed: 10641743]
85. Azzi A, Boscoboinik D, Clement S, Marilley D, Ozer N, Ricciarelli R, Tasinato A. Alpha-tocopherol as a modulator of smooth muscle cell proliferation. Prostaglandins Leukotrienes and Essential Fatty Acids 1997;57:507–514.
86. Azzi A, Ricciarelli R, Zingg JM. Non-antioxidant molecular functions of alpha-tocopherol (vitamin E). FEBS Lett 2002;519:8–10. [PubMed: 12023009]
87. Egger T, Hammer A, Wintersperger A, Goti D, Malle E, Sattler W. Modulation of microglial superoxide production by alpha-tocopherol in vitro: attenuation of p67(phox) translocation by a protein phosphatase-dependent pathway. J Neurochem 2001;79:1169–1182. [PubMed: 11752058]
88. Freedman JE, Farhat JH, Loscalzo J, Keaney JFJ. alpha-tocopherol inhibits aggregation of human platelets by a protein kinase C-dependent mechanism. Circulation 1996;94:2434–2440. [PubMed: 8921785]
89. Freedman JE, Keaney JF Jr. Vitamin E inhibition of platelet aggregation is independent of antioxidant activity. J Nutr 2001;131:374S–377S. [PubMed: 11160564]
90. Abdala-Valencia H, Cook-Mills JM. VCAM-1 signals activate endothelial cell protein kinase C {alpha} via oxidation. J Immunol 2006;177:6379–6387. [PubMed: 17056569]
91. Cantoni O, Boscoboinik D, Fiorani M, Stauble B, Azzi A. The phosphorylation state of MAP-kinases modulates the cytotoxic response of smooth muscle cells to hydrogen peroxide. FEBS Lett 1996;8:285–288. [PubMed: 8766717]
92. Gopalakrishna R, Gundimeda U. Antioxidant regulation of protein kinase C in cancer prevention. J Nutr 2002;132:3819S–3823S. [PubMed: 12468631]
93. Williams R, Spencer J, Rice-Evans C. Flavonoids: antioxidants or signalling molecules? Free Radic Biol Med 2004;36:838–849. [PubMed: 15019969]

94. Numakawa Y, Numakawa T, Matsumoto T, Yagasaki Y, Kumamaru E, Kunugi H, Taguchi T, Niki E. Vitamin E protected cultured cortical neurons from oxidative stressinduced cell death through the activation of mitogen-activated protein kinase and phosphatidylinositol 3-kinase. J Neurochem 2006;97:1191–1202. [PubMed: 16686696]

95. Piga R, Saito Y, Yoshida Y, Niki E. Cytotoxic effects of various stressors on PC12 cells: Involvement of oxidative stress and effect of antioxidants. Neurotoxicology 2007;28:67–75. [PubMed: 16942797]

96. Olguin-Martinez M, Mendieta-Condado E, Contreras-Zentella M, Escamilla JE, Aranda-Fraustro A, El-Hafidi M, Hernandez-Munoz R. Rate of oxidant stress regulates balance between rat gastric mucosa proliferation and apoptosis. Free Radic Biol Med 2006;41:1325–1337. [PubMed: 17015179]

97. Simamura E, Hirai KI, Shimada H, Koyama J, Niwa Y, Shimizu S. Furanonaphthoquinones Cause Apoptosis of Cancer Cells by Inducing the Production of Reactive Oxygen Species by the Mitochondrial Voltage-Dependent Anion Channel. Cancer Biol Ther Nov 19;2006 5(11)Epub ahead of print

98. Kline K, Yu W, Sanders BG. Vitamin E and breast cancer. J Nutr 2004;134:3458S–3462S. [PubMed: 15570054]

99. Wu D, Liu L, Meydani M, Meydani SN. Vitamin E Increases Production of Vasodilator Prostanoids in Human Aortic Endothelial Cells through Opposing Effects on Cyclooxygenase-2 and Phospholipase A2. J Nutr 2005;135:1847–1853. [PubMed: 16046707]

