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Anti-inflammatory properties of α - and γ -tocopherol

Elke Reiter^a, Qing Jiang^b, and Stephan Christen^{a,*}

^a Institute for Infectious Diseases, University of Berne, Friedbühlstrasse 51, CH-3010 Berne, Switzerland

^b Interdepartmental Nutrition Program, Department of Foods and Nutrition, Purdue University, West Lafayette, IN 47907, USA

Abstract

Natural vitamin E consists of four different tocopherol and four different tocotrienol homologues (α , β , γ , δ) that all have antioxidant activity. However, recent data indicate that the different vitamin E homologues also have biological activity unrelated to their antioxidant activity. In this review, we discuss the anti-inflammatory properties of the two major forms of vitamin E, α -tocopherol (α T) and γ -tocopherol (γ T), and discuss the potential molecular mechanisms involved in these effects. While both tocopherols exhibit anti-inflammatory activity *in vitro* and *in vivo*, supplementation with mixed (γ T-enriched) tocopherols seems to be more potent than supplementation with α T alone. This may explain the mostly negative outcomes of the recent large-scale interventional chronic disease prevention trials with α T and thus warrants further investigation.

Keywords

vitamin E; inflammation; redox signaling; protein kinase C; nuclear factor κ B; cyclooxygenase

1. Introduction

Vitamin E collectively refers to 8 different structurally-related tocopherols and tocotrienols that all possess antioxidant activity. In recent years it has become evident that the different forms of vitamin E also exhibit biological activity unrelated to their antioxidant activity, e.g., by modulating cell signaling processes. Here we review the current knowledge on the anti-inflammatory properties of α -tocopherol (α T) and γ -tocopherol (γ T), the two major forms of vitamin E in humans, and discuss the molecular mechanisms that may be responsible for this effect. Tocopherols and tocotrienols both possess a common chromanol ring that is methylated to different degrees at the 5' and 7' position, giving yield to the four different forms α , β , γ , and δ . While tocopherols have a saturated phytyl side chain, tocotrienols have an unsaturated isoprenoid side chain possessing three double bonds. Naturally occurring tocopherols contain three chiral centers with a configuration of R at position 2', 4' and 8'. Hence, natural tocopherols are *RRR*, whereas synthetic tocopherols are a mixture of all possible isomers (*all-rac*, previously incorrectly called *dl*). The reader is referred to the other chapters of this book for more details on the bioavailability (including definition of international units) and degradation

*Corresponding author. Tel.: +41 31 632 8707, fax +41 31 632 3550, E-mail addresses: elke.reiter@ifik.unibe.ch (E. Reiter), qjiang@purdue.edu (Q. Jiang), stephan.christen@ifik.unibe.ch (S. Christen).

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pathways of the various tocopherol forms. Tocotrienols are the subject of a special chapter in this book and are not discussed here.

Inflammatory diseases such as rheumatoid arthritis, asthma and hepatitis are a major cause of morbidity in humans. Chronic inflammation also contributes to the development of cancer (Balkwill and Mantovani 2001; Christen *et al.* 1999; Coussens and Werb 2002), cardiovascular disease (Libby 2002; Ross 1999), and neurodegenerative diseases (McGeer and McGeer 2001). While the anti-inflammatory effects of α T and γ T have been reviewed as separate entities (Jiang 2006; Meydani *et al.* 2005; Singh and Jialal 2004), this review specifically focuses on the comparative efficacy of the two major vitamin E forms and discusses the potential molecular mechanisms by which they act anti-inflammatory.

2. *In vivo* evidence for an anti-inflammatory effect of α - and γ T

2.1. Human studies

Aging is associated with enhanced oxidative stress and a decline in immune function. These changes may lead to an increase in the incidence and/or severity of microbial infections, autoimmune disorders, and degenerative diseases associated with chronic inflammation such as atherosclerosis, cancer, or neurodegenerative diseases such as Alzheimer's disease (Fulop *et al.* 2006). A marker of declined immune function in elderly is the decrease of IL-2, a cytokine important for the clonal expansion of T cells. A reduction in IL-2 levels leads to a decrease in clonal T cell expansion and thus to a decline in the specific immune response. Several studies have shown that supplementation of healthy elderly people with α T improves the overall immune response, as evidenced by an increased (i.e., restored) delayed-type hypersensitivity (DTH) reaction to various antigens, *in vitro* T cell proliferation, IL-2 production, and inhibition of PGE₂ formation (Table 1).

