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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 Cα. Moreover, we have demonstrated that α-tocopherol is an antagonist and γ-tocopherol is an agonist of PKCα through direct binding to a regulatory domain of PKCα. 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 Cα; Endothelial

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 Cα; 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

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 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

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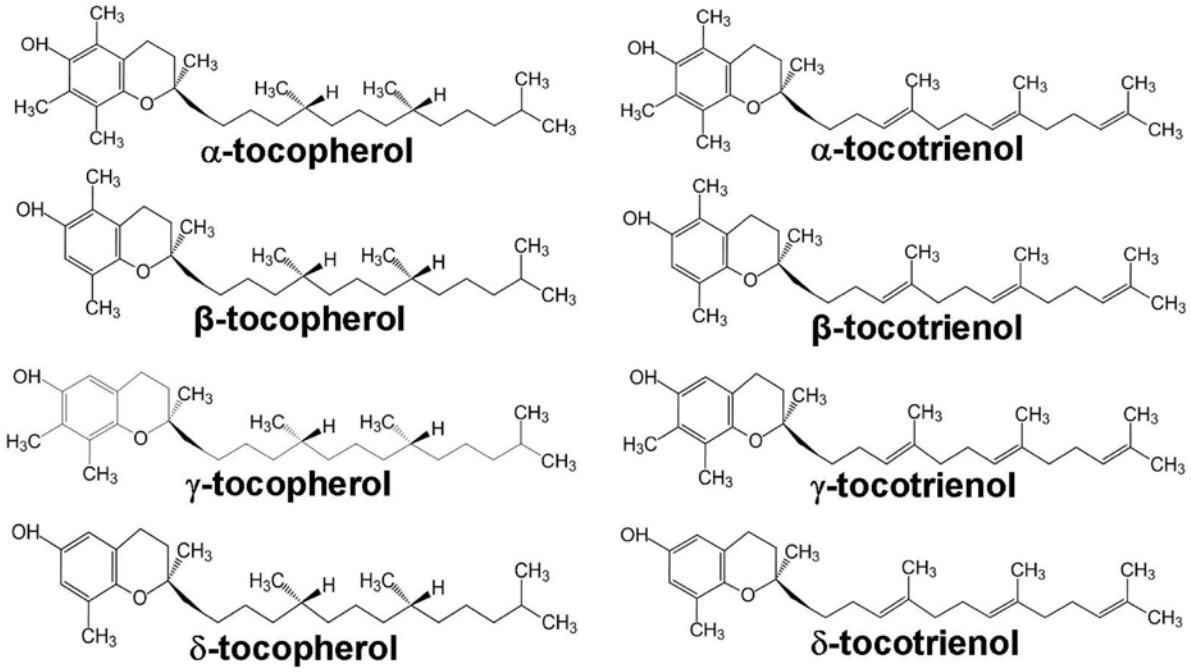

Figure 1: Natural R,R,R-tocopherols and R,R,R-tocotrienols. The isoforms differ in the number of methyl groups on the chromanol head group. The α-tocopherol isoform is the most abundant in tissues. The γ-tocopherol isoform is abundant in the diet but, in tissues, γ-tocopherol is 10 fold less abundant 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.

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 α4β1-integrin and ICAM-1 via the leukocyte ligand αLβ2. 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

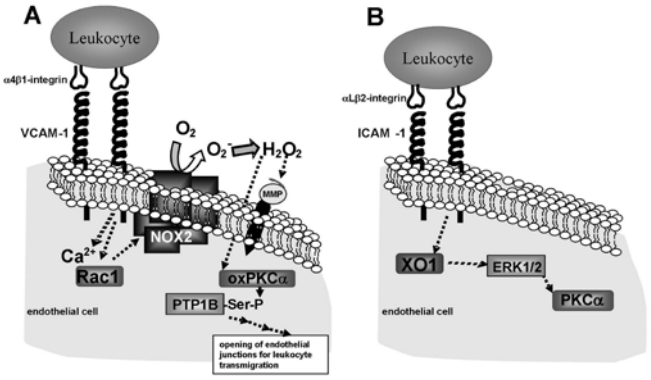

Figure 2: VCAM-1 and ICAM-1 signal transduction. A) Crosslinking of VCAM-1 activates calcium fluxes and Rac-1 which then activates endothelial cell NOX2. NOX2 catalyzes the production of superoxide that then dismutates to H_2O_2. VCAM-1 induces the production of only 1 μM H_2O_2. Within minutes of its 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 μm/sec to oxidize and transiently activate endothelial cell protein kinase C-α (PKCα). PKCα phosphorylates and activates protein tyrosine phosphatase 1B (PTP1B). PTP1B is not oxidized. These signals through reactive oxygen species (ROS), MMPs, PKCα, and PTP1B are required for VCAM-1-dependent leukocyte transendothelial migration. **B)** ICAM-1 activates XO, PLC, and ERK1/2 which then activates PKCα. PKCα is not oxidized during ICAM-1 signaling in endothelial cells.