100. Chakraborti T, Das S, Chakraborti S. Proteolytic activation of protein kinase Calpha by peroxynitrite in stimulating cytosolic phospholipase A2 in pulmonary endothelium: involvement of a pertussis toxin sensitive protein. Biochemistry 2005;44:5246–5257. [PubMed: 15794661]

101. Chakraborti S. Phospholipase A(2) isoforms: a perspective. Cell Signal 2003;15:637–665. [PubMed: 12742226]

102. Grau A, Ortiz A. Dissimilar protection of tocopherol isomers against membrane hydrolysis by phospholipase A(2). Chem Phys Lipids 1998;91:109–118. [PubMed: 9569615]

103. Eitenmiller, R.; Lee, J. Vitamin E: Food Chemistry, Composition, and Analysis. 530. Marcel Dekker, Inc; New York: 2004.

104. Cuschieri J, Bulger E, Biligren J, Garcia I, Maier RV. Vitamin E inhibits endotoxin-mediated transport of phosphatases to lipid rafts. Shock 2007;27:19–24. [PubMed: 17172975]

105. Schley PD, Brindley DN, Field CJ. (n-3) PUFA alter raft lipid composition and decrease epidermal growth factor receptor levels in lipid rafts of human breast cancer cells. J Nutr 2007;137:548–553. [PubMed: 17311938]

106. Moller S, Lauridsen C. Dietary fatty acid composition rather than vitamin E supplementation influence ex vivo cytokine and eicosanoid response of porcine alveolar macrophages. Cytokine 2006;35:6–12. [PubMed: 16914322]

107. Calder PC. Polyunsaturated fatty acids and inflammation. Prostaglandins Leukot Essent Fatty Acids 2006;75:197–202. [PubMed: 16828270]

108. Singh U, Devaraj S, Jialal I. Vitamin E, oxidative stress, and inflammation. Annu Rev Nutr 2005;25:151–174. [PubMed: 16011463]

109. Devaraj S, Jialal I. Alpha-tocopherol decreases interleukin-1beta release from activated human monocytes by inhibition of 5-lipoxygenase. Arterioscler Thromb Vasc Biol 1999;19:1125–1133. [PubMed: 10195945]

110. Devaraj S, Jialal I. Alpha-tocopherol decreases tumor necrosis factor-alpha mRNA and protein from activated human monocytes by inhibition of 5-lipoxygenase. Free Radic Biol Med 2005;38:1212–1220. [PubMed: 15808419]

111. Jiang Q, Elson-Schwab I, Courtemanche C, Ames BM. γ-tocopherol and its major metabolite, in contrast to α-tocopherol, inhibit cyclooxygenase activity in macrophages and epithelial cells. Proc Natl Acad Sci USA 2000;97:11494–11499. [PubMed: 11005841]

112. Jiang Q, Ames BN. Gamma-tocopherol, but not alpha-tocopherol, decreases proinflammatory eicosanoids and inflammation damage in rats. FASEB J 2003;17:816–822. [PubMed: 12724340]

113. Bookout AL, Jeong Y, Downes M, Yu RT, Evans RM, Mangelsdorf DJ. Anatomical profiling of nuclear receptor expression reveals a hierarchical transcriptional network. Cell 2006;126:789–799. [PubMed: 16923397]

