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Isoforms of Vitamin E Differentially Regulate PKC α and Inflammation: A Review

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Abstract

Vitamin E regulation of disease has been extensively studied but most studies focus on the α -tocopherol isoform of vitamin E. These reports indicate contradictory outcomes for anti-inflammatory functions of the α -tocopherol isoform of vitamin E with regards to animal and clinical studies. These seemingly disparate results are consistent with our recent studies demonstrating that purified natural forms of vitamin E have opposing regulatory functions during inflammation. In this review, we discuss that α -tocopherol inhibits whereas γ -tocopherol elevates allergic inflammation, airway hyperresponsiveness, leukocyte transendothelial migration, and endothelial cell adhesion molecule signaling through protein kinase C α . Moreover, we have demonstrated that α -tocopherol is an antagonist and γ -tocopherol is an agonist of PKC α through direct binding to a regulatory domain of PKC α . In summary, we have determined mechanisms for opposing regulatory functions of α -tocopherol and γ -tocopherol on inflammation. Information from our studies will have significant impact on the design of clinical studies and on vitamin E consumption.

Keywords

Tocopherol; Inflammation; Protein kinase C α ; Endothelial

Introduction

During inflammation, several mediators induce the expression of adhesion molecules on the endothelium. Mediators that induce expression of adhesion molecules include cytokines produced in the tissue, high levels of reactive oxygen species, high vascular fluid shear stress, or microbial stimulation of endothelial toll-like receptors [1-8]. The endothelial adhesion molecules mediate binding of leukocytes and then the bound leukocytes are recruited into tissues by chemokines/chemoattractants. The Specificity of leukocyte homing to tissues is regulated by the combination of chemokines in the microenvironment, adhesion molecules on the endothelium and leukocyte receptors for these chemokines and adhesion molecules [9]. Furthermore, the combination of vascular adhesion molecules expressed by

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an endothelial cell is dependent on the stimulant(s) for endothelial activation [10]. Thus, the microenvironment stimuli regulate the Specificity of leukocyte recruitment. The binding of leukocytes to the endothelium and the Specificity of these interactions have been reviewed [11-20].

Leukocyte binding to endothelial cell adhesion molecules initiate signaling cascades within endothelial cells that induce the opening of narrow vascular passageways through which the leukocytes migrate [19,21,22]. Leukocyte movement through these passageways is stimulated by chemokines that are produced by the endothelium and the tissue. If adhesion molecule-stimulated signal transduction is inhibited, leukocytes bind to the endothelium but do not complete transendothelial migration [23-25]. Such leukocytes often detach from the endothelium and start circulating in the blood as demonstrated by intravital microscopy. Thus, the endothelial cell adhesion molecules and their downstream signaling molecules are important targets for inhibiting leukocyte recruitment during inflammation. Two of these adhesion molecules vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) activate cell signaling through low levels of reactive oxygen species (ROS) [21,26,27]. Vitamin E isoforms have been found to play an important regulatory role in leukocyte recruitment [28].

Vitamin E Isoforms

Vitamin E is a lipid-soluble vitamin that consists of multiple natural and synthetic forms. The natural forms of vitamin E include α -tocopherol, β -tocopherol, γ -tocopherol, and δ -tocopherol as well as the tocotrienol forms of each of these [29,30] (Figure 1). The most abundant isoforms are α -tocopherol and γ -tocopherol. Plants synthesize the lipids tocopherols and tocotrienols from tyrosine and chlorophyll [31,32]. Then, these tocols are consumed in the diet from plant lipids. Mammals do not interconvert the tocopherol isoforms. Tocols are loaded in intestinal-formed chylomicrons. These chylomicrons are transported through the lymph to the thoracic duct to the blood and then to the liver, where the tocols are transferred to lipid particles. In the liver, α -tocopherol transfer protein preferentially transfers α -tocopherol to lipid particles, resulting in 10 fold higher α -tocopherol in tissues than γ -tocopherol [33]. At equal molar concentrations *in vitro*, it is reported that the α -tocopherol and γ -tocopherol isoforms and the tocotrienol forms have relatively similar capacity to scavenge reactive oxygen species (ROS) during lipid oxidation [29,34,35]. Thus, *in vivo*, there is likely more ROS scavenging by α -tocopherol than γ -tocopherol because it is at a 10 fold higher concentration in the tissues. In addition to scavenging ROS, γ -tocopherol, in contrast to α -tocopherol, also reacts with nitrogen species such as peroxynitrite forming 5-nitro- γ -tocopherol [36-38]. Reactive nitrogen species are induced by endotoxin or ozone [39,40]. Therefore, γ -tocopherol scavenging of reactive nitrogen species may be consistent with reports that supplementation with a mixture of tocopherols enriched for γ -tocopherol blocks acute endotoxin-stimulated or ozone-stimulated neutrophil inflammation in the lung [41-43]. Oxidized tocopherols are recycled by reduction by vitamin C [44-46]. Without reduction of vitamin E by vitamin C, vitamin E can act as ROS donor [47]. In mice, vitamin C is endogenously synthesized whereas humans must consume vitamin C [48]. Thus, some clinical studies have supplemented patients with both tocopherols and vitamin C [49-52]. *In vivo*, tocopherols are metabolized to carboxyethyl-hydroxychromans (CEHC) and excreted [30,53]. Importantly, besides the antioxidant capacity of the tocopherols, it has been reported that tocopherols also have non-antioxidant functions [28,29,54].

