Vitamin E: A Role in Signal Transduction

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Abstract

Vitamin E modulates the activity of several signal transduction enzymes with consequent alterations of gene expression. At the molecular level, vitamin E may directly bind to these enzymes and compete with their substrates, or it may change their activity by redox regulation. The translocation of several of these enzymes to the plasma membrane is regulated by vitamin E, suggesting the modulation of protein-membrane interactions as a common mechanism for vitamin E action. Enzyme-membrane interactions can be affected by vitamin E by interference with binding to specific membrane lipids or by altering cellular structures such as membrane microdomains (lipid rafts). Moreover, competition by vitamin E for common binding sites within lipid transport proteins may alter the traffic of lipid mediators and thus affect their signaling and enzymatic conversion. In this review, the main effects of vitamin E on enzymes involved in signal transduction are summarized and possible molecular mechanisms leading to enzyme modulation are evaluated.

Contents

INTRODUCTION

Vitamin E is an essential lipid-soluble molecule in higher eukaryotes with a regulatory role on signal transduction and gene expression. Vitamin E plays a central role in disease prevention by scavenging reactive oxygen species (ROS) and reactive nitrogen species (RNS), with consequent reduction of the formation and accumulation of damaged molecules such as membrane lipids, proteins, and nucleic acids. Thus, the cellular-signaling effects of vitamin E may be the result of protection from random modification by free radicals, or since some signal transduction enzymes are regulated by oxidation/reduction, vitamin E may alter their activity by specifically influencing their redox state. However, not all of the cellular effects of vitamin E can be explained by its antioxidant action, since the treatment of cells with each of the eight natural analogues of vitamin E (α-, β-, γ-, and δ-tocopherols and α-, β-, γ-, and δ-tocotrienols) often leads to different outcomes despite having essentially an equal ability to chemically scavenge free radicals (269). In other words, since each vitamin E analogue has a similar antioxidant chemical "soul," their different structural "bodies" must be responsible for their differential effects on signal transduction

ROS: reactive oxygen species

RNS: reactive nitrogen species

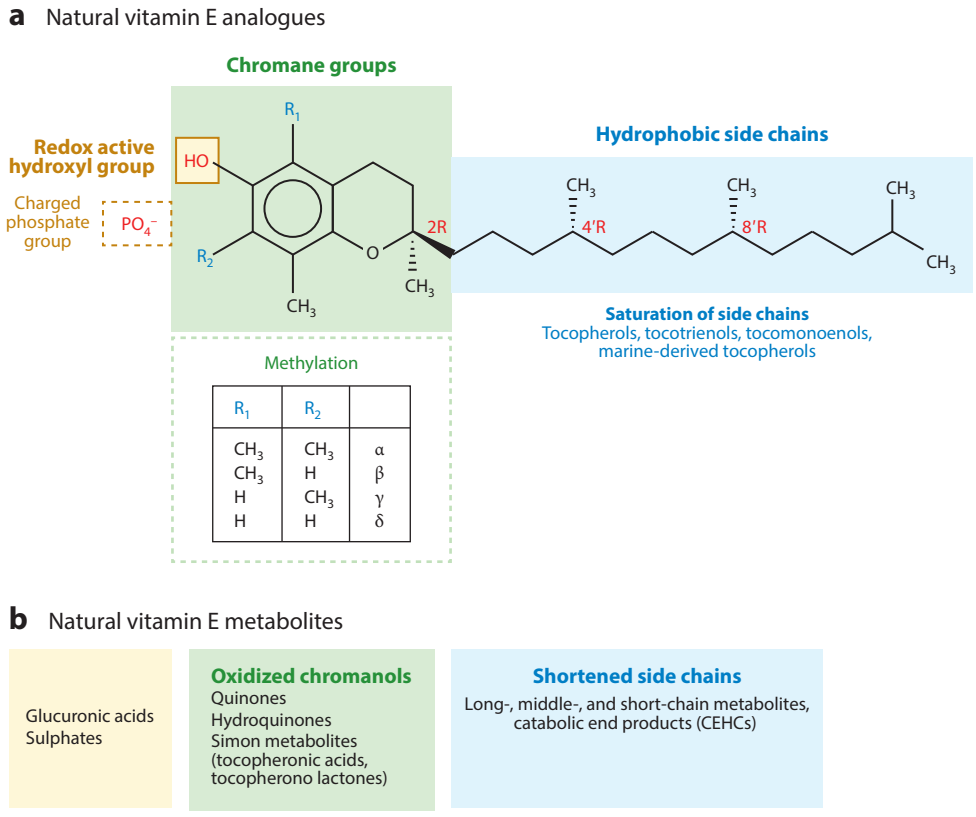

Figure 1

Chemical structures of natural vitamin E analogues and natural vitamin E metabolites. (*a*) Natural vitamin E is a composite of three functional and structural entities: (*i*) the hydrophobic side chain that anchors vitamin E in the plasma membrane [either a phytyl side chain (tocopherols, natural in the *RRR*- configuration) or an unsaturated isoprenoid side chain (tocotrienols, marine-derived tocopherols)]; (*ii*) the differently methylated chromane group (α-, β-, γ-, and δ-tocopherols and tocotrienols); and (*iii*) the redox-active hydroxyl group, which can become phosphorylated. (*b*) Natural vitamin E metabolites can have a shorter hydrophobic and/or hydroxylated side chain, an oxidized chromane group, and a modified hydroxyl group. Abbreviation: CEHCs, carboxyethyl hydroxychromans.

and gene expression (**Figure 1*a***). In these cases, the observed signaling effects may reflect specific interactions of vitamin E with enzymes, structural proteins, and transcription factors and/or result from vitamin E–induced alterations of the physical and structural properties of membrane lipid domains in which it is embedded.

Differences seen between the eight vitamin E analogues can also be explained by differential efficiency of cellular uptake, transport, intracellular distribution, and conversion to different metabolites (**Figure 1*b***) (250). Differential transport and metabolism are of particular relevance in animals and humans, in which *RRR*-α-tocopherol (αT) is selectively recognized and enriched by the liver α-tocopherol transfer protein (αTTP) and therefore in plasma and most tissues reaches much higher (~50x) concentrations than the other seven natural vitamin E analogues, which are less retained in the body, metabolized, and excreted (**Figure 2**) (118). Defective transport of αT is also the major reason for primary and secondary vitamin E deficiency disorders in humans

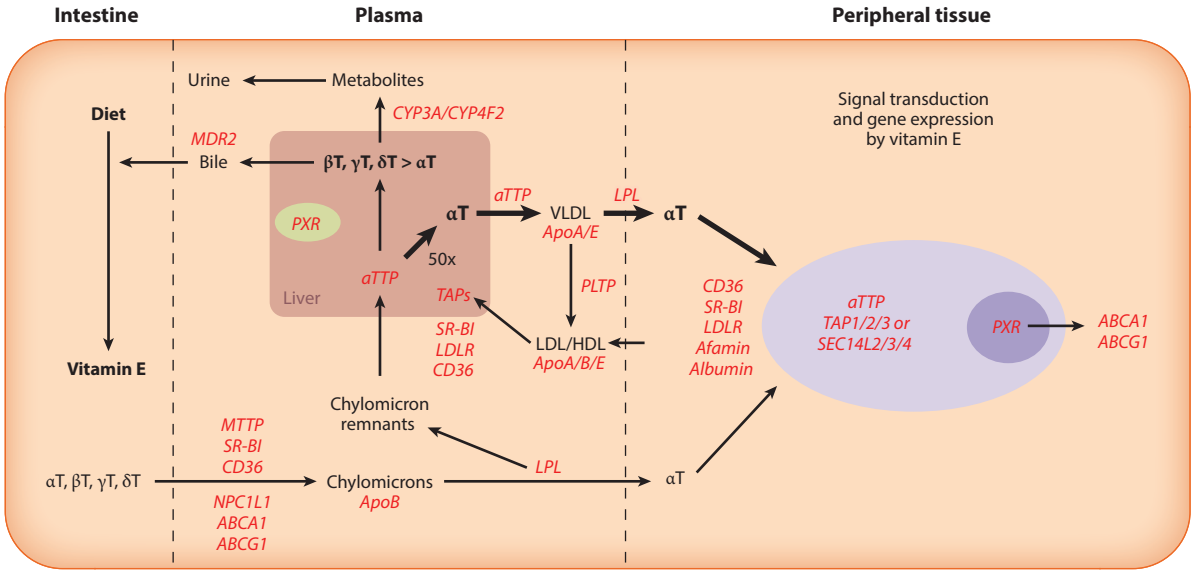

Figure 2

Genes involved in uptake, distribution, metabolism, and secretion play an important role in vitamin E bioavailability and bioactivity. The eight vitamin E analogues (α-, β-, γ-, and δ-tocopherols and α-, β-, γ-, and δ-tocotrienols) are present in the diet in different quantities. Uptake of vitamin E across the intestinal epithelium is mediated by transport proteins [microsomal triglyceride transfer protein (MTTP), Niemann-Pick C1-like 1 (NPC1L1), ATP-binding cassette transporter 1 (ABCA1), ATB-binding cassette transporter G1 (ABCG1), scavenger receptor class B type I (SR-BI), and scavenger receptor cluster of differentiation 36 (CD36)], after which it is secreted within chylomicrons to the bloodstream. Lipoprotein lipase (LPL) releases vitamin E from chylomicrons to peripheral tissues. Cellular uptake is facilitated by several proteins [SR-BI, CD36, low-density-lipoprotein receptor (LDLR), afamin, and albumin]. In cells, vitamin E is transported by α-tocopherol transfer protein (αTTP), tocopherol-associated protein (TAP)1/2/3 [also known as sec14-like (SEC14L)2/3/4], secreted by ABCA1 and ABCG1, and assembled with a certain vitamin E analogue selectivity into low-density and high-density lipoproteins (LDLs and HDLs). The liver takes up vitamin E from chylomicron remnants and LDL/HDL via SR-BI, CD36, and LDLR. In the liver, α-tocopherol (αT) is recognized by α-tocopherol transfer protein (αTTP) and incorporated into very-low-density lipoproteins (VLDLs), leading to an up to 50-fold enrichment of αT in plasma; the other vitamin E analogues (β-, γ-, and δ-tocopherol, excess αT, and the four tocotrienols) are metabolized by cytochromes P450 (CYP)3A/(CYP)4F2 and secreted in urine and bile involving multidrug resistance (MDR)2. Polymorphisms within these genes may play a role in vitamin E bioavailability and bioactivity and thus can also influence signaling and gene expression. Abbreviations: ApoB, apolipoprotein B; PXR, pregnane X receptor.

that result from mutations in a number of genes involved in vitamin E uptake and distribution (**Figure 2**) (241). Polymorphisms in these genes may contribute to the variability of the response of individuals to vitamin E supplementation (148, 274). Although most of the symptoms occurring during severe vitamin E deficiency, including fetal resorption and ataxia with vitamin E deficiency (AVED), have been traditionally attributed to its ability to prevent free radical damage, a role of vitamin E as a cofactor for enzymes or as an active lipid mediator involved in signal transduction and gene expression cannot be excluded at the present time. In fact, tissue- and cell-type selectivity of molecular events occurring during vitamin E deficiency suggest more specific mechanisms for vitamin E action than that of being a general antioxidant in the membranes.