114. Moore DD, Kato S, Xie W, Mangelsdorf DJ, Schmidt DR, Xiao R, Kliewer SA. International Union of Pharmacology. LXII. The NR1H and NR1I receptors: constitutive androstane receptor, pregnene X receptor, farnesoid X receptor alpha, farnesoid X receptor beta, liver X receptor alpha, liver X receptor beta, and vitamin D receptor. Pharmacol Rev 2006;58:742–759. [PubMed: 17132852]
115. Landes N, Pfluger P, Kluth D, Birringer M, Ruhl R, Bol GF, Glatt H, Brigelius-Flohe R. Vitamin E activates gene expression via the pregnane X receptor. Biochem Pharmacol 2003;65:269–273. [PubMed: 12504802]
116. Jones SA, Moore LB, Shenk JL, Wisely GB, Hamilton GA, McKee DD, Tomkinson NC, LeCluyse EL, Lambert MH, Willson TM, Kliewer SA, Moore JT. The pregnane X receptor: a promiscuous xenobiotic receptor that has diverged during evolution. Mol Endocrinol 2000;14:27–39. [PubMed: 10628745]
117. Michalik L, Auwerx J, Berger JP, Chatterjee VK, Glass CK, Gonzalez FJ, Grimaldi PA, Kadowaki T, Lazar MA, O'Rahilly S, Palmer CN, Plutzky J, Reddy JK, Spiegelman BM, Staels B, Wahli W. International Union of Pharmacology. LXI. Peroxisome proliferator-activated receptors. Pharmacol Rev 2006;58:726–741. [PubMed: 17132851]
118. Davies GF, McFie PJ, Khandelwal RL, Roesler WJ. Unique ability of troglitazone to up-regulate peroxisome proliferator-activated receptor-gamma expression in hepatocytes. J Pharmacol Exp Ther 2002;300:72–77. [PubMed: 11752099]
119. Chojkier M. Troglitazone and liver injury: in search of answers. Hepatology 2005;41:237–246. [PubMed: 15657914]
120. Masubuchi Y. Metabolic and non-metabolic factors determining troglitazone hepatotoxicity: a review. Drug Metab Pharmacokinet 2006;21:347–356. [PubMed: 17072088]
121. De Pascale MC, Bassi AM, Patrone V, Villacorta L, Azzi A, Zingg JM. Increased expression of transglutaminase-1 and PPARgamma after vitamin E treatment in human keratinocytes. Arch Biochem Biophys 2006;447:97–106. [PubMed: 16530159]
122. Sulzle A, Hirche F, Eder K. Thermally oxidized dietary fat upregulates the expression of target genes of PPARalpha in rat liver. J Nutr 2004;134:1375–1383. [PubMed: 15173399]
123. Wang Y, Botolin D, Xu J, Christian B, Mitchell E, Jayaprakasam B, Nair M, Peters J, Busik J, Olson LK, Jump DB. Regulation of hepatic fatty acid elongase and desaturase expression in diabetes and obesity. J Lipid Res. 2006
124. Xu J, Christian B, Jump DB. Regulation of rat hepatic L-pyruvate kinase promoter composition and activity by glucose, n-3 polyunsaturated fatty acids, and peroxisome proliferator-activated receptor-alpha agonist. J Biol Chem 2006;281:18351–18362. [PubMed: 16644726]
125. Zuo X, Wu Y, Morris JS, Stimmel JB, Leesnitzer LM, Fischer SM, Lippman SM, Shureiqi I. Oxidative metabolism of linoleic acid modulates PPAR-beta/delta suppression of PPAR-gamma activity. Oncogene 2006;25:1225–1241. [PubMed: 16288226]
126. Halliwell B. Oxidative stress in cell culture: an under-appreciated problem? FEBS Lett 2003;540:3–6. [PubMed: 12681474]
127. Davi G, Alessandrini P, Mezzetti A, Minotti G, Bucciarelli T, Costantini F, Cipollone F, Bon GB, Ciabattoni G, Patrono C. In vivo formation of 8-Epiprostaglandin F2 alpha is increased in hypercholesterolemia. Arterioscler Thromb Vasc Biol 1997;17:3230–3235. [PubMed: 9409316]
128. Davi G, Ciabattoni G, Consoli A, Mezzetti A, Falco A, Santarone S, Pennese E, Vitacolonna E, Bucciarelli T, Costantini F, Capani F, Patrono C. In vivo formation of 8-iso-prostaglandin F2 {alpha} and platelet activation in diabetes mellitus: effects of improved metabolic control and vitamin E supplementation. Circulation 1999;99:224–229. [PubMed: 9892587]
129. Meagher EA, Barry OP, Lawson JA, Rokach J, FitzGerald GA. Effects of vitamin E on lipid peroxidation in healthy persons. Jama 2001;285:1178–1182. [PubMed: 11231747]
130. Mastaloudis A, Leonard SW, Traber MG. Oxidative stress in athletes during extreme endurance exercise. Free Radic Biol Med 2001:911–922. [PubMed: 11585710]
131. Mastaloudis A, Morrow JD, Hopkins DW, Devaraj S, Traber MG. Antioxidant supplementation prevents exercise-induced lipid peroxidation, but not inflammation, in ultramarathon runners. Free Radic Biol Med 2004;36:1329–1341. [PubMed: 15110397]