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 Cα (PKCα) 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 PKCα through a mechanism that is different than VCAM-1 signaling in that PKCα is not oxidized during ICAM-1 signaling in endothelial cells (Figure 2B) [28,55]. In TNFα- 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 PKCα [55]. ICAM-1 crosslinking does not induce oxidative activation of PKCα, 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 PKCα, ICAM-1-induced ROS do not oxidize PKCα. In summary, ICAM-1 activates XO, PLC, and ERK1/2 which then activates PKCα. 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 PKCα [28]. Specifically, the VCAM-1-induced activation of PKCα is inhibited by α-tocopherol and this inhibition of PKCα 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 PKCα [98]. Briefly, co-factor-dependent activation of recombinant PKCα is increased by γ-tocopherol and is inhibited by α-tocopherol [98]. Oxidative activation of PKCα 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 PKCα or the C1a regulatory domain of PKCα but does not bind the control, DAG cofactor-independent enzyme, PKCζ [98]. α-tocopherol binding to PKCα or the PKCα-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 PKCα-C1a. Thus, α-tocopherol can function as an antagonist and γ-tocopherol can function as an agonist of PKCα.

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 PKCα without oxidation of PKCα [55]. ICAM-1 activation of PKCα but not the upstream signal ERK1/2 is inhibited by α-tocopherol [55]. The α-tocopherol inhibition of PKCα is ablated by the addition of γ-tocopherol [55]. Thus, ICAM-1 activation of PKCα is inhibited by α-tocopherol and this inhibition is ablated by γ-tocopherol. These data are consistent with PKCα 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 PKCα antagonist and agonist, respectively, during VCAM-1-activated and ICAM-1-activated signals. This emphasizes that tocopherols regulate multiple signal transduction pathways that activate PKCα 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 PKCα [27,99] and PKCα 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 PKCα [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 x 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

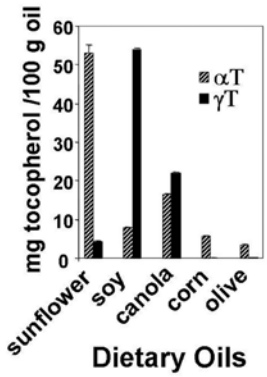

Figure 3: α-tocopherol and γ-tocopherol in dietary oils. Adapted from [28]. Tocopherols were extracted from dietary oils and measured by HPLC with an electrochemical detector.

Human Plasma:	γT (μM)	αT (μM)
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

Table 1: Human Plasma Tocopherol [139,140].

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, PKCα is activated by VCAM-1 and ICAM-1, albeit through different mechanisms. VCAM-1 and ICAM-1-activated PKCα is inhibited by α-tocopherol and increased by γ-tocopherol. Moreover, α-tocopherol and γ-tocopherol directly bind to PKCα 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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References