AVED: ataxia with vitamin E deficiency

Over the past two decades, the activity of many signal transduction enzymes has been described to be regulated by vitamin E, leading to alterations of cellular behavior such as proliferation, apoptosis, survival, inflammation, immunity, autophagocytosis, secretion, adhesion, migration, senescence, metastasis, gene expression, and differentiation. Most of these regulatory effects have been observed in cultured cells, and it is presently unknown whether they represent physiological

Table 1 Vitamin E modulates the enzymatic activity of specific enzymes involved in signal transduction (see text for references)

Enzyme class	Enzymes modulated by vitamin E
Protein kinases	Protein kinase C (PKC) Protein kinase B (PKB/Akt) Protein tyrosine kinases (PTKs)
Protein phosphatases	Protein phosphatase 2A (PP2A) Pleckstrin homology (PH) domain leucine-rich repeat protein phosphatase, isoform 1 (PHLPP1) Protein tyrosine phosphatase (PTP)
Lipid kinases	Diacylglycerol kinase (DAGK) Phosphatidylinositol-3-kinase alpha (PI3Kα) Phosphatidylinositol-3-kinase gamma (PI3Kγ)
Lipid phosphatases	5′- and 3′-Inositol polyphosphatases (SHIP)
Lipid metabolic enzymes	5-, 12-, and 15-lipoxygenases (5-, 12-, 15-LOX) Cyclooxygenase-2 (COX-2) Phospholipase A2 (PLA2)
Enzymes involved in cAMP metabolism	Adenylyl cyclase Phosphodiesterase

events occurring also in vivo and whether and how they are responsible for the essentiality of vitamin E. Recent research into the most prominent cellular effects has shed some light into the molecular signaling reactions modulated by vitamin E. Since signaling events are organized in networks, in many cases the observed regulatory effects of vitamin E may only represent secondary events, and a future challenge will be to identify a primary site for vitamin E signaling. This review focuses on currently emerging insights into the molecular mechanisms by which vitamin E influences signal transduction and gene expression.

REGULATION OF SIGNAL TRANSDUCTION AND GENE EXPRESSION BY VITAMIN E

The ability of vitamin E to modulate signal transduction and gene expression has been observed in numerous cell culture, plant, animal, and human studies. At the molecular level, vitamin E affects the activity of specific enzymes involved in signal transduction, such as protein kinases and phosphatases, lipid kinases and phosphatases, and other enzymes of lipid metabolism (**Table 1**). In some cases, vitamin E may also regulate the activity of these enzymes by changing their level of expression. Since signal transduction often involves activation and inactivation of several enzymes cross talking to each other in signaling networks and cascades, a regulatory effect observed after vitamin E treatment may also represent a secondary event occurring at a different site. Accordingly, regulatory effects of vitamin E on gene expression can also be explained as the result of primary signaling events that change the activity or expression level of specific transcription factors (199). The eight natural vitamin E analogues often affect signal transduction and gene expression with different potency, suggesting that these events are not the result of a general antioxidant action. In particular, activity differences become evident when the activities of the four tocopherols are compared to the four tocotrienols, e.g., on cell proliferation and angiogenesis (142, 218), but differences are also observed by comparing the α, β, γ, and δ isoforms of these vitamers (58, 146,

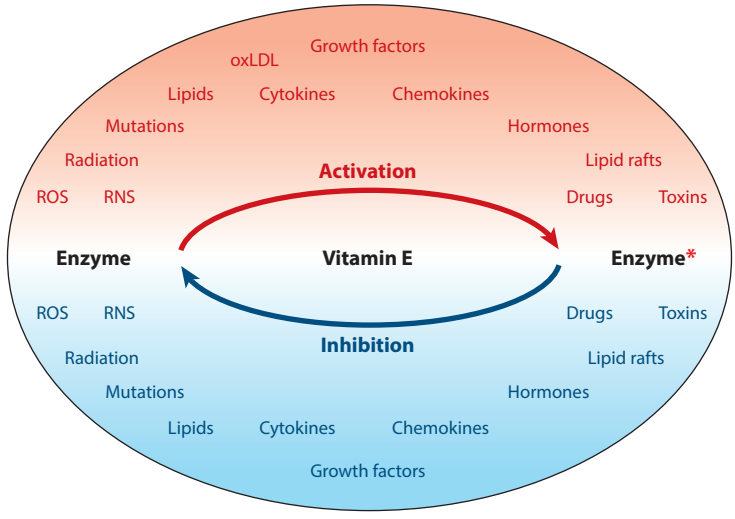

Figure 3

Modulation of the activity of signaling enzymes by vitamin E. Signal transduction occurs as a result of the activation or inactivation of signaling enzymes (**Table 1**) in response to various intracellular or extracellular triggers such as the ones shown in the periphery. As discussed in the text, vitamin E can interfere with the formation or action of these triggers and influences either directly or indirectly the activity of these enzymes and thus signal transduction and gene expression. Although both activating and inactivating effects of vitamin E have been observed, most of the regulatory effects of vitamin E described in the literature represent inhibitory effects that occur after signal transduction enzymes have been activated by various treatments (as marked by the red asterisk). Abbreviations: oxLDL, oxidized low-density lipoprotein; RNS, reactive nitrogen species; ROS, reactive oxygen species.

192, 230). Although vitamin E can both activate and inhibit signal transduction enzymes, most of the cellular effects described in the literature are inhibitory and occur after signal transduction has been activated by extracellular or intracellular triggers (**Figure 3**).

The activity of many transcription factors is regulated by vitamin E, and regulation of gene expression has been observed either at the level of individual genes or genome-wide as assessed with gene array experiments (81, 148, 161, 199, 247, 271, 276). In most of these cases the regulatory effects of vitamin E can be explained to be the result of the modulation of signal transduction enzymes involved in regulating the activity of specific transcription factors (**Table 2**). For some transcription factors, such as nuclear factor-kappa B (NFκB), nuclear factor erythroid 2–related factor 2 (NRF2), and peroxisome proliferator-activated receptor gamma (PPARγ), vitamin E analogue-specific regulatory effects have been observed (46, 58, 59, 110). Only a few cases have been described in which vitamin E is thought to interact with and activate directly a transcription factor, such as the pregnane X receptor transcription factor, which activates the expression of vitamin E metabolic enzymes, or the estrogen receptor beta (**Table 2**) (22, 24, 37, 123, 235). Regulatory effects of vitamin E on the expression of genes involved in cellular signaling can lead to the activation of further signaling cascades as a secondary response; e.g., the expression and release of growth factors and cytokines, such as transforming growth factor beta (TGFβ), interleukin (IL)-1β, IL-4, monocyte chemoattractant protein-1, endothelin, and CD95-ligand, ultimately may affect the activity of cell surface receptors and their signaling cascades. In the following sections, the main regulatory effects of the natural vitamin E analogues on enzymes involved in signal transduction are discussed; the cellular effects of vitamin E and the regulatory

Table 2 Major transcription factors that are modulated by vitamin E (see text for references)

Transcription factor	Effect of vitamin E
Peroxisome proliferator-activated receptor gamma (PPARγ)	Upregulation of expression and increase of activity by indirect mechanisms
Nuclear factor erythroid-derived 2-like 2 (NRF2)	Upregulation of expression by indirect mechanisms
Nuclear factor kappa B (NFκB)	Inhibition of activation by indirect mechanisms
RAR-related orphan receptor alpha (RORα)	Downregulated with vitamin E deficiency, indirect
Hypoxia-inducible factor 1 alpha (Hif1α)	Inhibition of activation by indirect mechanisms
Estrogen receptor beta (ERβ)	Direct binding of tocopherols and increase of activity
Pregnane X receptor (PXR)	Direct binding of tocopherols and increase of activity

effects of vitamin E on gene expression have been recently reviewed (25, 268) and therefore are only briefly addressed here.

Regulation of Protein Kinases and Phosphatases by Vitamin E

The first evidence that vitamin E plays a role in signal transduction came from studies of protein kinase C (PKC) (20, 132). Because αT inhibited PKC after stimulation with the phorbol ester phorbol 12-myristate 13-acetate (PMA) better than the other tocopherol analogues despite having essentially equal chemical antioxidant activity, nonantioxidant mechanisms were proposed as molecular mechanisms of action. Inhibition of PKC by αT led to a reduction of cell proliferation of many different cell types, including vascular smooth muscle cells (VSMCs), monocytes, macrophages, neutrophils, fibroblasts, mesangial cells, and brain cells, as well as of several cancer cell lines. The vitamin E analogue-specific inhibition of PKC also explained other cellular effects, such as the inhibition of endothelin secretion in endothelial cells (136) and the inhibition nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase assembly in monocytes and consequent lower superoxide production (31). In VSMCs, αT specifically inhibited the PKCα isoform (196, 227, 246), whereas in other cell types and in vivo, PKCδ was also inhibited (63, 172). In addition to the inhibition of cell proliferation by vitamin E, many cellular events, such as cell adhesion, migration, and inflammation, have been linked to the regulation of PKCα by vitamin E, and their different response to different vitamin E analogues suggests that they occur as a result of nonantioxidant mechanisms (reviewed in 39). As a nonantioxidant mechanism, differential binding of vitamin E analogues at the diacylglycerol (DAG)-binding site of PKCα-C1a have been proposed to explain their regulatory effects on leukocyte recruitment during inflammation (138). At the molecular level, higher activation of protein phosphatase 2A (PP2A) by αT when compared to other tocopherol analogues was also shown to inactivate PKC by dephosphorylation (57, 167, 195, 196).

In human mastocytoma cell (HMC)-1 cells, inhibition of cell proliferation by the four natural tocopherols occurred with different potency (δ > γ > α > β-tocopherol), and δ-tocopherol had even apoptotic effects at higher concentrations (111). In these cells, proliferation is driven by a constitutively active mutant c-kit tyrosine kinase receptor. In an analysis of the molecular mechanisms of vitamin E action in these cells, inhibition of cell proliferation by vitamin E was correlated to inhibition of protein kinase B (PKB/Akt) phosphorylation at Ser473, which occurred independently of effects on PKC or PP2A (111). In several other cancer cell lines, such as MCF-7 (Michigan Cancer Foundation-7) breast cancer cells, U937 myeloid leukemia cells, or

PMA: phorbol 12-myristate 13-acetate

VSMC: vascular smooth muscle cell

DAG: diacylglycerol

PI: phosphatidylinositol

PI3K: phosphatidylinositol-3-kinase

oxLDL: oxidized low-density lipoprotein

colon cancer cells, similar vitamin E analogue-specific proliferation-inhibitory effects were observed (51, 58, 111). Similar to the tocopherols, the tocotrienols inhibited PKB phosphorylation in breast cancer cells stimulated with epidermal growth factor as a result of a decrease in the relative intracellular levels of the phosphorylated forms of the phosphoinositide-dependent kinase 1 (PDK1), PKB, and glycogen synthase kinase 3 (α/β) (226); tocotrienols also reduced the activity of NFκB (214, 227). In breast cancer cells, the inhibitory effect of γ-tocotrienol (γTT) on PKB phosphorylation was again independent of phosphatases, which suggests alternative mechanisms (215). In pancreatic cancer cells, γTT and δTT had potent antiproliferative activity and induced apoptosis through inhibition of phosphatidylinositol (PI)-3-kinase (PI3K)/PKB and extracellular signal-regulated kinases (ERKs)/mitogen-activated protein kinases (MAPKs) via downregulation of human epidermal growth factor receptor/ErbB2 expression (220). In addition to cell proliferation, PKB plays an important role in a number of other cellular processes, such as cell migration, apoptosis, survival, secretion, and gene expression (85). PKB also regulates senescence and free radical production (50, 174), and as the "Warburg kinase," it influences the energy metabolism of normal and cancer cells (202). PKB has a wide range of cellular targets, and its increased activity can be found during not only tumorigenesis but also atherosclerosis (30, 263). In a model system for atherosclerotic foam cell formation as a result of increased uptake of oxidized low-density lipoproteins (oxLDLs) into THP-1 monocytes/macrophages, oxLDL-induced PKB phosphorylation at Ser473 was antagonized by αT, leading to reduced cellular lipid uptake by downregulating cluster of differentiation 36 (CD36) scavenger receptor expression and surface exposition via inhibition of the oxLDL/CD36/PKB/PPARγ signaling pathway (**Figure 4**) (48, 153, 197). In U937 macrophages, vitamin E prevented oxLDL-induced macrophage foam cell formation through modulating the activities of the oxidative stress–induced NFκB pathway and P-selectin expression (90).