Thus, supplementation of healthy elderly people with a relatively high dose of 800 mg/d α T for 4 weeks resulted in a three-fold increase in serum and peripheral blood mononuclear cell α T concentrations and a three-fold decrease in serum γ T concentration, while no changes were observed in the placebo group (Meydani *et al.* 1990). The DTH reaction was significantly increased in the α T-treated group, compared to both the placebo-treated group or the study group at baseline, both regarding the cumulative score (total diameter of induration of all positive reactions) and the antigen score (number of positive responses). Also, concanavalin A (Con A)-stimulated IL-2 production by isolated monocytes was significantly enhanced in the α T-supplemented group compared to cells at baseline. Furthermore, levels of the potent pro-inflammatory lipid mediator PGE₂ were significantly reduced in α T-supplemented phytohemagglutinin (PHA)-stimulated monocytes compared to placebo. This decrease in PGE₂ production might be responsible for the restoration of IL-2 production, as the former has been shown to suppress lymphocyte proliferation and IL-2 production (Goodwin and Webb 1980; Walker *et al.* 1983).

In another randomized controlled trial, Meydani *et al.* showed that the DTH response was significantly increased in healthy elderly people also by α T supplementation at a dose of 200 mg/d (Meydani *et al.* 1997). Furthermore, α T supplementation at this dose significantly boosted antibody titers to hepatitis B and tetanus vaccination compared to placebo. Immunoglobulin, T and B cell levels, were unaffected, however, as were antibody titers to diphtheria and pneumococcal vaccination.

Pallast *et al.* showed that 100 mg/d of α T for 24 weeks only partially restored the DTH response in elderly people (while they did not observe any effect at 50 mg/d). This partial restoration of the DTH response was accompanied by a trend toward higher IL-2 production in isolated PHA-stimulated peripheral blood mononuclear cells. Interestingly, IFN- γ production decreased and

IL-4 production increased in the groups receiving α T compared to baseline (but not placebo) (Pallast *et al.* 1999). In contrast, De Waart *et al.* could not find any beneficial effects in elderly people supplemented with 100 mg/d α T for 3 months, neither on ConA- or PHA-induced lymphocyte proliferation nor on antibody titers against common antigens such as milk protein (De Waart *et al.* 1997). It therefore appears that the immunostimulatory effect of α T occurs at supplementation doses >100 mg/d. It is noteworthy that at these levels, α T significantly depresses plasma/serum γ T concentrations. Whether γ T (either alone or in combination with α T) has a beneficial effect on the decline of immune function in elderly has not been investigated.

In a randomized controlled trial, Graat *et al.* examined the effect of α T supplementation on acute respiratory tract infections in well-nourished non-institutionalized elderly people (Graat *et al.* 2002). Participants were given four supplement regimens, *i.e.*, either 200 mg of *all rac*- α T acetate, a multivitamin-mineral preparation containing the recommended dietary allowance level for each component, combined all *rac*- α T plus multivitamin-minerals, or placebo, for a median period of 441 days. In this cohort, α T administration prompted no favorable effects on the incidence or severity of acute respiratory tract infections (upper and lower combined). Co-supplementation with the multivitamin-mineral preparation did also not result in a beneficial effect. However, when the two α T supplemented groups were combined, α T administration led to a significant *increase* in both the duration and severity of respiratory tract infections. It is possible, however, that this effect was confounded by a slightly higher number of active smokers in the α T-supplemented group. In another study in elderly nursing home residents, oral administration of 200 IU α T/d for 1 year showed no effect on incidence or duration for upper and lower respiratory tract infections. However, when supplemented with α T compared to placebo, there were significantly fewer people in the subgroup of subjects that reported at least one incidence of upper respiratory tract infection. Common colds also occurred less often in the α T supplemented group than in the placebo group (Meydani *et al.* 2004). The effect of α T supplementation on the incidence of respiratory tract infections was also analyzed in the subgroup of male smokers that participated in the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study (Hemila *et al.* 2002). Supplementation with 50 mg/d of α T for 4 years slightly reduced the incidence of common colds only in people ≥ 65 years of age. Although α T supplementation has a clear immunostimulatory effect in elderly people at doses >100 mg/d, the impact on respiratory tract infections (in which the adaptive immune response plays an important role) is relatively moderate. Elderly people that have low vitamin E levels probably benefit most from supplementation with α T. Whether high doses of α T may in fact exacerbate immunodeficiency in already α T-proficient elderly people also requires further investigation.