1. Marui N, Offermann MK, Swerlick R, Kunsch C, Rosen CA, et al. (1993) Vascular cell adhesion molecule-1 (VCAM-1) gene transcription and expression are regulated through an antioxidant-sensitive mechanism in human vascular endothelial cells. J Clin Invest 92: 1866-1874.
2. Hortelano S, López-Fontal R, Través PG, Villa N, Grashoff C, et al. (2010) ILK mediates LPS-induced vascular adhesion receptor expression and subsequent leucocyte trans-endothelial migration. Cardiovasc Res 86: 283-292.
3. Luo SF, Fang RY, Hsieh HL, Chi PL, Lin CC, et al. (2010) Involvement of MAPKs and NF-kappaB in tumor necrosis factor alpha-induced vascular cell adhesion molecule 1 expression in human rheumatoid arthritis synovial fibroblasts. Arthritis Rheum 62: 105-116.
4. Iademarco MF, McQuillan JJ, Rosen GD, Dean DC (1992) Characterization of the promoter for vascular cell adhesion molecule-1 (VCAM-1). J Biol Chem 267: 16323-16329.
5. Neish AS, Williams AJ, Palmer HJ, Whitley MZ, Collins T (1992) Functional analysis of the human vascular cell adhesion molecule 1 promoter. J Exp Med 176: 1583-1593.
6. O'Keeffe LM, Muir G, Piterina AV, McGloughlin T (2009) Vascular cell adhesion molecule-1 expression in endothelial cells exposed to physiological coronary wall shear stresses. J Biomech Eng 131: 081003.
7. Cassie S, Masterson MF, Polukoshko A, Viskovic MM, Tibbles LA (2004) Ischemia/reperfusion induces the recruitment of leukocytes from whole blood under flow conditions. Free Radic Biol Med 36: 1102-1111.
8. Reinhardt PH, Kubes P (1998) Differential leukocyte recruitment from whole blood via endothelial adhesion molecules under shear conditions. Blood 92: 4691-4699.
9. Lalor PF, Shields P, Grant A, Adams DH (2002) Recruitment of lymphocytes to the human liver. Immunol Cell Biol 80: 52-64.
10. Pober JS, Kluger MS, Schechner JS (2001) Human endothelial cell presentation of antigen and the homing of memory/effector T cells to skin. Ann N Y Acad Sci 941: 12-25.
11. Johnston B, Butcher EC (2002) Chemokines in rapid leukocyte adhesion triggering and migration. Semin Immunol 14: 83-92.
12. Marshall D, Haskard DO (2002) Clinical overview of leukocyte adhesion and migration: where are we now? Semin Immunol 14: 133-140.
13. Elangbam CS, Qualls CW Jr, Dahlgren RR (1997) Cell adhesion molecules--update. Vet Pathol 34: 61-73.
14. Ley K (1996) Molecular mechanisms of leukocyte recruitment in the inflammatory process. Cardiovasc Res 32: 733-742.
15. Springer TA (1994) Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell 76: 301-314.
16. Springer TA (1995) Traffic signals on endothelium for lymphocyte recirculation and leukocyte emigration. Annu Rev Physiol 57: 827-872.
17. Chan JR, Hyduk SJ, Cybulsky MI (2003) Detecting rapid and transient upregulation of leukocyte integrin affinity induced by chemokines and chemoattractants. J Immunol Methods 273: 43-52.
18. Laudanna C, Alon R (2006) Right on the spot. Chemokine triggering of integrin-mediated arrest of rolling leukocytes. Thromb Haemost 95: 5-11.
19. Muller WA (2009) Mechanisms of transendothelial migration of leukocytes. Circ Res 105: 223-230.
20. Laudanna C, Kim JY, Constantin G, Butcher E (2002) Rapid leukocyte integrin activation by chemokines. Immunol Rev 186: 37-46.
21. Matheny HE, Deem TL, Cook-Mills JM (2000) Lymphocyte migration through monolayers of endothelial cell lines involves VCAM-1 signaling via endothelial cell NADPH oxidase. J Immunol 164: 6550-6559.
22. Cook-Mills JM, Gallagher JS, Feldbush TL (1996) Isolation and characterization of high endothelial cell lines derived from mouse lymph nodes. In Vitro Cell Dev Biol Anim 32: 167-177.
23. Abdala-Valencia H, Earwood J, Bansal S, Jansen M, Babcock G, et al. (2007) Nonhematopoietic NADPH oxidase regulation of lung eosinophilia and airway hyperresponsiveness in experimentally induced asthma. Am J Physiol Lung Cell Mol Physiol 292: L1111-1125.
24. Keshavan P, Deem TL, Schwemberger SJ, Babcock GF, Cook-Mills JM, et al. (2005) Unconjugated bilirubin inhibits VCAM-1-mediated transendothelial leukocyte migration. J Immunol 174: 3709-3718.
25. Berdnikovs S, Abdala-Valencia H, Cook-Mills JM (2013) Endothelial cell PTP1B regulates leukocyte recruitment during allergic inflammation. Am J Physiol Lung Cell Mol Physiol 304: L240-L249.
26. Deem TL, Cook-Mills JM (2004) Vascular cell adhesion molecule 1 (VCAM-1) activation of endothelial cell matrix metalloproteinases: role of reactive oxygen species. Blood 104: 2385-2393.
27. Abdala-Valencia H, Cook-Mills JM (2006) VCAM-1 signals activate endothelial cell protein kinase Calpha via oxidation. J Immunol 177: 6379-6387.
28. Berdnikovs S, Abdala-Valencia H, McCary C, Somand M, Cole R, et al. (2009) Isoforms of vitamin E have opposing immunoregulatory functions during inflammation by regulating leukocyte recruitment. J Immunol 182: 4395-4405.
29. Zingg JM, Azzi A (2004) Non-antioxidant activities of vitamin E. Curr Med Chem 11: 1113-1133.
30. Brigelius-Flohé R, Traber MG (1999) Vitamin E: function and metabolism. FASEB J 13: 1145-1155.
31. Hunter SC, Cahoon EB (2007) Enhancing vitamin E in oilseeds: unraveling tocopherol and tocotrienol biosynthesis. Lipids 42: 97-108.
32. Schneider C (2005) Chemistry and biology of vitamin E. Mol Nutr Food Res 49: 7-30.

33. Wolf G (2006) How an increased intake of alpha-tocopherol can suppress the bioavailability of gamma-tocopherol. Nutr Rev 64: 295-299.

34. Yoshida Y, Saito Y, Jones LS, Shigeri Y (2007) Chemical reactivities and physical effects in comparison between tocopherols and tocotrienols: physiological significance and prospects as antioxidants. J Biosci Bioeng 104: 439-445.

35. Atkinson J, Epand RF, Epand RM (2008) Tocopherols and tocotrienols in membranes: a critical review. Free Radic Biol Med 44: 739-764.