Similarly, αT inhibits Tyk2 protein tyrosine kinase activity in oxLDL-stimulated macrophages (245), and tyrosine phosphorylation of JAK2, signal transducer and activator of transcription (STAT)1, and STAT3 in oxLDL-stimulated MRC5 fibroblasts is decreased by αT (137). Tyrosine phosphorylation of two major proteins (p120, p70) in angiotensin II–induced VSMC, as well as activation of ERK, was reduced by αT, whereas ERK activation by epidermal growth factor was unaffected (65). Relatedly, in HT4 hippocampal neuronal cells, glutamate-stimulated $pp60^{c-Src}$ tyrosine kinase activity was normalized by α-tocotrienol (αTT) but not by αT (213). In human neutrophils, tyrosine phosphorylation was decreased by α-tocopheryl succinate (αTS) as a result of activation of a protein tyrosine phosphatase (32). Inhibition of protein tyrosine kinase activity by tocopherols may also be involved in reducing PI3K activity, at least of class I and II PI3Ks that are regulated by tyrosine phosphorylation, which may also ultimately inhibit PKB (189).

Vitamin E also influences the MAPK/ERK signaling cascade (15, 52, 57, 160, 213). During lung tumorigenesis, urethane-induced activation of several members within the ERK signaling cascade (Ras, Raf, and Mek) was inhibited by treatment with vitamin E or α-tocopheryl oxybutyric acid, an ether derivative of vitamin E that cannot act as an antioxidant in vivo (264). In these cases, vitamin E may interfere with the formation of scaffolding complexes involving Ras, cRaf-1, and MEK as well as Erk-Ras-Raf complexes at the surface of endosomes (8, 200). Moreover, since cRaf-1 kinase membrane translocation and activation are promoted by phosphatitic acid, it appears possible that vitamin E inhibits these activities by interfering with membrane binding (8, 200).

In human peripheral mononuclear cells, αT stimulated the production of cyclic adenosine monophosphate by increasing adenylyl cyclase activity after engaging G-coupled prostaglandin EP2 and EP4 receptors, and attenuated proinflammatory cytokine and chemokine production (207). At least in vitro, the hydrolysis of cyclic adenosine monophosphate by cyclic nucleotide phosphodiesterases is stimulated by the phosphorylated form of vitamin E (206).

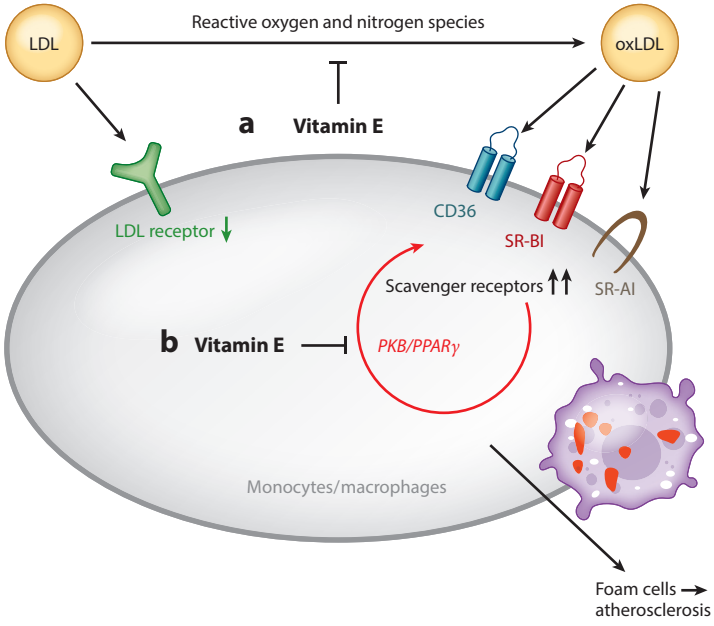

Figure 4

Mechanisms of regulation of the CD36 scavenger receptor by vitamin E. During inflammation, low-density lipoproteins (LDLs) can become modified by reactive oxygen and reactive nitrogen species to form oxidized LDL (oxLDL). Whereas the LDL receptor is downregulated by its ligand LDL, the cluster of differentiation 36 (CD36) scavenger receptor is upregulated by its ligand oxLDL via activation of protein kinase B (PKB) and peroxisome proliferator-activated receptor gamma (PPARγ), leading to excess uptake of lipids and foam cell formation and ultimately to atherosclerosis. (*a*) As a lipid-soluble antioxidant, vitamin E can interfere with oxidation of LDL and thus reduce upregulation of CD36, lipid accumulation, and foam cell formation. (*b*) Vitamin E can also inhibit activation of PKB and PPARγ by oxLDL, leading to lower expression of CD36, reduced CD36-mediated signaling, and reduced lipid uptake and lipid-mediated signaling (see also **Figure 6**). Similar events may occur with other scavenger receptors (SRs) such as SR-BI and SR-A1.

Contrary to the above-described inhibitory effects of vitamin E on PKB and ERK1/2, a stimulatory effect was observed in cultured cortical neurons treated with αT and γT as well as with αTT and γTT, leading to induction of the antiapoptotic B-cell lymphoma-2 (Bcl-2) protein and increased survival in response to H_2O_2; these findings suggest that the response to vitamin E can be modulated by cell-type-specific regulatory mechanisms (176). Increased cell proliferation was also observed with naive T cells after supplementation of aged subjects with vitamin E; lower cyclooxygenase 2 (COX-2) activity resulted in increased IL-2 production and decreased prostaglandin E2 (PGE2) levels (141). Moreover, vitamin E modulated the expression of several cell-cycle control proteins and restored age-dependent decline of immune synapse formation in $CD4^+$ T cells by modifying signaling and gene expression, which explains the immune-stimulatory effect of vitamin E in the elderly (150). A small increase of proliferation was also observed with αT and γT in THP-1 monocytes, whereas βT had no effect and δT was inhibitory (278). Similarly, αT increased proliferation of hematopoietic stem/progenitor cells, leading to bone marrow hyperplasia. In addition, differentiation to the granulocytic/monocytic lineage and ERK1/2 activation in response to IL-3 stimulation were enhanced by αT, whereas basal phosphorylation of ERK1/2, PKC, and STAT-5 was decreased (175).

Regulation of Lipid Kinases and Phosphatases by Vitamin E

An alternative mechanism for the inhibition of PKC by vitamin E was proposed to occur during diabetes, in which PKC is activated by increased levels of the lipid DAG (121). In this situation, αT counteracts high-glucose-induced DAG levels by stimulating diacylglycerol kinase (DAGK), thus removing DAG by phosphorylating it and converting it to phosphatitic acid and ultimately leading to lower PKC activity (238).

Another lipid kinase that is affected by vitamin E is PI3Kγ (113, 279). Regulation of PI3Kγ activity by vitamin E occurs in an analogue-dependent manner and is modulated by tocopherol-associated proteins 1, 2, and 3 [TAP1, TAP2, and TAP3, also known as sec14-like (SEC14L) proteins 2, 3, and 4, respectively] (275, 279). These proteins associate with and inhibit PI3Kγ activity in vitro (113) and become activated by vitamin E–mediated lipid exchange and/or dissociation of the inactive complex (275, 279). Likewise, the mouse TAP1 protein competes with p85 for binding to PI3Kα, with consequent inhibition of PKB activity and prostate tumor formation (169); however, in this case αT was not able to further affect the inhibitory effect of TAP1 on PI3Kα and PKB. In cultured cortical neurons, αT, αTT, γT, and γTT prevented cell death induced by oxidative stress through the activation of MAPK and PI3K and upregulation of Bcl-2 (176). Similarly, in U937 cells, the oxysterol 7-ketocholesterol induced apoptosis and phospholipidosis and suppressed PI3K, PDK1, and PKB activity, events that were reversed by vitamin E (244). In contrast, in neoplastic mammary epithelial cells, but not in normal cells, γTT induced apoptosis by inhibiting Pl3K/PDK1/PKB mitogenic signaling and NFκB transcriptional activity independent of a change in phosphatase and tensin homolog (pTEN) or PP2A activity (228).

Regulation of Lipid Metabolic Enzymes by Vitamin E

As a hydrophobic molecule, vitamin E may compete at the active site of lipid metabolic enzymes involved in the production of specific messenger lipids or in lipids involved in defining the physical and structural properties of membranes. In fact, vitamin E modulates several enzymes involved in generating lipid messengers, such as phospholipase A2 (PLA2) (33, 187); 5-, 12-, and 15-lipoxygenase (5-, 12-, and 15-LOX) (49, 80, 114, 191); and COX-2 (1); in some of these cases, direct binding of vitamin E to the enzyme has been described. In ionophore-stimulated human blood neutrophils or differentiated HL-60 cells, the four vitamin E analogues differentially inhibited the formation of the inflammatory lipid mediator leukotriene B(4) [LTB(4)]. δT suppressed cytosolic Ca^{2+} increase and/or LTB(4) formation triggered by ionophores, sphingosine 1-phosphate, and lysophosphatidic acid without inhibiting 5-LOX, whereas the long-chain vitamin E metabolite 13'-carboxychromanol decreased cellular LTB(4) production as result of its strong inhibition of the 5-LOX activity (105). Similar regulatory effects have been observed with the tocotrienols, which have cholesterol-lowering properties. A lower cholesterol level can change the membrane composition and the structure of membrane microdomains, leading to suppression of cell proliferation and induction of apoptosis in cancer cells. At the molecular level, the tocotrienols modulate cholesterol biosynthesis by influencing the mevalonate-cholesterol pathway, which possibly also affects the biosynthesis of cholesterol-derived steroid hormones (reviewed in 181, 212). αTT reduces cholesterol levels by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity and its mRNA translation, and by decreasing the secretion of apolipoprotein B (apoB) and enhancing its proteasomal degradation (184, 186, 252). Several isoprenoids inhibit HMG-CoA reductase synthesis and accelerate reductase degradation in a manner similar to that of αTT, which suggests that these effects are mainly mediated by the unsaturated isoprenoid side chain of the tocotrienols (147).

MOLECULAR MECHANISMS OF VITAMIN E–MEDIATED SIGNAL TRANSDUCTION AND GENE EXPRESSION

LTP: lipid transport protein

VEGF: vascular endothelial growth factor

It is difficult to explain the above-described effects of vitamin E on signal transduction and gene expression by a single molecular mechanism. Because vitamin E is a hydrophobic molecule, it occurs most of the time in cellular membranes and lipid vesicles, where it can act as an antioxidant molecule preventing lipid peroxidation. However, the different membranes in a cell are also primary sites for lipid-mediated signal transduction, and many enzymes require for their activity the interaction with specific membrane lipids. These lipids are either synthesized locally or transported from other sites by lipid transport proteins (LTPs), which bring them to membranes, enzymes, receptors, or structural proteins where they induce signaling. Some of the LTPs are also known to bind vitamin E, and competition between vitamin E and messenger lipids for common binding sites may alter the transport and signaling function of the LTP. Moreover, the presence of vitamin E in membrane domains may change their composition and structure and thus affect their signaling function (10, 125). As described in the following sections, recent research into these different possibilities of signal transduction has revealed as a common and perhaps even primary regulatory mechanism of vitamin E action the modulation of enzyme-membrane interaction.