132. Mastaloudis A, Yu TW, O'Donnell RP, Frei B, Dashwood RH, Traber MG. Endurance exercise results in DNA damage as detected by the comet assay. Free Radic Biol Med 2004;36:966–975. [PubMed: 15059637]

133. Mastaloudis A, Traber MG, Carstensen K, Widrick JJ. Antioxidants did not prevent muscle damage in response to an ultramarathon run. Med Sci Sports Exerc 2006;38:72–80. [PubMed: 16394956]

134. Bruno RS, Rainakrishnan R, Montine TJ, Bray TM, Traber MG. alpha-Tocopherol disappearance is faster in cigarette smokers and is inversely related to their ascorbic acid status. American Journal Of Clinical Nutrition 2005;81:95–103. [PubMed: 15640466]

135. Kolleck I, Witt W, Wissel H, Sinha P, Rustow B. HDL and vitamin E in plasma and the expression of SR-BI on lung cells during rat perinatal development. Lung 2000;178:191–200. [PubMed: 10960554]

136. Devaraj S, Hugou I, Jialal I. Alpha-tocopherol decreases CD36 expression in human monocyte-derived macrophages. J Lipid Res 2001;42:521–527. [PubMed: 11290823]

137. Ricciarelli R, Zingg JM, Azzi A. Vitamin E reduces the uptake of oxidized LDL by inhibiting CD36 scavenger receptor expression in cultured aortic smooth muscle cells. Circulation 2000;102:82–87. [PubMed: 10880419]

138. Adachi H, Tsujimoto M. Endothelial scavenger receptors. Prog Lipid Res 2006;45:379–404. [PubMed: 16712941]

139. Zhang Y, Ahmed AM, McFarlane N, Capone C, Boreham DR, Truant R, Igdoura SA, Trigatti BL. Regulation of SR-BI-mediated selective lipid uptake in Chinese hamster ovary-derived cells by protein kinase signaling pathways. J Lipid Res 2007;48:405–416. [PubMed: 17079793]

140. Peretti N, Delvin E, Sinnett D, Marcil V, Garofalo C, Levy E. Asymmetrical regulation of scavenger receptor class B type I by apical and basolateral stimuli using Caco-2 cells. J Cell Biochem 2007;100:421–433. [PubMed: 16927335]

141. Klein A, Deckert V, Schneider M, Dutrillaux F, Hammann A, Athias A, Le Guern N, Pais de Barros JP, Desrumaux C, Masson D, Jiang XC, Lagrost L. Alpha-tocopherol modulates phosphatidylserine externalization in erythrocytes. relevance in phospholipid tansfer protein-deficient mice. Arterioscler Thromb Vasc Biol. 2006

142. Tanito M, Yoshida Y, Kaidzu S, Chen ZH, Cynshi O, Jishage K, Niki E, Ohira A. Acceleration of age-related changes in the retina in alpha-tocopherol transfer protein null mice fed a Vitamin E-deficient diet. Invest Ophthalmol Vis Sci 2007;48:396–404. [PubMed: 17197560]

143. Stillwell W, Wassall SR. Docosahexaenoic acid: membrane properties of a unique fatty acid. Chem Phys Lipids 2003;126:1–27. [PubMed: 14580707]

Table

Chemical Assessments of the Antioxidant Abilities of Tocopherols in Organic Solution and Aqueous Suspension of Phospholipids