36. Christen S, Woodall AA, Shigenaga MK, Southwell-Keely PT, Duncan MW, et al. (1997) gamma-tocopherol traps mutagenic electrophiles such as NO(X) and complements alpha-tocopherol: physiological implications. Proc Natl Acad Sci USA 94: 3217-3222.

37. Patel A, Liebner F, Netscher T, Mereiter K, Rosenau T (2007) Vitamin E chemistry. Nitration of non-alpha-tocopherols: products and mechanistic considerations. J Org Chem 72: 6504-6512.

38. Wolf G (1997) gamma-Tocopherol: an efficient protector of lipids against nitric oxide-initiated peroxidative damage. Nutr Rev 55: 376-378.

39. He Y, Franchi L, Núñez G (2013) The protein kinase PKR is critical for LPS-induced iNOS production but dispensable for inflammasome activation in macrophages. Eur J Immunol .

40. Fakhrzadeh L, Laskin JD, Laskin DL (2004) Ozone-induced production of nitric oxide and TNF-alpha and tissue injury are dependent on NF-kappaB p50. Am J Physiol Lung Cell Mol Physiol 287: L279-285.

41. Hernandez ML, Wagner JG, Kala A, Mills K, Wells HB, et al. (2013) Vitamin E, Î³-tocopherol, reduces airway neutrophil recruitment after inhaled endotoxin challenge in rats and in healthy volunteers. Free Radic Biol Med .

42. Wiser J, Alexis NE, Jiang Q, Wu W, Robinette C, et al. (2008) In vivo gamma-tocopherol supplementation decreases systemic oxidative stress and cytokine responses of human monocytes in normal and asthmatic subjects. Free Radic Biol Med 45: 40-49.

43. Wagner JG, Harkema JR, Jiang Q, Illek B, Ames BN, et al. (2009) Gamma-tocopherol attenuates ozone-induced exacerbation of allergic rhinosinusitis in rats. Toxicol Pathol 37: 481-491.

44. Huang J, May JM (2003) Ascorbic acid spares alpha-tocopherol and prevents lipid peroxidation in cultured H4IIE liver cells. Mol Cell Biochem 247: 171-176.

45. Halpner AD, Handelman GJ, Harris JM, Belmont CA, Blumberg JB (1998) Protection by vitamin C of loss of vitamin E in cultured rat hepatocytes. Arch Biochem Biophys 359: 305-309.

46. Buettner GR (1993) The pecking order of free radicals and antioxidants: lipid peroxidation, alpha-tocopherol, and ascorbate. Arch Biochem Biophys 300: 535-543.

47. Carr AC, Zhu BZ, Frei B (2000) Potential antiatherogenic mechanisms of ascorbate (vitamin C) and alpha-tocopherol (vitamin E). Circ Res 87: 349-354.

48. Tsao CS, Young M (1990) Enzymatic formation of ascorbic acid in liver homogenate of mice fed dietary ascorbic acid. In Vivo 4: 167-169.

49. Sienra-Monge JJ, Ramirez-Aguilar M, Moreno-Macias H, Reyes-Ruiz NI, Del Río-Navarro BE, et al. (2004) Antioxidant supplementation and nasal inflammatory responses among young asthmatics exposed to high levels of ozone. Clin Exp Immunol 138: 317-322.

50. Sesso HD, Buring JE, Christen WG, Kurth T, Belanger C, et al. (2008) Vitamins E and C in the prevention of cardiovascular disease in men: the Physicians' Health Study II randomized controlled trial. JAMA 300: 2123-2133.

51. Hernandez M, Zhou H, Zhou B, Robinette C, Crissman K, et al. (2009) Combination treatment with high-dose vitamin C and alpha-tocopherol does not enhance respiratory-tract lining fluid vitamin C levels in asthmatics. Inhal Toxicol 21: 173-181.

52. Robinson I, de Serna DG, Gutierrez A, Schade DS (2006) Vitamin E in humans: an explanation of clinical trial failure. Endocr Pract 12: 576-582.

53. Jiang Q, Christen S, Shigenaga MK, Ames BN (2001) gamma-tocopherol, the major form of vitamin E in the US diet, deserves more attention. Am J Clin Nutr 74: 714-722.

54. Azzi A, Stocker A (2000) Vitamin E: non-antioxidant roles. Prog Lipid Res 39: 231-255.

55. Abdala-Valencia H, Berdnikovs S, Cook-Mills JM (2012) Vitamin E isoforms differentially regulate intercellular adhesion molecule-1 activation of PKCÎ± in human microvascular endothelial cells. PLoS One 7: e41054.

56. Hakugawa J, Bae SJ, Tanaka Y, Katayama I (1997) The inhibitory effect of anti-adhesion molecule antibodies on eosinophil infiltration in cutaneous late phase response in Balb/c mice sensitized with ovalbumin (OVA). J Dermatol 24: 73-79.