Vitamin E Isoforms Regulate Adhesion Molecule Signaling Through ROS During Inflammation

Vitamin E has been used to regulate inflammatory diseases that involve VCAM-1- and ICAM-1-mediated leukocyte recruitment. VCAM-1 and ICAM-1 signal transduction occurs through ROS and protein kinase C, both of which are regulated by vitamin E [28,55]. However, there are contradictory outcomes of vitamin E administration in patients with asthma and atherosclerosis as well as animal models of inflammation. These clinical and experimental studies have primarily focused on analysis of one form of vitamin E, α -tocopherol, even though multiple forms of vitamin E are present in the studies. We have recently demonstrated that vitamin E isoforms have opposing functions and that these opposing functions [28] and the reported contradictory outcomes of the previous studies are consistent with the combination of vitamin E isoforms that were present in these previous studies.

VCAM-1 and ICAM-1 function in disease and infections

VCAM-1 and ICAM-1, which signal through ROS, have a regulatory role in peripheral tissue inflammation in several diseases. In these diseases, there are different leukocyte cell types that bind to VCAM-1 via the leukocyte ligand $\alpha 4 \beta 1$ -integrin and ICAM-1 via the leukocyte ligand $\alpha L \beta 2$. This is, at least in part, a result of leukocyte specific chemokine activation of integrins to their integrin high affinity conformation [11,17,18,20]. Blocking VCAM-1 by intravenous injection of anti-VCAM-1 blocking antibodies inhibits eosinophil and mast cell precursor recruitment in asthma models [56-63], severity and onset of atopic dermatitis [64], T cell infiltration into the intestine in inflammatory bowel disease [65], T cell infiltration into the brain in an experimental model of multiple sclerosis [66,67], CD8⁺ T cell, monocyte and dendritic cell infiltration into the brain during lymphocytic choriomeningitis virus (LCMV) infections [68], and monocyte recruitment, carotid neointimal formation and inflammation in cardiovascular disease [69-76]. ICAM-1 is important for leukocyte recruitment in several diseases including atherosclerosis and T cell recruitment in an experimental model of multiple sclerosis [77-80]. In summary, VCAM-1 and ICAM-1 function in allergic and infection-induced inflammation.

VCAM-1 and ICAM-1 signaling through ROS

VCAM-1 activates intracellular signals in endothelial cells (Figure 2A). These signals are transient and occur within minutes, consistent with the transient, rapid nature of leukocyte transendothelial migration. Activation of VCAM-1 stimulates calcium channels, intracellular calcium release, and the small molecular weight G protein Rac1 for the activation of the NADPH oxidase NOX2 [21,81]. VCAM-1 does not activate other enzymes that generate reactive oxygen species [21]. The activated NOX2 generates superoxide that then dismutates to hydrogen peroxide (H_2O_2), generating approximately 1 μM H_2O_2 during VCAM-1 signaling [81,82]. This concentration is relatively low as compared to the 50-200 μM H_2O_2 produced by macrophages or neutrophils in tissues [83,84]. It is also much lower than the exogenous 100-1000 μM H_2O_2 used in studies on oxidative damage [85-89]. These differences in H_2O_2 levels are important in understanding functions of oxidation as we and others reported that 1 μM H_2O_2 and >50 μM H_2O_2 have opposing effects on signal transduction [26,27,90,91]. During VCAM-1 signaling, the 1 μM H_2O_2 oxidizes the pro-domain of matrix metalloproteinases (MMPs), causing autocatalytic cleavage of the pro-domain and activation of endothelial cell-associated MMPs within minutes [26] (Figure 2A). During VCAM-1 signaling, the H_2O_2 also diffuses through cell membranes at 100 μm /second [92]. In contrast, superoxide has a relatively low diffusion rate across membranes [92]. During VCAM-1 signal transduction, the 1 μM H_2O_2 directly oxidizes and transiently