Antioxidant Effects of Vitamin E and Redox Regulation of Enzymes Involved in Signal Transduction

The scavenging of free radicals in the membrane by vitamin E can affect the activity of signaling lipids and signal transduction enzymes by preventing their random destruction. In membranes, vitamin E reduces oxidation of specific messenger lipids involved in signal transduction (236), reduces the formation of oxLDL and of oxLDL-induced signal transduction (**Figure 4**), or prevents the formation of nitrated fatty acids that serve as cell-signaling, gene-regulatory, and inflammatory mediators (36, 42, 211). Moreover, because the activity of several signal transduction enzymes can be modulated by oxidation and reduction, redox-active molecules such as vitamin E or vitamin C (L-ascorbic acid) may influence their activity by generally reducing the levels of RNS and ROS, by specifically scavenging them when bound to these enzymes or by regulating their production (reviewed in 13, 64). In certain situations, such as in the absence of coantioxidants in lipoproteins, vitamin E can also act as a pro-oxidant and initiate a chain reaction of lipid peroxidation, possibly generating oxidized lipids with a regulatory function on signal transduction and gene expression (242). The predominant location of vitamin E in membranes may limit redox regulation to specific situations, such as after enzyme activation and translocation to the plasma membrane. Alternatively, the production of ROS can be influenced by vitamin E, e.g., by interfering with the assembly and activation of NADPH-oxidase (31, 246). Increased ROS production is often observed after exposure to chemical and physical stressors, such as heat, cold, radiation, drugs, toxic compounds, bacteria, and inflammatory triggers, and it is not surprising that in these situations vitamin E has often been reported to alleviate their damaging or regulatory effects on redox signaling (25, 109, 236). Moreover, free radical scavenging may affect signaling cascades in which the production of free radicals is integral to the signaling process, e.g., the activation of growth factor receptors by their ligands, such as insulin, vascular endothelial growth factor (VEGF), angiotensin II, endothelin, and platelet-derived growth factor, and cytokines such as tumor necrosis factor alpha and IL-1β (reviewed in 64, 96, 122).

The activity of a number of signaling enzymes is regulated by the redox state, but in most cases it remains to be elucidated whether vitamin E affects signaling enzymes and thus plays a role in their regulation. Oxidative activation of PKCα in vitro is prevented by αT at a tenfold

lower concentration when compared to γ-tocopherol (γT) and involves direct binding of vitamin E to the enzyme (138). Likewise, PKB is regulated via reversible oxidation of an intramolecular disulfide bridge in an activation loop, and activation of PKB by ROS can be prevented by vitamin E in human bronchial epithelial cells (BEAS-2B) (182). Several protein tyrosine phosphatase family members are physiologically regulated by reversible oxidation and reduction (140). The tumor suppressor phosphatase PTEN, which dephosphorylates phosphatidylinositol-(3,4,5)-trisphosphate (PI345P) and thus reduces PI3K-induced signal transduction (7), is inactivated by oxidation of critical cysteines within the active site, leading to net activation of growth factor–stimulated signal transduction involving the PI3K/PKB pathway (13, 127). Similarly, the protein tyrosine phosphatase 1B is temporarily inactivated by a burst of ROS occurring after epidermal growth factor stimulation (12). However, at least in HMC-1 mast cells (111) and in neoplastic mammary epithelial cells, neither activation of PTEN nor of PP2A by vitamin E is responsible for the inhibition of PKB by vitamin E, which suggests other mechanisms of action (215, 228). Among the Ser/Thr phosphatases that use catalytic mechanisms different from protein tyrosine phosphatases, only calcineurin, also called protein phosphatase 2B, and possibly protein phosphatase 1 are affected by free radicals, and it remains to be determined whether vitamin E can regulate their activity (222).

Direct Binding of Vitamin E and Modulation of Enzymes Involved in Signal Transduction

Vitamin E can affect the synthesis and concentration of messenger lipids in the plasma membrane by directly binding to specific signal transduction enzymes, such as protein and lipid kinases, phosphatases, phospholipases, and other enzymes involved in the biosynthesis of phospholipids. Direct binding of vitamin E to PKCα or the PKCα-C1a domain was demonstrated using a competition assay; in these experiments, the binding of a synthetic vitamin E derivative [NBD (7-nitrobenz-2-oxa-1,3-diazole)-tagged αT] could be blocked by DAG, αT, γT, and retinol, but not by cholesterol or phosphatidylserine (138). Direct binding of vitamin E to enzymes involved in generating lipid messengers also occurs with PLA2 (33, 187); 5-, 12-, and 15-LOX (49, 80, 114, 191); and COX-2 (1) (see also **Table 4**). These enzymes are modulated in a vitamin E analogue-specific manner, suggesting that each tocopherol and tocotrienol analogue binds to them with different affinity. Direct binding of αT to 5-LOX not only inhibits the enzymatic activity but also affects membrane translocation via the inhibition of tyrosine phosphorylation (126). Thus, in addition to modulating the enzymatic activity of these enzymes by binding to their active site, vitamin E may interfere with the enzyme activation/membrane translocation process or may act as a competitive inhibitor.

Direct Binding of Vitamin E to Transport Proteins Involved in Signal Transduction

Several proteins have been identified that are involved in vitamin E analogue-specific uptake and transport and therefore are able to bind vitamin E at least transiently (**Table 3** and **Figure 2**) (272). Since in most cases these proteins also bind other ligands, it remains to be determined to what degree binding of vitamin E affects the action of these other ligands, their cellular distribution, and their associated signaling functions.

Vitamin E is absorbed from micelles in the intestine by scavenger receptor class B type I (SR-BI) and CD36, which absorb vitamin E into intestinal enterocytes (75, 95, 190). Absorbed vitamin E is assembled together with other lipids into chylomicrons, which transport vitamin E to peripheral

Table 3 Vitamin E–binding and transport proteins (see text for references)

Vitamin E–binding proteins	Molecular activity
Alpha-tocopherol transfer protein (αTTP)	Binding of tocopherols and selective enrichment of α-tocopherol in plasma, with consequent higher tissue concentrations
Niemann-Pick-C1-like 1 (NPC1L1)	Binding of tocopherols and intracellular transport
Tocopherol-associated protein 1 (TAP1, SEC14L2)	Binding of tocopherols and intracellular transport; possible role in cellular signaling; possible role in plasma lipid levels
Tocopherol-associated protein 2 (TAP2, SEC14L3)	Binding of tocopherols and intracellular transport; possible role in cellular signaling
Tocopherol-associated protein 3 (TAP3, SEC14L4)	Binding of tocopherols and intracellular transport; possible role in cellular signaling
Afamin	Binding and transport of α-tocopherol in cerebrospinal fluid
Albumin	Binding of α-tocopherol in plasma; possible role in tissue distribution
Phospholipid transfer protein (PLTP)	Binding of tocopherols and transfer between lipoproteins and tissues
Scavenger receptor class B type I (SR-BI)	Transport of vitamin E and other ligands; signaling
Scavenger receptor cluster of differentiation 36 (CD36)	Transport of vitamin E and other ligands; signaling
ATP-binding cassette transporter A1 (ABCA1)	Transport of vitamin E and other ligands
ATP-binding cassette transporter G1 (ABCG1)	Transport of vitamin E and other ligands

tissues, where it is released by lipoprotein lipase. Up to this stage, each vitamin E analogue is taken up with equal efficiency, and thus how much of each analogue is transported depends on the amounts present in the diet. However, because the different vitamin E analogues use the same route of uptake, they compete with each other in their uptake, distribution, and metabolism in the body (240), which ultimately may also affect vitamin E analogue-specific signaling and gene expression.

In plasma, the level of αT is much (50 times) higher than that of any of the other analogues, and several proteins have been reported to influence the plasma and tissue levels of the eight vitamin E analogues (**Table 3**) (reviewed in 118, 159, 198, 274). The main protein influencing plasma vitamin E levels, αTTP, mediates the enrichment of αT by the liver, the assembly into very-low-density lipoprotein (VLDL), and the delivery to the circulation, whereas the other tocopherol analogues are preferentially channeled into metabolism and secretion (28, 83, 208). αTTP deficiency leads to vitamin E deficiency in the body with the symptoms of ataxia with vitamin E deficiency and increased atherosclerotic lesion formation in apoE$^{-/-}$ knockout mice (231). In these vitamin E–deficient animals, vitamin E supplementation prevents atherosclerosis independent of reducing lipid oxidation in the vessel wall (225). By using gene expression arrays, expression changes of entire gene networks were observed in αTTP$^{-/-}$ knockout mice, suggesting that the level of vitamin E is an important factor for setting the expression level of many genes, most likely as a result of the modulation of signal transduction (74).

Vitamin E transport in plasma occurs mainly in lipoproteins [chylomicrons, VLDL, LDL, and high-density lipoprotein (HDL)], from which it is released to tissues after binding to their receptors (e.g., LDLr, SR-BI, CD36) by lipoprotein lipase. The plasma phospholipid transfer protein enhances the vitamin E exchange between these lipoproteins and between lipoproteins and cells (120, 125). As a result of a decreased vitamin E exchange, phospholipid transfer protein–deficient mice show reduced vitamin E content and elevated lipofuscin, cholesterol oxides, and cellular peroxides in tissues such as brain (47), as well as increased cholesterol accumulation in macrophages (177); however, delayed formation of conjugated dienes occurs as a result of increased

VLDL: very-low-density lipoprotein

HDL: high-density lipoprotein

plasma levels of vitamin E in circulating apoB-containing lipoproteins at the expense of the vascular wall (104).

HDL-associated vitamin E is transported by SR-BI to hepatocytes, to type II pneumocytes, and across the brain capillary endothelial cells forming the blood-brain barrier (77, 117, 133, 134). SR-BI-deficient mice have a lower tissue uptake of αT that may impair the signaling function of vitamin E and contribute to the reproductive, cardiovascular, and neurodegenerative pathologies observed in these animals (134). SR-BI is involved in a number of other functions, such as cholesterol homeostasis, cholesterol flow, membrane lipid transport, membrane domain assembly, uptake of hepatitis C virus, phagocytosis of apoptotic cells, and platelet functions, and it remains to be clarified whether vitamin E affects these events as well (216).

Other tocopherol-binding proteins, including TAP1, TAP2, TAP3 (SEC14L2, SEC14L3, SEC14L4) (113, 166, 188, 277), NPC1L1 (163), afamin (248), and albumin (61, 180), have been described, but as discussed below, their role in vitamin E tissue transport, activity, metabolism, and signaling remains to be further elucidated (reviewed in 274, 277).

Modulation of Membrane-Protein Interaction and Protein Translocation to the Plasma Membrane by Vitamin E and Signal Transduction

Because many enzymes that are affected by vitamin E become active at the plasma membrane, the modulation of enzyme translocation to the plasma membrane was suggested to be a common theme for the regulation of signal transduction by vitamin E (**Table 4**) (267, 268). Similarly, because enzyme translocation is often targeted to specific membrane microdomains such as lipid rafts, vitamin E was proposed to affect signaling by influencing these membrane domains (125). As described below, for some enzymes vitamin E may either activate or inhibit the mechanisms of membrane translocation, whereas for others it may interfere with the binding of the enzyme to specific lipids in the membrane.