57. Chin JE, Hatfield CA, Winterrowd GE, Brashler JR, Vonderfecht SL, et al. (1997) Airway recruitment of leukocytes in mice is dependent on alpha4-integrins and vascular cell adhesion molecule-1. Am J Physiol 272: L219-229.

58. Sagara H, Matsuda H, Wada N, Yagita H, Fukuda T, et al. (1997) A monoclonal antibody against very late activation antigen-4 inhibits eosinophil accumulation and late asthmatic response in a guinea pig model of asthma. Int Arch Allergy Immunol 112: 287-294.

59. Boyce JA, Mellor EA, Perkins B, Lim YC, Luscinskas FW (2002) Human mast cell progenitors use alpha4-integrin, VCAM-1, and PSGL-1 E-selectin for adhesive interactions with human vascular endothelium under flow conditions. Blood 99: 2890-2896.

60. Abonia JP, Austen KF, Rollins BJ, Joshi SK, Flavell RA, et al. (2005) Constitutive homing of mast cell progenitors to the intestine depends on autologous expression of the chemokine receptor CXCR2. Blood 105: 4308-4313.

61. Abonia JP, Hallgren J, Jones T, Shi T, Xu Y, et al. (2006) Alpha-4 integrins and VCAM-1, but not MAdCAM-1, are essential for recruitment of mast cell progenitors to the inflamed lung. Blood 108: 1588-1594.

62. Hallgren J, Jones TG, Abonia JP, Xing W, Humbles A, et al. (2007) Pulmonary CXCR2 regulates VCAM-1 and antigen-induced recruitment of mast cell progenitors. Proc Natl Acad Sci USA 104: 20478-20483.

63. Alcaide P, Jones TG, Lord GM, Glimcher LH, Hallgren J, et al. (2007) Dendritic cell expression of the transcription factor T-bet regulates mast cell progenitor homing to mucosal tissue. J Exp Med 204: 431-439.

64. Chen L, Lin SX, Amin S, Overbergh L, Maggiolino G, et al. (2010) VCAM-1 blockade delays disease onset, reduces disease severity and inflammatory cells in an atopic dermatitis model. Immunol Cell Biol 88: 334-342.

65. Soriano A, Salas A, Salas A, Sans M, Gironella M, et al. (2000) VCAM-1, but not ICAM-1 or MAdCAM-1, immunoblockade ameliorates DSS-induced colitis in mice. Lab Invest 80: 1541-1551.

66. Baron JL, Madri JA, Ruddle NH, Hashim G, Janeway CA Jr (1993) Surface expression of alpha 4 integrin by CD4 T cells is required for their entry into brain parenchyma. J Exp Med 177: 57-68.

67. Allavena R, Noy S, Andrews M, Pullen N (2010) CNS elevation of vascular and not mucosal addressin cell adhesion molecules in patients with multiple sclerosis. Am J Pathol 176: 556-562.

68. Ou R, Zhang M, Huang L, Flavell RA, Koni PA, et al. (2008) Regulation of immune response and inflammatory reactions against viral infection by VCAM-1. J Virol 82: 2952-2965.

69. Iiyama K, Hajra L, Iiyama M, Li H, DiChiara M, et al. (1999) Patterns of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 expression in rabbit and mouse atherosclerotic lesions and at sites predisposed to lesion formation. Circ Res 85: 199-207.

70. Qu Y, Shi X, Zhang H, Sun W, Han S, et al. (2009) VCAM-1 siRNA reduces neointimal formation after surgical mechanical injury of the rat carotid artery. J Vasc Surg 50: 1452-1458.

71. Barringhaus KG, Phillips JW, Thatte JS, Sanders JM, Czarnik AC, et al. (2004) Alpha4beta1 integrin (VLA-4) blockade attenuates both early and late leukocyte recruitment and neointimal growth following carotid injury in apolipoprotein E (-/-) mice. J Vasc Res 41: 252-260.

72. Linhartova K, Sterbakova G, Racek J, Cerbak R, Porazikova K, et al. (2009) Linking soluble vascular adhesion molecule-1 level to calcific aortic stenosis in patients with coronary artery disease. Exp Clin Cardiol 14: e80-83.

73. Huo Y, Hafezi-Moghadam A, Ley K (2000) Role of vascular cell adhesion molecule-1 and fibronectin connecting segment-1 in monocyte rolling and adhesion on early atherosclerotic lesions. Circ Res 87: 153-159.

74. Briscoe DM, Yeung AC, Schoen FJ, Allred EN, Stavrakis G, et al. (1995) Predictive value of inducible endothelial cell adhesion molecule expression for acute rejection of human cardiac allografts. Transplantation 59: 204-211.

75. Yamaguchi M, Suwa H, Miyasaka M, Kumada K (1997) Selective inhibition of

vascular cell adhesion molecule-1 expression by verapamil in human vascular endothelial cells. Transplantation 63: 759-764.