activates intracellular protein kinase C α (PKC α) in endothelial cells [27] that then induces phosphorylation and activation of protein tyrosine phosphatase 1B (PTP1B) [93]. Interestingly, the PTP1B which has an oxidizable cysteine in its catalytic domain is not oxidized during VCAM-1 signaling in endothelial cells [93], indicating specificity of targets for oxidation by the low concentrations of reactive oxygen species generated during VCAM-1 signaling. Importantly, the signals in Figure 2A function in regulation of VCAM-1-dependent leukocyte transendothelial migration *in vitro* and *in vivo* [21,23,24,26-28,81,93,94].

ICAM-1 activates the generation of ROS and PKC α through a mechanism that is different than VCAM-1 signaling in that PKC α is not oxidized during ICAM-1 signaling in endothelial cells (Figure 2B) [28,55]. In TNF α -stimulated human microvascular endothelial cells, ICAM-1 crosslinking activates xanthine oxidase (XO) which generates ROS for the activation of ERK1/2 [55]. ERK1/2 then induces activation of PKC α [55]. ICAM-1 crosslinking does not induce oxidative activation of PKC α , although it was dependent on ICAM-1-induced XO-generated ROS in endothelial cells [55]. Thus, in contrast to VCAM-1 signaling that induces oxidative activation of PKC α , ICAM-1-induced ROS do not oxidize PKC α . In summary, ICAM-1 activates XO, PLC, and ERK1/2 which then activates PKC α . These signals are required for ICAM-1-dependent leukocyte transendothelial migration [95-97].

Vitamin E isoform specific regulation of VCAM-1 signaling and ICAM-1 signaling

We reported that, *in vitro*, natural d- α -tocopherol blocks whereas natural d- γ -tocopherol elevates VCAM-1-dependent lymphocyte transmigration at physiological concentrations [28]. Moreover, treatment with γ -tocopherol ablates the inhibition by α -tocopherol such that the lymphocyte transmigration is the same as the vehicle-treated control [28]. This occurs at physiological tocopherol concentrations; in tissues, γ -tocopherol is at 1/10 the concentration of α -tocopherol [28]. Briefly, γ -tocopherol, at 1/10 the concentration of α -tocopherol, ablates the effects of α -tocopherol. These regulatory functions of the tocopherols on lymphocyte transmigration are through a direct effect of the tocopherols on endothelial cells because pretreatment of the endothelial cells with α -tocopherol or γ -tocopherol overnight inhibits and elevates, respectively, lymphocyte transmigration *in vitro* without affecting lymphocyte-endothelial cell adhesion [28]. In contrast, pretreatment of the lymphocytes with physiological concentrations of tocopherols has no effect on VCAM-1-dependent lymphocyte transmigration [28]. The γ -tocopherol-induced elevation of transendothelial migration is VCAM-1-dependent since anti-VCAM-1 blocking antibodies inhibit lymphocyte transmigration [28]. We reported that the tocopherols at physiological levels modulate endothelial function during VCAM-1-dependent transmigration by altering VCAM-1-induced oxidative activation of endothelial cell PKC α [28]. Specifically, the VCAM-1-induced activation of PKC α is inhibited by α -tocopherol and this inhibition of PKC α by α -tocopherol is ablated by γ -tocopherol [28]. In summary, the α -tocopherol and γ -tocopherol have opposing regulatory functions on VCAM-1 signaling during leukocyte transmigration *in vitro*.