	Rates					Antioxidant Efficiencies			Fetal Resorption Assay
	$k_s \cdot 10^{-3} M^{-1} s^{-1}$	$k_s \cdot 10^{-5} M^{-1} s^{-1}$	$k_5 \cdot 10^{-4} M^{-1} s^{-1}$	$K_1 \cdot 10^{-4} M^{-1} s^{-1}$	$k_1 \cdot 10^{-4} M^{-1} s^{-1}$				
	[52]	**[55]**	**[51]**	**[50]**	**[50]**	**[53]**	**[53]**	**[54]**	**[56]**
	EtOH	**Triton Micelle**		***IAS***	***LKEPR***	***0.10 M HDTBr***	***0.10 M SDS***	***0.5 M SDS***	***% of all rac α-tocopheryl acetate***
Footnotes:	***{a}***	***{b}***	***{c}***	***{d}***	***{d}***	***{e}***	***{e}***	***{f}***	***{g}***
						Conjugated dienes	***Conjugated dienes***	**O_2*uptake***	
α-Tocopherol	5.12	5.12	235 ± 50	320	260	1770 ± 90	1830 ± 50	1000	80
β-Tocopherol	2.24	1.05	166 ± 33	130		970 ± 140	950 ± 30	590	45
γ-Tocopherol	2.42	1.00	159 ± 42	140	70	1020 ± 40	980 ± 60	590	13
δ-Tocopherol	1	0.149	65 ± 13	44	33	530 ± 30	570 ± 50	240	<0.4
Tocol	0.56	0.0353							
PMC			214 ± 81	380		2850 ± 100	2880 ± 120	4050	
Trolox				110		8300 ± 360	1320 ± 90	2970	

PMC = 2,3,5,7,8-pentamethylchromanol, Tocol = 2-methyl-2-(4,8,12-trimethyltridecyl)chroman-6-ol, Trolox = 6-Hydroxy-2,5,7,8- tetramethylchroman-2-carboxylic acid

{a} k_S is the second-order rate constant (in ethanol) for the H-atom abstraction of the various chromanols by the stable phenoxy radical of 3,5-di-*tert*-butyl-4'-methoxybiphenyl-4-ol at 25°C.

{b} k_S is the second-order rate constant (in 5% Triton X-100 micelles) for the H-atom abstraction of the various chromanols by the stable phenoxy radical of 3,5-di-tert-butyl-4'-methoxybiphenyl-4-ol at 25°C.

{c} k_5 is the second order rate constant for the H-atom abstraction of the various chromanols with peroxyradicals produced by the 2,2′-azo-bis(isobutyronitrile) (AIBN) initiated oxidation of styrene in chlorobenzene solution at 30°C

{d} k_1 is the second order rate constant for the H-atom abstraction of the various chromanols with peroxyradicals produced by AIBN initiated oxidation of styrene in chlorobenzene solution at 30° C; the so-called *inhibited autoxidation of styrene* (IAS) method essentially similar to that used in [51]. The *laser kinetic electron paramagnetic resonance* (LKEPR) method uses a laser pulse to decompose di-*tert*-butyl ketone in the presence of oxygen to quickly produce peroxyl radicals whose decay rate due to H-atom abstraction of the chromanols could be monitored by EPR.

{e and f} Antioxidant Efficiencies (AE) are defined as k_{inh}/k_p where k_{inh} is the second order rate constant for the reaction of peroxyl radicals from linoleic acid with the chromanols and k_p is the second order propagation rate constant for the autoxidation of linoleic acid. Consequently, the AE values are without units. Linoleic acid was provided in hexadecyltrimethylammonium bromide (HDTBr) or sodium dodecylsulfate (SDS) supported micelles in the presence of the radical initiator 2,2′-azobis(2- amidinopropane) (ABAP) at pH 7.4. Rates of reactions were monitored by following conjugated diene formation at 234 nm [53] or O_2-uptake [54].

{g} Biological activities of orally provided chromanols are presented relative to *all-rac*-α-tocopheryl acetate (100%).