76. Kavanagh DP, Durant LE, Crosby HA, Lalor PF, Frampton J, et al. (2010) Haematopoietic stem cell recruitment to injured murine liver sinusoids depends on (alpha) 4 (beta)1 integrin/VCAM-1 interactions. Gut 59: 79-87.

77. Ebisawa M, Bochner BS, Georas SN, Schleimer RP (1992) Eosinophil transendothelial migration induced by cytokines. I. Role of endothelial and eosinophil adhesion molecules in IL-1 beta-induced transendothelial migration. J Immunol 149: 4021-4028.

78. Hohki G, Terada N, Hamano N, Kitaura M, Nakajima T, et al. (1997) The effects of eotaxin on the surface adhesion molecules of endothelial cells and on eosinophil adhesion to microvascular endothelial cells. Biochem Biophys Res Commun 241: 136-141.

79. Steffen BJ, Butcher EC, Engelhardt B (1994) Evidence for involvement of ICAM-1 and VCAM-1 in lymphocyte interaction with endothelium in experimental autoimmune encephalomyelitis in the central nervous system in the SJL/J mouse. Am J Pathol 145: 189-201.

80. Cotran RS, Mayadas-Norton T (1998) Endothelial adhesion molecules in health and disease. Pathol Biol (Paris) 46: 164-170.

81. Cook-Mills JM, Johnson JD, Deem TL, Ochi A, Wang L, et al. (2004) Calcium mobilization and Rac1 activation are required for VCAM-1 (vascular cell adhesion molecule-1) stimulation of NADPH oxidase activity. Biochem J 378: 539-547.

82. Tudor KS, Hess KL, Cook-Mills JM (2001) Cytokines modulate endothelial cell intracellular signal transduction required for VCAM-1-dependent lymphocyte transendothelial migration. Cytokine 15: 196-211.

83. DeLeo FR, Quinn MT (1996) Assembly of the phagocyte NADPH oxidase: molecular interaction of oxidase proteins. J Leukoc Biol 60: 677-691.

84. Cook-Mills JM, Wirth JJ, Fraker PJ (1990) Possible roles for zinc in destruction of Trypanosoma cruzi by toxic oxygen metabolites produced by mononuclear phagocytes. Adv Exp Med Biol 262: 111-121.

85. Hogg N, Browning J, Howard T, Winterford C, Fitzpatrick D, et al. (1999) Apoptosis in vascular endothelial cells caused by serum deprivation, oxidative stress and transforming growth factor-beta. Endothelium 7: 35-49.

86. Hermann C, Zeiher AM, Dimmeler S (1997) Shear stress inhibits H2O2-induced apoptosis of human endothelial cells by modulation of the glutathione redox cycle and nitric oxide synthase. Arterioscler Thromb Vasc Biol 17: 3588-3592.

87. Ballinger SW, Patterson C, Yan CN, Doan R, Burow DL, et al. (2000) Hydrogen peroxide- and peroxynitrite-induced mitochondrial DNA damage and dysfunction in vascular endothelial and smooth muscle cells. Circ Res 86: 960-966.

88. Usatyuk PV, Natarajan V (2004) Role of mitogen-activated protein kinases in 4-hydroxy-2-nonenal-induced actin remodeling and barrier function in endothelial cells. J Biol Chem 279: 11789-11797.

89. Lee HS, Namkoong K, Kim DH, Kim KJ, Cheong YH, et al. (2004) Hydrogen peroxide-induced alterations of tight junction proteins in bovine brain microvascular endothelial cells. Microvasc Res 68: 231-238.

90. Rajagopalan S, Meng XP, Ramasamy S, Harrison DG, Galis ZS (1996) Reactive oxygen species produced by macrophage-derived foam cells regulate the activity of vascular matrix metalloproteinases in vitro. Implications for atherosclerotic plaque stability. J Clin Invest 98: 2572-2579.

91. Fialkow L, Chan CK, Downey GP (1997) Inhibition of CD45 during neutrophil activation. J Immunol 158: 5409-5417.

92. Mathai JC, Sitaramam V (1994) Stretch sensitivity of transmembrane mobility of hydrogen peroxide through voids in the bilayer. Role of cardiolipin. J Biol Chem 269: 17784-17793.

93. Deem TL, Abdala-Valencia H, Cook-Mills JM (2007) VCAM-1 activation of endothelial cell protein tyrosine phosphatase 1B. J Immunol 178: 3865-3873.

94. Pero RS, Borchers MT, Spicher K, Ochkur SI, Sikora L, et al. (2007) Galphai2-mediated signaling events in the endothelium are involved in controlling leukocyte extravasation. Proc Natl Acad Sci U S A 104: 4371-4376.

95. Oh HM, Lee S, Na BR, Wee H, Kim SH, et al. (2007) RKIKK motif in the intracellular domain is critical for spatial and dynamic organization of ICAM-1: functional implication for the leukocyte adhesion and transmigration. Mol Biol Cell 18: 2322-2335.