Given that α -tocopherol and γ -tocopherol have similar anti-oxidant capacity but opposing functions in VCAM-1 signaling, it suggests that tocopherols also have non-antioxidant functions. We recently reported a mechanism by which α -tocopherol and γ -tocopherol have opposing functions during regulation of PKC α [98]. Briefly, co-factor-dependent activation of recombinant PKC α is increased by γ -tocopherol and is inhibited by α -tocopherol [98]. Oxidative activation of PKC α is inhibited by α -tocopherol at a 10 fold lower concentration than γ -tocopherol [98]. In binding studies, α -tocopherol directly binds to full-length PKC α .

or the C1a regulatory domain of PKC α but does not bind the control, DAG cofactor-independent enzyme, PKC ζ [98]. α -tocopherol binding to PKC α or the PKC α -C1a domain is blocked by diacylglycerol and retinol but not by cholesterol or phosphatidylserine (PS) [98]. In summary, α -tocopherol and γ -tocopherol bind the diacylglycerol binding site on PKC α -C1a. Thus, α -tocopherol can function as an antagonist and γ -tocopherol can function as an agonist of PKC α .

α -tocopherol and γ -tocopherol have opposing functions during ICAM-1 signaling in endothelial cells *in vitro*. ICAM-1 activates XO, PLC, and ERK1/2 which then activates PKC α without oxidation of PKC α [55]. ICAM-1 activation of PKC α but not the upstream signal ERK1/2 is inhibited by α -tocopherol [55]. The α -tocopherol inhibition of PKC α is ablated by the addition of γ -tocopherol [55]. Thus, ICAM-1 activation of PKC α is inhibited by α -tocopherol and this inhibition is ablated by γ -tocopherol. These data are consistent with PKC α antagonist and agonist functions of α -tocopherol and γ -tocopherol, respectively, during ICAM-1 signaling in endothelial cells. Thus, α -tocopherol and γ -tocopherol can function as a PKC α antagonist and agonist, respectively, during VCAM-1-activated and ICAM-1-activated signals. This emphasizes that tocopherols regulate multiple signal transduction pathways that activate PKC α during leukocyte recruitment.

Vitamin E Isoform Specific Regulation of Leukocyte Recruitment *In Vivo*

We reported that, *in vivo*, supplemental doses of α -tocopherol and γ -tocopherol, that result in physiological tissue levels of these tocopherols, have opposing regulatory functions on recruitment of leukocytes to the lung during allergic inflammation [28]. In a model of allergic inflammation, animals are sensitized by intraperitoneal administration of chicken egg ovalbumin (OVA) in adjuvant and then the lung is challenged with OVA in saline [28]. During allergic inflammation in the lung, eosinophil migration is dependent on VCAM-1 whereas the other leukocytes can migrate on the adhesion molecule ICAM-1 [57,58]. However, both VCAM-1 and ICAM-1 signal through PKC α [27,99] and PKC α can be regulated by tocopherols [100,101]. In our studies *in vivo*, we focused on supplementation with tocopherols after OVA antigen sensitization to determine whether tocopherols modulate the OVA antigen-challenge phase [28]. This is important because patients are already sensitized. Supplementation with tocopherols after OVA-sensitization and during OVA-challenge raised tissue tocopherols 5-7 fold higher than mice consuming control rodent chow; this does not affect body weight or lung weight [28,102]. Consistent with the *in vitro* studies with tocopherol regulation of leukocyte migration, d- γ -tocopherol supplementation elevates leukocyte accumulation in the bronchoalveolar lavage and lung tissue in response to OVA challenge [28]. In contrast, d- α -tocopherol supplementation inhibits OVA-induced lung inflammation. Moreover, *in vivo*, this physiological level of d- γ -tocopherol, at only 10% the tissue concentration of d- α -tocopherol, ablates the anti-inflammatory benefit of the d- α -tocopherol isoform in response to OVA challenge [28]. Furthermore, the levels of tocopherols in this study do not alter numbers of blood eosinophils, indicating that eosinophils were available for recruitment. The opposing functions of purified d- α -tocopherol or d- γ -tocopherol *in vivo* is not through modulation of expression of several cytokines, chemokines, or vascular adhesion molecules which regulate inflammation because these were not altered by tocopherol supplementation [28]. This modulation of leukocyte infiltration in allergic inflammation, without alteration of adhesion molecules, cytokines or chemokines, is similar to several previous reports of *in vivo* inhibition of lung inflammation by inhibition of intracellular signals in endothelial cells [23,24,94]. In summary, α -tocopherol and γ -tocopherol supplementation *in vivo* have opposing regulatory function on allergic inflammation that is, at least in part, by regulation of VCAM-1 and ICAM-1 activation of PKC α [28]. The opposing functions of tocopherol

isoforms have important implications for the interpretation of clinical studies and animal studies of vitamin E regulation of inflammation.