In general, enzymes interact differently with the plasma membrane; therefore, several possible mechanisms for modulating protein-membrane interactions and enzyme activation by vitamin E can be envisioned. In most cases, the translocation of enzymes to the plasma membrane is dependent on binding to specific messenger lipids and/or scaffold proteins known to play an important role in signal transduction (232). Specific domains in proteins recognize specific messenger lipids and become attracted to the plasma membrane, where they become active (reviewed in 35, 92). Upon membrane interaction, lipid binding, and membrane insertion, many enzymes undergo a conformational change. Each enzyme has different structural requirements for activation by protein-membrane interaction; these requirements involve specific and nonspecific protein-membrane interactions and electrostatic as well as conformational switches that are induced by phosphorylation, by binding of lipid messengers, or by membrane integration (35, 92, 145). Specific domains in proteins—such as the C1/C2-domains in PKC; the pleckstrin-homology domain in PKB/Akt, PDK1, or pleckstrin homology domain leucine-rich repeat protein phosphatase, isoform 1 (PHLPP1); the FYVE domain in EEA1; or the PX domain in $p40^{phox}$—recognize specific messenger lipids at the plasma membrane, where they become active and where vitamin E may have a regulatory influence (reviewed in 35, 92). Moreover, some enzymes recognize only one specific lipid, some require more than one, some either dimerize or require binding to additional proteins, some need Ca^{2+} or Zn^{2+}, and some need to be phosphorylated (reviewed in 35, 92).

As discussed below, recent studies suggest that vitamin E can compete at these domains with messenger lipids and thus change the activity of signal transduction enzymes by preventing or enhancing their translocation and interaction with membranes in an analogue-specific manner. Moreover, vitamin E in the plasma membrane may change the energetics and kinetics determining

Table 4 Inhibition of membrane-protein interactions and enzyme translocation to the plasma membrane—a common theme for vitamin E action? (See text for references)

Enzymes modulated by vitamin E analogues	Domain (bound lipids)	Active at plasma membrane	Direct binding of vitamin E to enzyme	Enzyme activity change by vitamin E	Modulation of enzyme translocation to the plasma membrane by vitamin E
Enzymes inhibited by vitamin E					
Protein kinase C alpha (PKCα)	C1 and C2 domain (DAG)	Yes	No Yes	Inhibition Inhibition	Inhibition
Protein kinase B (PKB, Akt)	PH domain [PI(34)P and PI(345)P]	Yes	No	Inhibition Stimulation Inhibition	Inhibition Stimulation by high concentrations of *RRR*-α-tocopherol (αT) and γ-tocopherol (γT) Inhibition by stimulation of PH domain leucine-rich repeat protein phosphatase, isoform 1 phosphatase
Protein tyrosine kinases (PTK) ($pp60^{c-Src}$, Tyk2)	Not known	Yes	Not known	Inhibition	Not known
Phospholipase A2 (PLA2)	C2 domain	Yes	Yes	Inhibition	Not known
Cyclooxygenase 2 (COX2)	Transmembrane	Yes	Yes	Inhibition	Not known
5-, 12- and 15-Lipoxygenase (5-, 12-, and 15-LOX)	PLAT-LOX-domain	Yes	Yes	Inhibition	Not known
Nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase	PX domain [PI(3)P]	Yes	No	Inhibition	Inhibition is indirect, via inhibition of phosphorylation of subunits p47 and p67 by PKC
Enzymes activated by vitamin E					
PH domain leucine-rich repeat protein phosphatase, isoform 1 (PHLPP1)	PH domain (vitamin E)	Yes	Not known	Stimulation	Stimulation, leading to inactivation of PKB/Akt by dephosphorylation
Phospho-serine/threonine phosphatase 2A (PP2A)	Not known	Not known	No	Stimulation	Dephosphorylation of PKC by PP2A may occur at the plasma membrane
Phospho-tyrosine phosphatase	Not known	Not known	Not known	Stimulation	Not known
Src homology (SH)-related complex (SRC) homology 2 domain-containing inositol-5-phosphate (SHIP) phosphatase	Not known	Not known	Not known	Stimulation	Inhibition by α-tocopherol succinate; activation of PKB; inhibition of MAPK and tumor necrosis factor alpha gene expression
Diacylglycerol kinase alpha (DAGKα)	Atypical C1 domain (PA)	Yes	No	Stimulation	Stimulation, via induction of tyrosine phosphorylation

Table adapted from Reference 267.

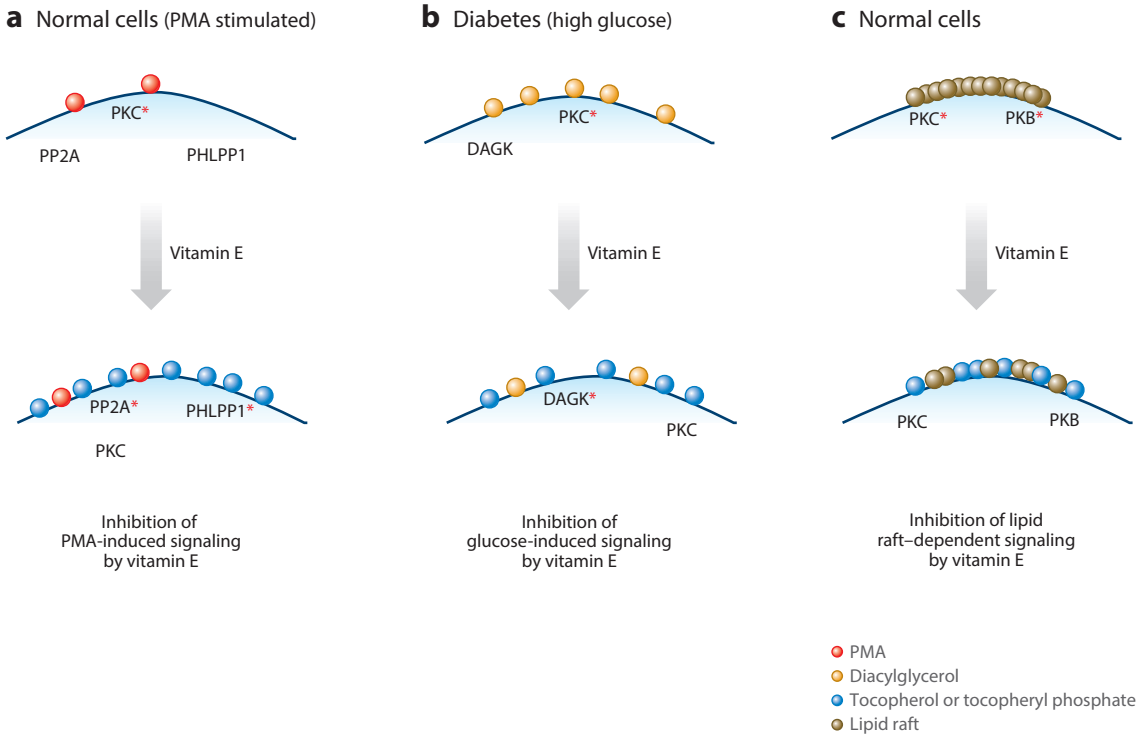

Figure 5

Mechanisms of regulation of protein kinase C (PKC) by vitamin E. (*a*) In normal cells, e.g., cells stimulated by the phorbol ester phorbol 12-myristate 13-acetate (PMA), PKC is activated by phosphorylation and translocation to the plasma membrane. Vitamin E binds to PKC and inactivates it or activates a phosphatase [such as protein phosphatase 2A (PP2A) or pleckstrin homology domain leucine-rich repeat protein phosphatase, isoform 1 (PHLPP1)] that dephosphorylates PKC and reduces its activity. (*b*) In the diabetic condition, high glucose leads to increased levels of diacylglycerol (DAG) in the plasma membrane, which results in activation of PKC. Vitamin E activates DAG kinase (DAGK), which phosphorylates DAG to form phosphatitic acid, thus reducing the level of cellular DAG and leading to a lower activation of PKC and consequent inhibition of cell proliferation. (*c*) The ability of vitamin E to change the composition and physical characteristics of plasma membrane structures such as lipid rafts can influence signaling enzymes that are influenced by these microdomains, such as PKC and PKB.

the nonspecific and specific interactions of enzymes with membranes. In addition, enzymes may exist that contain domains that can specifically recognize vitamin E in the plasma membrane. These domains may serve as enzyme anchors at the plasma membrane and may either activate their own signaling cascade or may interfere with the signaling of other enzymes.

The translocation of PKCα to the plasma membrane is inhibited by tocopherols and tocotrienols after activation by epidermal growth factor in normal mammary epithelial cells (227). PKCα gets activated by phosphorylation and then translocates to the plasma membrane, where its C2 domain binds phosphoinositite phosphates in a Ca^{2+}-dependent manner and exposes its DAG-binding C1 domain. Inhibition of PKCα by αT occurs either by direct binding to the enzyme (138) or via stimulation of PP2A and dephosphorylation (57, 167, 195, 196) (**Figure 5*a***). Cofactor-dependent activation of recombinant PKCα was increased by γT and was inhibited by αT, leading to opposing effects on leukocyte migration across endothelial cells by regulating vascular cell-adhesion molecule-1 activation. Moreover, whereas the PKCα-C1b domain did not bind to vesicles containing tocopherols and phosphatidyl serine, PKCα-C2 domain binding was

enhanced by tocopherols, indicating a domain-specific interaction of PKCα with vitamin E analogues embedded in membranes. Dephosphorylation of active PKCα by αT most likely occurs after translocation of PP2A to the plasma membrane, given that a complex between PKCα and PP2A has been detected (21). As a secondary event of PKCα inhibition by αT, the assembly of the active NADPH-oxidase at the plasma membrane is inhibited, which may prevent chronic inflammatory processes, including inflammatory bowel disease, scleroderma, liver fibrosis, and neurodegeneration (56, 76). In monocytes, PKCα inhibition by αT attenuates the NADPH-oxidase subunit $p47^{(phox)}$ membrane translocation and phosphorylation (31, 246). In microglia cells, αT inactivates PKC via a phosphatase-mediated pathway (PP1 or PP2A) and, as a consequence, blocks the phosphorylation-dependent translocation of the NADPH-oxidase subunit $p67^{(phox)}$ to the plasma membrane (56). Alternatively, the assembly of NADPH-oxidase by vitamin E may also be affected by vitamin E as the result of modulation of PI3K, which is involved in the recruitment of the subunits to the plasma membrane via their PX domains, which specifically bind PI3P and PI4P (221).

Membrane translocation for DAGKα (**Figure 5*b***) is induced by αT, αTS, trolox, and troglitazone, and at high concentrations also by α-tocopheryl acetate; it is triggered by phosphorylation at Tyr334 and possibly also involves protein-membrane interactions mediated by the atypical C1 domain (67). Activation of DAGK by αT lowers high-glucose-induced DAG levels by phosphorylating DAG and removing it by generating phosphatitic acid, which leads to lower PKC activity (238).

Activation of signal transduction occurs after binding of specific ligands to their receptors at the plasma membrane, which preferentially occurs at specific membrane microdomains such as lipid rafts. Thus, since vitamin E can change the composition of these plasma membrane structures (10, 125), it may modulate the signaling function of these receptors and change the activity of enzymes such as PKC, DAGK, and PKB by influencing lipid rafts (**Figure 5*c***) (185).