96. Sumagin R, Sarelius IH (2010) Intercellular adhesion molecule-1 enrichment near tricellular endothelial junctions is preferentially associated with leukocyte transmigration and signals for reorganization of these junctions to accommodate leukocyte passage. J Immunol 184: 5242-5252.

97. Greenwood J, Amos CL, Walters CE, Couraud PO, Lyck R, et al. (2003) Intracellular domain of brain endothelial intercellular adhesion molecule-1 is essential for T lymphocyte-mediated signaling and migration. J Immunol 171: 2099-2108.

98. McCary CA, Yoon Y, Panagabko C, Cho W, Atkinson J, et al. (2012) Vitamin E isoforms directly bind PKCÎ± and differentially regulate activation of PKCÎ±. Biochem J 441: 189-198.

99. Etienne-Manneville S, Manneville JB, Adamson P, Wilbourn B, Greenwood J, et al. (2000) ICAM-1-coupled cytoskeletal rearrangements and transendothelial lymphocyte migration involve intracellular calcium signaling in brain endothelial cell lines. J Immunol 165: 3375-3383.

100. Clément S, Tasinato A, Boscoboinik D, Azzi A (1997) The effect of alpha-tocopherol on the synthesis, phosphorylation and activity of protein kinase C in smooth muscle cells after phorbol 12-myristate 13-acetate down-regulation. Eur J Biochem 246: 745-749.

101. Munteanu A, Zingg JM, Azzi A (2004) Anti-atherosclerotic effects of vitamin E--myth or reality? J Cell Mol Med 8: 59-76.

102. Meydani SN, Shapiro AC, Meydani M, Macauley JB, Blumberg JB (1987) Effect of age and dietary fat (fish, corn and coconut oils) on tocopherol status of C57BL/6Nia mice. Lipids 22: 345-350.

103. McCary CA, Abdala-Valencia H, Berdnikovs S, Cook-Mills JM (2011) Supplemental and highly elevated tocopherol doses differentially regulate allergic inflammation: reversibility of Î±-tocopherol and Î³-tocopherol's effects. J Immunol 186: 3674-3685.

104. Wagner KH, Kamal-Eldin A, Elmadfa I (2004) Gamma-tocopherol--an underestimated vitamin? Ann Nutr Metab 48: 169-188.

105. Suchankova J, Voprsalova M, Kottova M, Semecky V, Visnovsky P (2006) Effects of oral alpha-tocopherol on lung response in rat model of allergic asthma. Respirology 11: 414-421.

106. Wagner JG, Jiang Q, Harkema JR, Ames BN, Illek B, et al. (2008) Gamma-tocopherol prevents airway eosinophilia and mucous cell hyperplasia in experimentally induced allergic rhinitis and asthma. Clin Exp Allergy 38: 501-511.

107. Okamoto N, Murata T, Tamai H, Tanaka H, Nagai H (2006) Effects of alpha tocopherol and probucol supplements on allergen-induced airway inflammation and hyperresponsiveness in a mouse model of allergic asthma. Int Arch Allergy Immunol 141: 172-180.

108. Mabalirajan U, Aich J, Leishangthem GD, Sharma SK, Dinda AK, et al. (2009) Effects of vitamin E on mitochondrial dysfunction and asthma features in an experimental allergic murine model. J Appl Physiol 107: 1285-1292.

109. Kelly FJ, Mudway I, Blomberg A, Frew A, Sandström T (1999) Altered lung antioxidant status in patients with mild asthma. Lancet 354: 482-483.

110. Kalayci O, Besler T, Kilinç K, Sekerel BE, Saraçlar Y (2000) Serum levels of antioxidant vitamins (alpha tocopherol, beta carotene, and ascorbic acid) in children with bronchial asthma. Turk J Pediatr 42: 17-21.

111. Shvedova AA, Kisin ER, Kagan VE, Karol MH (1995) Increased lipid peroxidation and decreased antioxidants in lungs of guinea pigs following an allergic pulmonary response. Toxicol Appl Pharmacol 132: 72-81.

112. Devereux G, Seaton A (2005) Diet as a risk factor for atopy and asthma. J Allergy Clin Immunol 115: 1109-1117.

113. Weiss ST (1997) Diet as a risk factor for asthma. Ciba Found Symp 206: 244-257: discussion 253-7.

114. Troisi RJ, Willett WC, Weiss ST, Trichopoulos D, Rosner B, et al. (1995) A prospective study of diet and adult-onset asthma. Am J Respir Crit Care Med 151: 1401-1408.

115. Dow L, Tracey M, Villar A, Coggon D, Margetts BM, et al. (1996) Does dietary intake of vitamins C and E influence lung function in older people? Am J Respir Crit Care Med 154: 1401-1404.

116. Smit HA, Grievink L, Tabak C (1999) Dietary influences on chronic obstructive lung disease and asthma: a review of the epidemiological evidence. Proc Nutr Soc 58: 309-319.