In our report that supplemental doses of the α -tocopherol and γ -tocopherol isoforms of vitamin E decrease and increase, respectively, lung inflammation [28], the supplemental doses of tocopherol raised plasma tocopherol 5 fold [28]. However, in this previous study, the reported 2mg/day dose for tocopherols were suspended for only a couple of minutes before administration to the animals [28]. We then found that with only a couple of minutes of suspension time, the final tocopherol suspension is actually at a 0.2 mg/day dose because complete suspension of tocopherols in the vehicle ethoxylated castor oil requires at least 20 minutes as determined by HPLC [103]. Moreover, we recently reported that the completely suspended 0.2 mg tocopherol/day (as determined by HPLC) raises the plasma tocopherol 5 fold and is comparable to the 5 fold increase in plasma tocopherol in our previous report [28]. Furthermore, the 0.2 mg dose of tocopherols subcutaneously administered daily during OVA challenge demonstrated the anti-inflammatory and pro-inflammatory regulatory functions of α -tocopherol and γ -tocopherol, respectively [103] as in our previous report [28]. In summary, we define completely suspended 0.2 mg tocopherol treatment/day as “supplemental tocopherol treatment” since it raises plasma tocopherols 5 fold. Importantly, this information on suspension of tocopherol affects interpretations of reports on vitamin E in which tocopherols were suspended in oil vehicles because incomplete suspension can result in different dose-dependent experimental outcomes [103]. We have reported that tocopherol regulation of inflammation is partially reversible by supplemental levels of tocopherols but fully reversible by highly-elevated levels ($10 \times$ supplemental levels) of tocopherols [103]. In summary, natural d- α -tocopherol and natural d- γ -tocopherol differ in structure by only one methyl group but, at physiological tissue concentrations, these tocopherols have opposing regulatory effects on leukocyte recruitment, VCAM-1 signal transduction and ICAM-1 signal transduction [28].

New Interpretations for Reports on Tocopherol Regulation of Inflammation in Experimental Models

Our data on regulation of inflammation by supplementation of tocopherol isoforms alter interpretations of animal studies with tocopherol modulation of inflammation. Many reports with animal studies indicate that vitamin E was administered to animals but the form, source, and purity of tocopherols are often not reported. Furthermore, the tissue levels of tocopherol isoforms after administration are sometimes not determined. Another source of confounding factors in studies is the lack of consideration for tocopherol isoforms that are present in the oils in animal and human diets or in the oil vehicles used for delivery of the tocopherols. We and others have determined the levels of α -tocopherol and γ -tocopherol in dietary oils (Figure 3) [28,53,104]. In rodent studies, rodent chow contains α -tocopherol but low to no γ -tocopherol. However, in some reports for allergic inflammation, α -tocopherol is administered in oil vehicles that contain other tocopherol isoforms [105] and our interpretation of this study is that γ -tocopherol in the soy oil vehicle antagonized the function of the α -tocopherol that was administered. In another report, γ -tocopherol in tocopherol-stripped corn oil was administered daily by gavage to rats two weeks after one OVA sensitization and then the rats received two OVA challenges but there were predominantly neutrophils in the lung tissue rather than the expected predominant eosinophil infiltration after several OVA challenges [106]. It has also been reported by Okamoto et al. [107] that in mice fed α -tocopherol starting 2 weeks before sensitization with OVA, there is a reduction in the number of eosinophils in the bronchoalveolar lavage. In addition, Mabalirajan et al. [108] reported that oral administration of 0.4 mg α -tocopherol/mouse/day in ethanol after sensitization blocked OVA-induced lung inflammation [108]. Thus, conflicting reports of tocopherol regulation of OVA-induced inflammation are likely

outcomes of differences in isoforms of tocopherols present in the studies from diet, administration, and oil vehicles.

Clinical Implications for Vitamin E Regulation of Lung Inflammation

It is reported that patients with mild asthma have reduced α -tocopherol, reduced ascorbic acid, and increased glutathione in airway fluid but these patients have normal blood levels of tocopherol and ascorbic acid (vitamin C) [109]. In other studies, asthmatic patients had reduced sera α -tocopherol and ascorbic acid even during the asymptomatic periods of asthma [110]. In animal studies, the α -tocopherol and ascorbic acid are decreased in broncoalveolar lavage of guinea pigs sensitized with OVA [111]. Therefore, it has been suggested that supplementation with vitamins E and C may regulate lung inflammation.