The activity of PKB and translocation to the plasma membrane is inhibited by tocopherols in HMC-1, which leads to the inhibition of cell proliferation (**Figure 6*a***); after inhibition of the mutant constitutively active c-kit tyrosine kinase in these cells, PKB translocation induced by nerve growth factor beta is inhibited as well (111), indicating that the translocation process is not dependent on a specific membrane receptor but rather on the lipid mediator synthesized. The PKB activation and inhibition process offers several molecular targets that could be influenced by vitamin E (210, 257). PKB translocation to the plasma membrane occurs by means of the pleckstrin homology domain, which binds PI34P and PI345P, produced by activated PI3K, an enzyme that is known to be modulated by vitamin E (113, 275, 279). A similar domain is found in PDK1, a kinase that phosphorylates Thr308, leading to partial activation of PKB. Full activation requires phosphorylation of PKB at Ser473 by PDK2, and several enzymes have been proposed to have this activity (ataxia telangiectasia mutated, DNA-dependent protein kinase, integrin-linked kinase, PKCα, PKCβ, and the rictor-mTOR complex).

Most human cancers show activation of the PI3K/PKB signaling pathway due to genomic aberrations in several regulatory genes (30). A recent study established that the phosphatase PHLPP1 dephosphorylates and inactivates PKB in cancer cells. PHLPP1 binds via the PH-domain to αT and γT and translocates to the plasma membrane, leading to dephosphorylation and inactivation of PKB (**Figure 6*b***) (88). High concentrations of vitamin E also trigger the translocation of PDK1 and PKB to the plasma membrane (**Figure 6*c***), which suggests that the overall PKB activity is the result of a balance of membrane translocation and activation of PDK1/2, PKB, and PHLPP1. Interestingly, PHLPP1 levels are reduced in several cancer cell lines that have elevated PKB phosphorylation (69), possibly making them less susceptible to the antiproliferative effects of the tocopherols. PHLPP1 also dephosphorylates the conventional and novel PKC isoforms

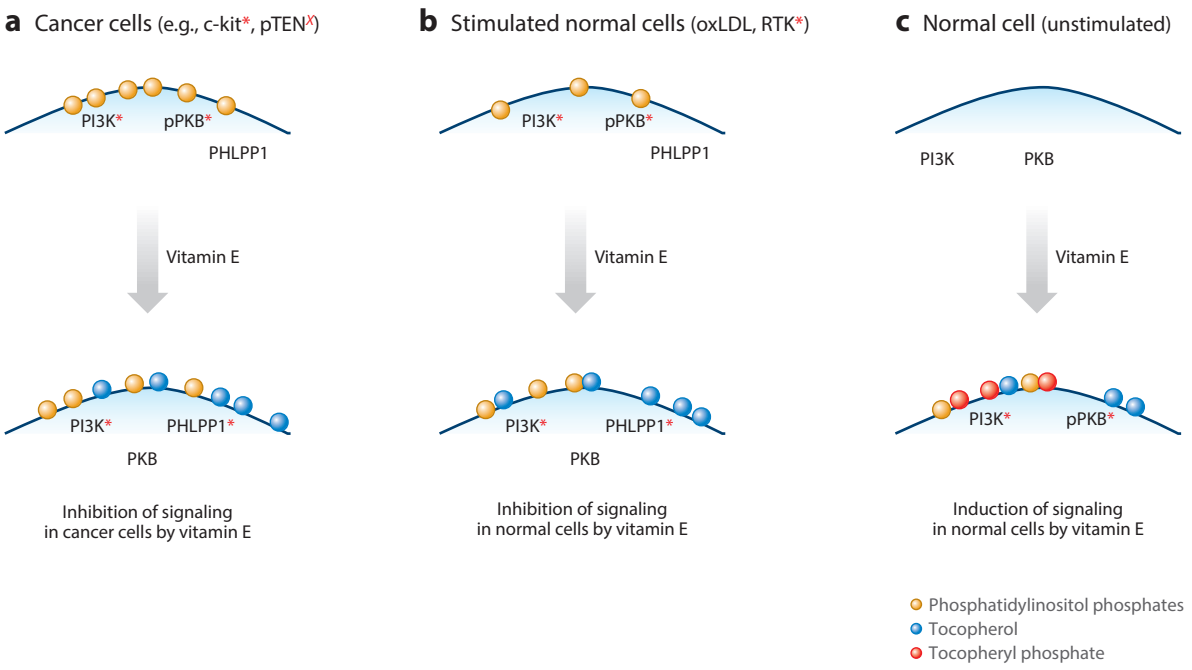

Figure 6

Mechanisms of regulation of protein kinase B (PKB/Akt) by vitamin E. (*a*) In cancer cells, e.g., with mutant and activated c-kit tyrosine kinase or inactivated phosphatase and tensin homolog (pTEN) phosphatase, or (*b*) in normal cells that were stimulated with growth factors or oxidized low-density lipoprotein (oxLDL) and have activated receptor tyrosine kinases (RTKs), phosphatidylinositol-3-kinase (PI3K) and prosurvival protein kinase B (pPKB) are active and located at the plasma membrane; treatment with vitamin E leads to recruitment of the phosphatase PHLPP1 to the membrane that dephosphorylates pPKB and inactivates it. (*c*) In normal unstimulated cells, PKB is inactive and located in the cytosol; treatment with *RRR*-α-tocopherol (αT) and more so with α-tocopheryl phosphate (αTP) leads to activation of PI3Kγ, which produces phosphorylated phosphatidylinositol and leads to translocation of PKB to the plasma membrane and activation via phosphorylation by phosphoinositide-dependent kinase (PDK)1/2 (for details, see **Figure 7**).

and renders them susceptible to degradation (29), which suggests that activation of PHLPP1 by tocopherols may play a role in PKCα and PKCδ regulation as well (see **Figure 5**). Activation of PHLPP1 by tocopherols may modulate other physiological processes in which it is involved, such as cell survival/apoptosis, circadian rhythm, memory formation, and T cell development (254).

As described below, several additional enzymes involved in generating lipid messengers at the plasma membrane are modulated by vitamin E, but in most cases it is unclear whether vitamin E acts only as a competitive inhibitor with their substrate lipids or also interferes with the enzyme activation/membrane translocation process. For PLA2, inhibition occurs by direct binding of αT to the enzyme, as suggested by cocrystallization of αT and PLA2 (33). The activity toward lamellar fluid membranes is best inhibited by αT, whereas β-, γ-, and δ-tocopherol inhibit to a lower extent (79). Inhibition of platelet PLA2 by vitamin E reduces arachidonate release from the membrane phospholipids and its subsequent metabolism to biologically active eicosanoids (55, 187). By an unknown mechanism, αT also enhances the release of prostacyclin from human endothelial cells via stimulation of PLA2 (237). COX2 enzyme activity is inhibited by the involvement of γT in competitive binding to the enzyme (101–103). Vitamin E renders COX-2 more sensitive to inhibition by aspirin by as yet unknown mechanisms (1). COX2-catalyzed synthesis of PGE2 plays an important role in inflammation and its associated diseases, such as cancer and vascular

heart disease. 5-LOX enzyme activity is inhibited by αT, but not βT, leading to a reduction in the release of the proinflammatory cytokine IL-1β (49). 5- and 15-LOX are both inhibited by direct binding of αT, and it is not known whether this interferes with translocation to the plasma membrane (80, 191); at least for 5-LOX, membrane translocation may also be affected via the inhibition of tyrosine kinases (126). Glutamate-induced cell death in primary cortical neurons is blocked by nanomolar concentrations of αTT, which directly binds to 12-LOX, blocking the access of the natural substrate arachidonic acid (114).

Modulation of Signal Transduction by Vitamin E by Changing Plasma Membrane Properties

Alternative mechanisms by which the translocation of signal transduction enzymes to the plasma membrane can be modulated by vitamin E have been described, such as by influencing vesicle transport, recycling signaling receptors, influencing the intracellular Ca^{2+} concentration, or by changing the plasma membrane stability, structure, fluidity, or curvature (23). In ethanol-treated cerebral VSMC, vitamin E reduced the cellular Ca^{2+} concentration, what may affect the translocation of several Ca^{2+}-dependent enzymes to the plasma membrane (35, 92, 266). An effect of vitamin E on Ca^{2+} levels may also influence the accessibility of messenger and structural lipids in the membrane to signaling enzymes, e.g., via proteins like the myristoylated alanine-rich C-kinase substrate (MARCKS) and growth associated protein 43 (GAP43), which determine the free PI(45)P concentration by sequestering it by means of electrostatic interactions and by releasing it upon Ca^{2+}-dependent signaling (139).

αT is not randomly distributed throughout the phospholipid bilayer of biological membranes, and as compared with other isomers, it shows a propensity to associate with lipid rafts (10, 125). Vitamin E may modulate the local concentration of messenger lipids, their spacial clustering in membrane microdomains (e.g., in lipid rafts), or their transbilayer asymmetry, and thus change the ability of several enzymes to interact with these membrane structures and to become activated. Apoptotic effects of cholesterol oxides, in particular 7-ketocholesterol, are prevented by vitamin E (αT but not by γT) by changing the presence of 7-ketocholesterol in sphingolipid/cholesterol-enriched lipid raft domains (**Figure 5*c***) (125, 204). Interestingly, 7-ketocholesterol-induced apoptosis was mediated by dephosphorylation/inactivation of PKB, and in this case αT but not γT prevented the PKB-dephosphorylation. Vitamin E also influences the transbilayer asymmetry of phospholipids in the plasma membrane, e.g., by inhibiting the externalization of phosphatidylserine in erythrocytes with consequent reduced procoagulant properties (115). Similar to that, vitamin E inhibits hemolysis induced by hemin by increasing the stability of erythrocytes membranes in a nonantioxidant manner (251).

Modulation of Signal Transduction by Vitamin E by Changing the Surface Exposition of Membrane Receptors

Vitamin E can also change the expression, cell surface exposition, and activity of membrane receptors. The CD36 scavenger receptor mediates signal transduction and gene expression of several ligands (e.g., long-chain fatty acids, myristic acid) (62) and also acts together with Toll-like receptor 2/6 as a signaling receptor (e.g., for oxLDL, β-amyloid, thrombospondin, hexarelin, collagen, *Staphylococcus aureus*, and *Plasmodium falciparum*) (60, 224, 239). CD36, in its function as a fatty acid transporter (FAT), also mediates the uptake of long-chain fatty acids, myristic acid, and oxidized lipids that are involved in signaling and gene expression via activation of PPARγ (60, 97, 224, 239). The ability of vitamin E to reduce CD36 mRNA and protein expression (**Figure 4**)