117. Tabak C, Smit HA, Räsänen L, Fidanza F, Menotti A, et al. (1999) Dietary factors and pulmonary function: a cross sectional study in middle aged men from three European countries. Thorax 54: 1021-1026.

118. Shahar E, Hassoun G, Pollack S (2004) Effect of vitamin E supplementation on the regular treatment of seasonal allergic rhinitis. Ann Allergy Asthma Immunol 92: 654-658.

119. Friebele E (1996) The attack of asthma. Environ Health Perspect 104: 22-25.

120. Vollmer WM, Osborne ML, Buist AS (1998) 20-year trends in the prevalence of asthma and chronic airflow obstruction in an HMO. Am J Respir Crit Care Med 157: 1079-1084.

121. van Schayck CP, Smit HA (2005) The prevalence of asthma in children: a reversing trend. Eur Respir J 26: 647-650.

122. Aït-Khaled N, Enarson D, Bousquet J (2001) Chronic respiratory diseases in developing countries: the burden and strategies for prevention and management. Bull World Health Organ 79: 971-979.

123. Bousquet J, Bousquet PJ, Godard P, Daures JP (2005) The public health implications of asthma. Bull World Health Organ 83: 548-554.

124. Devereux G (2007) Early life events in asthma--diet. Pediatr Pulmonol 42: 663-673.

125. Pearson PJ, Lewis SA, Britton J, Fogarty A (2004) Vitamin E supplements in asthma: a parallel group randomised placebo controlled trial. Thorax 59: 652-656.

126. Misso NL, Brooks-Wildhaber J, Ray S, Vally H, Thompson PJ (2005) Plasma concentrations of dietary and nondietary antioxidants are low in severe asthma. Eur Respir J 26: 257-264.

127. Schünemann HJ, Grant BJ, Freudenheim JL, Muti P, Browne RW, et al. (2001) The relation of serum levels of antioxidant vitamins C and E, retinol and carotenoids with pulmonary function in the general population. Am J Respir Crit Care Med 163: 1246-1255.

128. Gazdík F, Gvozdjáková A, Nádvorníková R, Repická L, Jahnová E, et al. (2002) Decreased levels of coenzyme Q(10) in patients with bronchial asthma. Allergy 57: 811-814.

129. Schett G, Kiechl S, Bonora E, Zwerina J, Mayr A, et al. (2009) Vascular cell adhesion molecule 1 as a predictor of severe osteoarthritis of the hip and knee joints. Arthritis Rheum 60: 2381-2389.

130. Nahrendorf M, Keliher E, Panizzi P, Zhang H, Hembrador S, et al. (2009) 18F-4V for PET-CT imaging of VCAM-1 expression in atherosclerosis. JACC Cardiovasc Imaging 2: 1213-1222.

131. Libby P, DiCarli M, Weissleder R (2010) The vascular biology of atherosclerosis and imaging targets. J Nucl Med 51 Suppl 1: 33S-37S.

132. Jordan JM, De Roos AJ, Renner JB, Luta G, Cohen A, et al. (2004) A case-control study of serum tocopherol levels and the alpha- to gamma-tocopherol ratio in radiographic knee osteoarthritis: the Johnston County Osteoarthritis Project. Am J Epidemiol 159: 968-977.

133. Brand C, Snaddon J, Bailey M, Cicuttini F (2001) Vitamin E is ineffective for symptomatic relief of knee osteoarthritis: a six month double blind, randomised, placebo controlled study. Ann Rheum Dis 60: 946-949.

134. Dietrich M, Traber MG, Jacques PF, Cross CE, Hu Y, et al. (2006) Does gamma-tocopherol play a role in the primary prevention of heart disease and cancer? A review. J Am Coll Nutr 25: 292-299.

135. Siekmeier R, Steffen C, März W (2007) Role of oxidants and antioxidants in atherosclerosis: results of in vitro and in vivo investigations. J Cardiovasc Pharmacol Ther 12: 265-282.

136. Munteanu A, Zingg JM (2007) Cellular, molecular and clinical aspects of vitamin E on atherosclerosis prevention. Mol Aspects Med 28: 538-590.

137. Meydani M (2004) Vitamin E modulation of cardiovascular disease. Ann N Y Acad Sci 1031: 271-279.

138. Dutta A, Dutta SK (2003) Vitamin E and its role in the prevention of atherosclerosis and carcinogenesis: a review. J Am Coll Nutr 22: 258-268.

139. Cook-Mills JM, Marchese ME, Abdala-Valencia H (2011) Vascular cell adhesion molecule-1 expression and signaling during disease: regulation by reactive oxygen species and antioxidants. Antioxid Redox Signal 15: 1607-1638.

140. Cook-Mills JM, McCary CA (2010) Isoforms of vitamin E differentially regulate inflammation. Endocr Metab Immune Disord Drug Targets 10: 348-366.