Reports of clinical studies on vitamin E primarily focus on the α -tocopherol isoform without adjustment for the dietary contribution of γ -tocopherol to the outcomes of these studies. For interpretation of the clinical studies, it is especially important to take into consideration the dietary contribution of tocopherol isoforms because γ -tocopherol is more abundant in western diets. The average plasma concentration of α -tocopherol is the same among many countries [104]. However, the American diet is rich in γ -tocopherol found in soy oil, the major form of vegetable oil in the United States. In contrast, γ -tocopherol is low in other oils (sunflower and olive oil) commonly used in some of the European countries (Figure 3) [28,53,104]. However, as countries assume western lifestyles, diets change including increased consumption of soybean oil [112]. Consistent with this, in the United States and the Netherlands, the average plasma γ -tocopherol level is 2-6 times higher than that reported for 6 European countries including Italy (Table 1) [104]. This fold increase in plasma γ -tocopherol is similar to fold increase in plasma γ -tocopherol in the animal studies [28] in which γ -tocopherol elevated allergic inflammation and γ -tocopherol opposed the anti-inflammatory functions of α -tocopherol, even at 1/10 the concentration of α -tocopherol.

In clinical studies involving asthma patients, it is reported that α -tocopherol supplementation of asthmatic patients is beneficial in Italy and Finland but disappointingly not beneficial for asthmatic patients in studies in the United States or the Netherlands [113-117]. These clinical outcomes are consistent with an interpretation that there is little benefit of α -tocopherol for inflammation in the presence of 2-6 fold elevation in plasma γ -tocopherol in people in the United States and the Netherlands (Table 1). Therefore, differences in outcome of the clinical reports on vitamin E modulation of asthma in European countries and the United States may, in part, reflect the opposing regulatory functions of α - and γ -tocopherol forms of vitamin E consumed in diets and supplements. In Israel, it is reported that vitamin E supplementation reduces nasal symptoms of seasonal ragweed allergic rhinitis, although the form and purity of vitamin E and the contents of the placebo were not indicated [118]. Although there are many other differences regarding the environment and genetics of the people in these countries, the clinical data are consistent with the animal studies demonstrating opposing functions of the tocopherol isoforms on leukocyte recruitment [28].

It has also been suggested that changes in environmental factors including vitamin E consumption may contribute to the increased incidence of asthma. The incidence of asthma in several countries including the United States and the Netherlands has dramatically increased in the last 40 years [119-121]. It is thought that there are environmental factors contributing to this increase since it is too rapid for genetic changes. The prevalence of asthma is higher in the United States than Western Europe or Mediterranean countries [122]. The World Health Organization has reported that the prevalence of asthma from 1950 to the present has increased in many countries including countries with high rates of asthma,

intermediate rates of asthma or low rates of asthma [123]. The increases in prevalence occur as countries assume western lifestyles [123]. The dietary changes in the United States in the last 40 years with increased consumption of γ -tocopherol in vegetable oil may, in part, be a contributing factor to changes in asthma prevalence. In addition, in a Scottish cohort, it is reported that reduced maternal intake of vitamin E (likely referring to α -tocopherol) is associated with increased asthma and wheezing in children up to 5 years old [124]. Then in this same report, it was discussed that from 1967 to 2004, there was a significant increase in vegetable oil intake by Scottish [124], which we interpret as indicative of an increase in dietary γ -tocopherol since vegetable oil (soybean oil) is rich in γ -tocopherol (Figure 3). In a study in the United Kingdom, α -tocopherol administration in soybean oil to asthmatics did not have benefit for asthmatics [125]. This is consistent with the interpretation that the γ -tocopherol in soybean oil ablates the benefit of α -tocopherol supplementation. In addition, the isoforms of vitamin E in the patients are not indicated in these studies [125]. In summary, since α -tocopherol levels and other antioxidants are low in asthmatics [109-111,126-128] and since α -tocopherol can reduce inflammation, an increase in physiological levels of α -tocopherol in the presence of low γ -tocopherol may be necessary to promote optimal health in asthmatics in combination with other regimens to treat inflammation. In addition, the opposing tocopherol isoforms are also consistent with outcomes of vitamin E studies in osteoarthritis and atherosclerosis [69,72,129-138] as we previously reviewed [139,140].