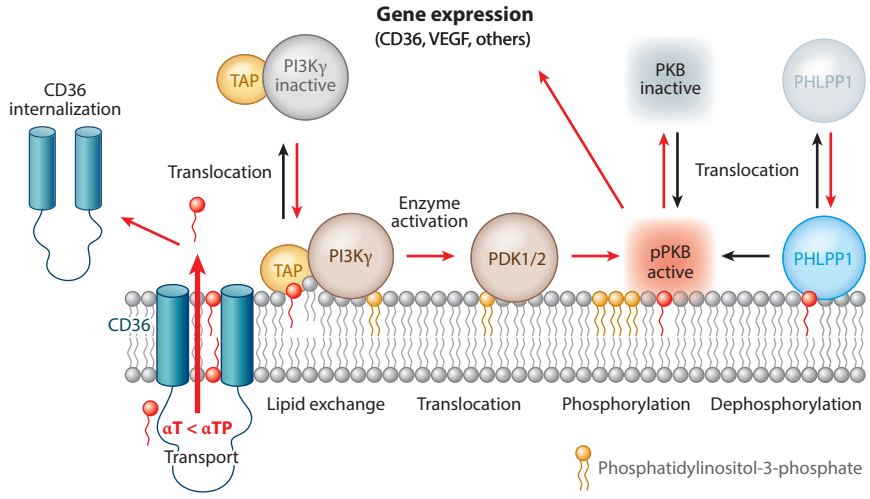

Figure 7

Model of the modulation of the phosphatidylinositol-3-kinase/protein kinase B (PI3Kγ/PKB) signaling pathway by vitamin E via human tocopherol-associated protein (hTAP)-mediated lipid exchange and enzyme translocation to the plasma membrane. *RRR*-α-tocopherol (αT) and α-tocopheryl phosphate (αTP) are taken up via internalization and transport by cluster of differentiation 36 (CD36) or scavenger receptor class B type I (SR-BI). Internalized αT, and more so αTP, can activate PI3Kγ via hTAP-mediated lipid exchange, leading to phosphorylation of phosphatidylinositols, translocation of PKB to the plasma membrane, and activation of PKB through phosphorylation by PDK1/2, ultimately leading to the modulation of gene expression [e.g., of CD36, vascular endothelial growth factor (VEGF)]. High amounts of αT and αTP in the plasma membrane later lead to translocation and activation of pleckstrin homology domain leucine-rich repeat protein phosphatase, isoform 1 (PHLPP1) to the plasma membrane, dephosphorylation of prosurvival protein kinase B (pPKB), and inactivation of pPKB (see also **Figure 6**). Note that internalization of CD36 by αT and αTP also reduces the ability of CD36 to recognize its ligands, such as oxidized low-density lipoprotein (oxLDL), at the cellular surface, with consequent lower oxLDL-triggered activation of PKB and gene expression (see also **Figure 4**). Red arrows indicate stimulation by vitamin E.

(22, 155) as well as to reduce CD36 cell surface exposition (278, 279) suggests that vitamin E removes active CD36 from the plasma membrane, with consequent reduced exposure, transport, and signaling of these ligands (**Figure 7**). In line with that, lipid uptake and signaling to PKB are increased by the CD36 ligand oxLDL, and vitamin E can reduce the activation of PKB (153), which suggests that changes in CD36 expression and localization can contribute to the regulatory effects of αT on lipid transport, signal transduction, and gene expression. Oxidized lipids taken up via CD36 may also activate CD36 and VEGF expression via activation of PPARγ (94, 193). Similarly, the inhibitory effects of thrombospondin on angiogenesis—effects that are mediated by CD36—could be influenced by vitamin E by changing the presence and activity of CD36 at the plasma membrane (43). Since CD36 has been recently implicated in coordinating intracellular cholesterol crystal formation leading to NLPR3 inflammasome activation and induction of IL-1β expression (215a), a reduction of CD36 at the cellular surface by αTP may also explain the anti-inflammatory effects of αTP in hypercholesterolemic rabbits (128).

Moreover, endocytosis and endosomal sorting of several receptors, including receptor tyrosine kinases, are regulated by the ubiquitin-proteasome system (84, 135). Vitamin E modulates the activity of the ubiquitin-proteasome system, and therefore it may affect endocytosis and the activity and expression of signaling receptors (152, 156, 223). Accordingly, PP2A translocates to the plasma membrane upon inhibition of the proteasome and ubiquitination, which suggests that

activation of PP2A by vitamin E may be a consequence of its regulatory effects on the ubiquitin-proteasome system (255). In T cell lymphoma Jurkat cells, increased surface expression of Fas and FasL occurred upon treatment with γTT but not αTT by an unknown mechanism, and γTT inhibited cell proliferation and induced apoptosis, increased mitochondrial ROS production, elevated activation of c-Jun N-terminal kinase (JNK), and elevated suppression of ERK and p38 MAPK; in contrast, normal human peripheral blood mononuclear cells were not affected (258).

Modulation of Transport and Conversion of Lipids to Signaling Mediators by Vitamin E

Vitamin E can compete with messenger lipids for binding to several LTPs (**Table 3**) and can modulate their transport, local concentration, spatial clustering, and accessibility to enzymes and membrane microdomains (e.g., in lipid rafts) or their asymmetric presence in the plasma membrane bilayer (reviewed in 10, 253). Further molecular mechanisms by which vitamin E can affect signal transduction are based on a lipid exchange model described for other lipids and recently visualized with the crystal structures of the closest SEC14p homolog—the *Saccharomyces cerevisiae* Sfh1a (209)—as well as with human αTTP (11, 71, 93, 119). In this model, the SEC14-domain-mediated lipid transfer and exchange of phosphatidylcholine against phosphatidylinositol results in activation of PI4K and secretion and trafficking of lipid raft proteins (44, 209). In mice and humans, the related SEC14L2/3/4 proteins [human tocopherol-associated protein 1/2/3 (hTAP1/2/3)] interact directly with PI3K and modulate its activity in vitro and in vivo (113, 169). The in vitro activities of PI3Kγ and PI3Kα are inhibited by hTAP1, most likely by forming an inactive hTAP1/PI3K heterodimer (113, 169, 275, 279). The binding of PI to hTAP1 is reversed by αT, and more so by α-tocopheryl phosphate (αTP), and leads to stimulation of PI3Kγ activity, which suggests that αT and αTP promote dissociation of the inactive complex and/or the release of sequestered PI from hTAP1 for subsequent presentation to the kinase by means of a heterotypic lipid exchange mechanism (275, 279). Thus, modulation of PI3K by hTAPs may affect gene expression in a vitamin E–dependent manner, e.g., through an impact on the PI3K/PKB signal-transduction pathway by transporting these ligands to specific enzymes such as cytosolic PI3Kγ or to membrane sites accessible for regulating PI3K/PKB/PHLPP1 (**Figure 7**) (88, 89, 153, 278).

In addition to αT and PI, the hTAPs bind in vitro to several other ligands, such as squalene, phosphatidylinositol-3,4,5-phosphate, phosphatidylcholine, and phosphatidylserine, and the exchange with vitamin E could affect their transport and signaling pathways as well (reviewed in 205). Whether similar signaling events also contribute to the regulation of the biosynthesis of cholesterol by TAP1/SEC14L2 by regulating squalene epoxidation via stimulation of squalene transport and presentation to squalene epoxidase remains to be investigated (149, 219). Accordingly, αT may stimulate in vitro squalene epoxidase activity, possibly by forcing the release of squalene and/or facilitating its presentation to the enzymes (113, 149, 169, 183, 219).

Phosphorylated Vitamin E and Signal Transduction

The naturally occurring phosphorylated analogue of αT, αTP, which is present in foods and tissues in amounts of nmol/g of extracted material (72, 162, 165, 278), may be more potent in modulating some of the above events involving lipid transport and enzyme translocation to the plasma membrane because it is negatively charged and more similar to phosphorylated messenger lipids; therefore, αTP is better able to modulate specific and nonspecific protein-membrane interactions (reviewed in 272). The biological function of αTP is not clear to date; it may act as a cofactor for enzymes, as a ligand of a receptor or a transcription factor, or as a "second

messenger" in the membrane, capable of exerting regulatory effects (reviewed in 165, 280). Only small amounts of αTP are formed from αT by a putative αT kinase in cultured cells, plasma, and animal tissues, and an αTP phosphatase or esterase has been postulated for the dephosphorylation reaction (72, 108, 162, 165, 171). Interestingly, hTAP1 stimulated αT kinase activity in primary human coronary artery cells, which indicates possible hTAP-mediated αT exchange with an as yet unknown lipid (275, 279).

CEHC: carboxyethyl hydroxychroman

In THP-1 monocytes and cultured cortical neurons, αTP stimulated the PI3K and/or PKB signaling pathway, a pathway that is downregulated by αT, which suggests that αTP acts as an active lipid mediator that influences signal transduction and gene expression (111, 153, 176). Activation of PI3Kγ/PKB by αTP increases the expression of a number of genes, such as *VEGF* (275, 279), insulin-induced gene 1 (*INSIG1*), sestrin 2 (*SESN2*), and tribbles homolog 3 (*TRB3*) (129, 279), and the molecular mechanisms involved are currently being elucidated. Induction of PI3Kγ activity by αTP involves hTAP1/SEC14L2-mediated lipid exchange (279). The induction of VEGF expression by αTP suggests that it may be involved in angiogenesis and vasculogenesis (reviewed in 280). In vivo, αTP reduces atherosclerotic lesions as result of lower expression of cytokines and CD36 scavenger receptor in hypercholesterolemic rabbits (128, 164).

Vitamin E Metabolites and Signal Transduction

Several natural vitamin E metabolites with cellular effects have been identified by in vitro experiments (**Figure 1*b***), but because they occur at rather low concentrations (nM) in plasma and tissue, it is still unclear whether in vivo they play also a role in signal transduction and gene expression (reviewed in 100, 235, 250). The long-chain metabolites, such as alpha-13′-hydroxychromanol and alpha-13′-carboxychromanol, reduce oxLDL-induced lipid accumulation in human macrophages in vitro, probably owing to a reduction in phagocytosis of oxLDL (249). These effects appeared to be independent of oxLDL uptake by CD36 because the long-chain metabolites stimulated expression of CD36. The main metabolites of vitamin E, the carboxyethyl hydroxychromans (CEHCs), can act as bioactive compounds that bind to and modulate the activity of nuclear receptors, transcription factors, membrane channels, and enzymes (reviewed in 27, 28). The accumulation of CEHCs can mediate anti-inflammatory and antioxidative effects or have other regulatory properties (78, 82, 86). Whereas the metabolite of γT (γ-CEHC) inhibits the 70 pS potassium channel and therefore has natriuretic activity, the analogous αT metabolite (α-CEHC) shows no inhibition, which implies that the mechanisms are nonantioxidant (157). In activated macrophages and epithelial cells, γ-CEHC inhibits COX-2 and PGE2 synthesis, events that could change signal transduction and gene expression (102, 103). Similarly, in carrageenan-induced inflammation in male Wistar rats, γ-CEHC reduced PGE2 synthesis at the site of inflammation and inhibited LTB(4) formation, a potent chemotactic agent synthesized by 5-LOX of neutrophils (101). In prostate cancer cells, γ-CEHC exerts inhibitory effects on cyclin D1 expression, with parallel retardation of cell proliferation (68). Interestingly, the inhibition of cyclin D1 expression by γ-CEHC is competed for by α-CEHC, which again suggests a nonantioxidant mechanism that is dependent on the different structure of these metabolites. In contrast, both α-CEHC and γ-CEHC inhibited microglial PGE2 and nitrite production and reduced iNOS mRNA and protein expression; thus, both are effective in reducing these cytokine-stimulated inflammatory processes (78). In phorbol-ester-stimulated neutrophils, superoxide anion production was inhibited not only by α-tocopherol but also by γ- and δ-tocopherol as well as by α-, γ-, and δ-CEHC at physiological concentrations (243). This effect was mediated by the inhibition of the membrane translocation and activation of PKC, which is the key event in phorbol-ester-induced signaling (**Figure 5**). Importantly, CEHCs were stronger inhibitors of PKC as compared with intact

tocopherol precursors, and the gamma forms of both tocopherol and CEHC showed the highest inhibitory activities, which is suggestive of nonantioxidant mechanisms. Accordingly, tocopherols, but not CEHCs, directly inhibited the fully activated NADPH oxidase, but none of the test compounds was able to directly scavenge superoxide anions when measured in a cell-free system.