Concluding Remarks

Vitamin E regulation of disease has been extensively studied in humans, animal models and cell systems. Most of these studies focus on the α -tocopherol isoform of vitamin E. These reports indicate contradictory outcomes for anti-inflammatory functions of the α -tocopherol isoform of vitamin E, especially with regards to clinical studies of asthma and atherosclerosis. These seemingly disparate clinical results are consistent with our recently reported unrecognized properties of isoforms of vitamin E. Specifically, we reported that supplementation with physiological levels of purified natural forms of the vitamin E isoforms α -tocopherol and γ -tocopherol has opposing regulatory functions during inflammation such that α -tocopherol is anti-inflammatory and γ -tocopherol is pro-inflammatory. During leukocyte recruitment, PKC α is activated by VCAM-1 and ICAM-1, albeit through different mechanisms. VCAM-1 and ICAM-1-activated PKC α is inhibited by α -tocopherol and increased by γ -tocopherol. Moreover, α -tocopherol and γ -tocopherol directly bind to PKC α and function as an antagonist and agonist, respectively. In summary, the differential regulation of inflammation by isoforms of vitamin E provide a basis towards designing drugs and diets that more effectively modulate inflammatory pathways and improve health.

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Abbreviations

BAL	Bronchoalveolar Lavage
CEHC	Carboxyethyl-hydroxychroman
ICAM-1	Intercellular Adhesion Molecule-1
LCMV	Lymphocytic Choriomeningitis Virus
MMP	Matrix Metalloproteinase
OVA	Chicken egg ovalbumin
PKCα	Protein Kinase C α
PTP1B	Protein Tyrosine Phosphatase 1B
ROS	Reactive Oxygen Species
VCAM-1	Vascular Cell Adhesion Molecule-1
TIMP	Tissue Inhibitor of Metalloproteinase
TNFα	Tumor Necrosis Factor-alpha
αTTP	Alpha-Tocopherol Transfer Protein

Figure 1. Natural R,R,R-tocopherols and R,R,R-tocotrienols

The isoforms differ in the number of methyl groups on the chromanol head group. The α -tocopherol isoform is the most abundant in tissues. The γ -tocopherol isoform is abundant in the diet but, in tissues, γ -tocopherol is 10 fold less abundant than α -tocopherol because of preferential transfer of α -tocopherol in the liver by α -TTP. The other forms of tocopherols and tocotrienols are less abundant in the diet and in tissues than α -tocopherol and γ -tocopherol.

Figure 2. VCAM-1 and ICAM-1 signal transduction

A) Crosslinking of VCAM-1 activates calcium fluxes and Rac-1 which then activates endothelial cell NOX2. NOX2 catalyzes the production of superoxide that then dismutates to H_2O_2 . VCAM-1 induces the production of only $1 \mu\text{M}$ H_2O_2 . Within minutes of its production, H_2O_2 activates endothelial cell-associated matrix metalloproteinases (MMPs) that degrade extracellular matrix and endothelial cell surface receptors in cell junctions. H_2O_2 also diffuses through membranes at $100 \mu\text{m}/\text{sec}$ to oxidize and transiently activate endothelial cell protein kinase C- α (PKC α). PKC α phosphorylates and activates protein tyrosine phosphatase 1B (PTP1B). PTP1B is not oxidized. These signals through reactive oxygen species (ROS), MMPs, PKC α , and PTP1B are required for VCAM-1-dependent leukocyte transendothelial migration. **B)** ICAM-1 activates XO, PLC, and ERK1/2 which then activates PKC α . PKC α is not oxidized during ICAM-1 signaling in endothelial cells.

Figure 3. α -tocopherol and γ -tocopherol in dietary oils

Adapted from [28]. Tocopherols were extracted from dietary oils and measured by HPLC with an electrochemical detector.

Table 1

Human Plasma Tocopherol [139,140].

Human Plasma:	γ T (μ M)	α T (μ M)
USA (4 reports)	2.5	22
	5.4	22
	5.2	27
	7	20
Netherlands	2.3	25
France	1.2	26
Italy	1.2	24
Austria	1.4	21
Ireland	1.8	26
Spain (2 reports)	1.7	27
	1.7	27
Lithuania	1.6	22
China (3 reports)	1.4	19
	2.4	19
		22
Japan (2 reports)	1.7	23
	2.0	23