Gene Polymorphisms as Determinants of Regulatory Effects of Vitamin E on Signal Transduction and Gene Expression

Supplementation with vitamin E for the prevention of diseases such atherosclerosis, cancer, neurodegeneration, and nonalcoholic steatohepatitis has been the focus of many studies, and depending on the study, both positive and negative effects have been reported (reviewed in 38, 41, 154, 194, 270). An increased all-cause mortality with high doses of vitamin E supplementation was detected in some meta-analyses of clinical studies (16, 143), although this was not confirmed in later studies (3, 70). The often mixed outcome of vitamin E supplementation has been explained by several factors, such as the dose and duration of supplementation, the high levels of vitamin E already present at baseline, the various presence of other phytochemicals and micronutrients in the diet, or specific environmental and pathophysiological circumstances that deplete vitamin E to various degrees, such as inflammation, infection, smoking, or ultraviolet irradiation (201, 203).

Polymorphisms in genes involved in the uptake and distribution of vitamin E, such as *αTTP*, *bTAPs*, *CD36*, *SR-BI*, and ATP-binding cassette transporter 1 (*ABCA1*) (**Figure 2** and **Table 3**), have been suggested to play a role for the different responsiveness of different individuals to vitamin E supplementation (18, 26, 54, 99, 148, 274). As described above, these genes also play a role in vitamin E–mediated signal transduction and gene expression, and therefore polymorphisms in these genes may alter the response to vitamin E in cells and tissues (112, 124, 273, 274, 277). Polymorphisms in *αTTP* and *bTAP1* have been associated with elevated prostate cancer risk (259) resulting either from altered plasma vitamin E concentration, from regulatory effects of these proteins on signaling and gene expression in the prostate (151, 168, 277), or possibly from modulating the biosynthesis of cholesterol/steroid biosynthesis by regulating squalene epoxidase or HMG-CoA reductase (106, 112, 169, 268, 273). Similarly, polymorphisms detected in cytokine genes that are regulated by vitamin E may render patients more (or less) responsive to the anti-inflammatory effects of vitamin E supplementation (14). Activity differences between natural vitamin E analogues observed in animal and human studies indicate that their regulatory effects on signaling and gene expression observed in cells also play a role in animals and humans (2, 40, 262, 276).

Polymorphisms in the *CD36/FAT* scavenger receptor gene can influence the levels of vitamin E in plasma and possibly also in tissues (124, 197). These CD36 polymorphisms as well as gene variants resulting from alternative splicing may affect the ability of CD36 to modulate signal transduction and gene expression and thus affect the responsiveness to vitamin E (5, 34, 60, 131, 224, 239, 281), which may ultimately translate into an altered risk for diseases such as atherosclerosis (130) and metabolic syndrome (173). Similar to CD36, polymorphisms in SR-BI (19), ABCA1, and ATP-binding cassette transporter G1 (ABCG1) may influence vitamin E levels in plasma and tissues (66, 75, 158, 170, 179, 180).

Polymorphisms in a number of other genes such as haptoglobin have been implicated in the bioavailability and bioactivity of vitamin E (uptake, distribution, oxidation, metabolism, and molecular action) (reviewed in 270, 274). Diabetic patients with the haptoglobin 2-2 allele are at risk for cardiovascular disease since they have reduced plasma vitamin E and C levels as a result of increased oxidation. In addition to the scavenging of free radicals, other mechanisms may play a role in the higher risk for atherosclerosis in individuals with the haptoglobin 2-2 allele, as indicated by the finding that the combination of αT with L-ascorbic acid did not have any further protective

effect and even mitigated the effect of αT on HDL oxidation (9). Thus, the haptoglobin polymorphisms may be important determinants for the preventive effects of vitamin E by influencing the vitamin E concentration in plasma and tissues, with consequent changes in signal transduction and gene expression (17, 144). Likewise, the apoE gene influences not only the plasma lipid profile, inflammatory cytokine expression, and the level of free radicals but also the plasma and tissue distribution of vitamin E, and polymorphisms in this gene have been associated with a lower retention of vitamin E in tissues but higher levels in the circulation (91).

Taken together, gene polymorphisms in vitamin E regulatory genes may explain the difficulty in providing clear evidence for beneficial effects in vitamin E supplementation studies (18). A strategy to increase the benefits of vitamin E may be to personalize vitamin E supplementation by selecting certain subpopulations of individuals with a specific genotype, such as diabetic patients with the haptoglobin 2-2 allotype (17, 144).

Synthetic Vitamin E Analogues and Signal Transduction

As outlined above, the eight natural vitamin E analogues and their metabolites differently influence signal transduction and gene expression, and the mechanism involved depends very much on the associated enzyme, suggesting that binding sites and molecular interactions are specific to the analogue. Chemical modifications within these natural vitamin E analogues may enhance or inhibit these regulatory interactions of vitamin E or may generate novel molecules targeting additional enzymes. Many vitamin E derivatives have been synthesized, and in most cases they have apoptotic effects at higher concentrations (269). Because synthetic vitamin E derivatives, when modified at the hydroxyl group, are not able to act as chemical free radical scavengers, their regulatory effects may give important insights into the structural components required for the signaling effects of vitamin E.

The best studied synthetic vitamin E derivative, α-tocopheryl succinate (αTS), directly binds to the antiapoptotic Bcl-xL/Bcl-2 proteins and disrupts the binding of the Bak BH3 peptide, leading to increased caspase-dependent apoptosis and reduction of prostate cancer cell proliferation (217). Apoptosis by αTS is also induced by targeting the ubiquinone-binding site of mitochondrial complex II (53). αTS and αTP recently were shown to bind to cytochrome c and activate its peroxidase activity by causing structural rearrangements (261). In NIH3T3 cells, αTS inhibits proliferation and induces apoptosis by decreasing oncogenic Ras protein levels and suppressing the levels of prosurvival protein kinase B (pPKB) and pErk1/2 as well as the expression of the transcriptional targets of oncogenic Ras such as c-Myc, cyclin D1, and E2F1 (52). αTS and its stable ether analogue, α-tocopheryl oxybutyric acid, inhibit cell proliferation and induce apoptosis in breast and prostate cancer cells by targeting the PKB and JNK signaling pathway, and βTS interfered at the membrane with PKB phosphorylation (4, 260). In contrast, PKB is activated by αTS in endotoxin-induced THP-1 monocytes as result of inhibition of translocation of the Src homology (SH)-related (SRC) complex homology 2 domain-containing inositol-5-phosphate (SHIP) phosphatase to lipid rafts but leads to inhibition of MAPK and tumor necrosis factor alpha gene expression (45). A similar regulatory effect may occur with the synthetic phosphatidylinositol ether lipid analogue, which strongly affects protein-membrane interaction and thus inhibits PKB membrane translocation, phosphorylation, and kinase activity (73). PKB phosphorylation at the plasma membrane is also inhibited by synthetic short-chain vitamin E derivatives via activation of the phosphatase PHLPP1, as also occurs less efficiently with αT (88).

The ester linkage in αTS can be cleaved by cellular esterases, and a nonhydrolysable ether-linked αT derivative has been synthesized, the *RRR*-α-tocopherol ether-linked acetic acid analogue (α-TEA), or 2,5,7,8-tetramethyl-2R-(4R,8R,12-trimethyltridecyl)chroman-6-yloxy

acetic acid (6). α-TEA has increased apoptotic activity compared to αTS in several cancer cell lines and decreases tumor burden and metastasis in mouse cancer models (116). α-TEA modulates cellular signaling by activating JNK and its substrate c-Jun, and mediates a conformational change in Bax, triggering cleavage of Bid and caspases-8, -9, and -3 (265). α-TEA also decreases phosphorylation of PKB and ERK1/2 and lowers the cellular FLICE-like inhibitory protein and survivin protein levels (265). In breast cancer cell lines, α-TEA suppressed constitutively active basal levels of pPKB, pERK, pmTOR, and their downstream targets as well as induced apoptosis involving insulin receptor substrate-1/PI3K and JNK (234). In tamoxifen-resistant breast cancer cells, α-TEA disrupted cholesterol-rich microdomains and reduced prosurvival mediators pPKB, pmTOR, and pERK1/2, phosphorylated form of estrogen receptor-α (Ser-167 and Ser-118) and induced mitochondria-dependent apoptosis via an endoplasmic reticulum stress–triggered prodeath pJNK/CHOP/DR5 amplification loop (233). In prostate cancer cells, α-TEA treatment increased Fas and Fas ligand mRNA and protein levels as well as the levels of cell surface membrane Fas and induced apoptosis via increasing a prolonged, elevated level of activated (phosphorylated) JNK and its substrate c-Jun (98).

The vitamin E analogue EPC-K1 (L-ascorbic acid 2-[3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethytridecyl)-2H-1-benzopyran-6-yl-hydrogen-phosphate] potassium salt) is a composite molecule between αTP and L-ascorbate (vitamin C) linked by a phosphodiester bond. A number of cellular events are affected by EPC-K1, most likely as result of a change in signal transduction. EPC-K1 has anti-inflammatory properties, it protects against ischemia/reperfusion injury and lipid peroxidation (reviewed in 154, 280), and it reduces nitric oxide-induced neurotoxicity by preventing apoptosis and mitochondrial dysfunction in cerebellar granule cells (256). Furthermore, EPC-K1 modulates NFκB and the glucocorticoid receptor via redox regulation (87, 178), inhibits phospholipase A2 activity, and stimulates endothelial nitric oxide production that leads to endothelium-dependent relaxation (229). EPC-K1 also prevents 6-hydroxydopamine-induced dopamine depletion in mouse striatum by increasing the activity of superoxide dismutase and catalase (107).

CONCLUSIONS AND FUTURE DIRECTIONS

Although vitamin E was discovered nearly a century ago as a molecule essential for reproduction in rodents, its physiological role for human health and its molecular and cellular actions are still under investigation. Over the past two decades, many studies have clearly shown that the function of vitamin E goes beyond that of a lipid-soluble antioxidant molecule and that the benefits of vitamin E do rely on additional regulatory effects. Cell culture and animal experiments have revealed that vitamin E modulates signal transduction and gene expression, and the molecular mechanisms involved are beginning to be resolved. Moreover, these studies have revealed that each natural vitamin E analogue has different biological effects that cannot be explained only by their antioxidant action and their differences in bioavailability. These effects are more likely the result of analogue-specific transport and interaction with enzymes, receptors, transcription factors, and membrane microdomains. It remains to be elucidated to what degree these regulatory effects represent physiological events that also occur in vivo, whether and to what degree the regulatory effects are responsible for the essentiality of vitamin E, and which of the effects correspond to pharmacological effects of vitamin E analogues at high concentrations. Recent vitamin E supplementation studies showing differences between natural vitamin E analogues for human health indicate that the regulatory effects that are mostly observed in cells and animals may also play a role in humans (2, 40). The elucidation of the molecular mechanisms and

cellular events modulated by each of the natural vitamin E analogues will help in defining novel biomarkers for vitamin E requirements and in designing novel derivatives with improved activity.

SUMMARY POINTS

1. The eight vitamin E analogues differently modulate signal transduction and gene expression involving antioxidant and nonantioxidant molecular mechanisms.
2. Vitamin E can modulate the activity of signal transduction enzymes by directly binding to them or by influencing their redox regulation.
3. In membranes, vitamin E can modulate signal transduction by preventing the oxidation of lipids or by modulating the structure and composition of membrane lipid domains (lipid rafts).
4. Vitamin E can compete with lipid mediators for common binding sites within lipid transport proteins and can modulate their traffic, enzymatic conversion, and signaling function.
5. The modulation of enzyme-membrane interaction is a common theme by which vitamin E affects signal transduction and gene expression.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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