The observation that asthma patients or subjects with other allergies have reduced levels of α T has prompted several studies to test the potential benefits of α T to the alleviation of allergic symptoms, in which Th2 cytokines such as IL-4 and IL-5 are thought to play a major role. However, supplementation of 112 patients with documented hay fever with 800 mg/day α T for 2.5 months failed to decrease the percentage of days with serious symptom or medication use during pollen season compared to placebo control; only a small effect on certain nasal symptoms was noted (Shahar *et al.* 2004). In another double-blind, placebo-controlled trial, supplementation of allergic rhinitis patients with 400 IU α T/day for four weeks also had no effect on symptom severity. Furthermore, α T supplementation had no effect on serum levels of specific IgE antibodies or lipid peroxides in response to allergen provocation (Montano Velazquez *et al.* 2006).

The prevention of cardiovascular disease (CVD) is one of the major areas in which vitamin E, primarily α T, has drawn a lot of attention in the past decade. While it has long been believed that atherosclerosis and associated CVD is a consequence of oxidative modifications of circulating lipoproteins (LDL in particular), which has been shown to be inhibited by α T in

numerous *in vitro* studies, it is becoming more evident that atherosclerosis is a consequence of complex interaction between lipid metabolism, vascular function and inflammation (Libby 2002; Ross 1999). In a set of studies in both healthy subjects and diabetes patients (which are at higher risk for CVD), α T supplementation was found to have an anti-inflammatory effect on monocytes isolated from supplemented subjects and on *in vivo* markers of inflammation. Thus, Devaraj *et al.* showed that resting or LPS-stimulated monocytes isolated from normal healthy people supplemented with 1200 IU/d α T for 8 weeks produced less superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), lipid peroxides, and IL-1 β compared to either baseline or following washout of the supplement (Devaraj *et al.* 1996). Stimulated monocytes from α T-supplemented people also displayed a diminished adhesion to vascular endothelial cells *in vitro*. In another study, the same investigators tested the effect of α T supplementation on inflammatory parameters in patients suffering from type 2 diabetes. Monocytes isolated from these patients exhibited higher levels of $O_2^{\bullet -}$ production, IL-1 β release, and endothelial cell adhesion upon stimulation with LPS. Supplementation with 1200 IU/d α T for 3 months inhibited the enhanced LPS-induced $O_2^{\bullet -}$ production, IL-1 β release, and endothelial cell adhesion to approximately the same low levels as in normal healthy controls (Devaraj and Jialal 2000a). α T supplementation also markedly decreased the increased plasma levels of the soluble endothelial cell adhesion molecules VCAM-1, ICAM-1, and E-selectin in diabetic patients. The same authors also reported that α T supplementation decreases plasma levels of C-reactive protein (CRP) in both patients with type 2 diabetes and normal healthy subjects, and that this decrease in CRP is associated with a reduction in LPS-induced IL-6 production in isolated monocytes (Devaraj and Jialal 2000b). CRP is a hepatic acute phase protein induced by IL-6 and a clinically important marker of ongoing inflammation and predictor of cardiovascular events in humans.

The effect of α T supplementation on plasma CRP and soluble adhesion molecules in type 2 diabetes patients was also studied by other investigators. Thus, supplementation with 800 IU/d of α T for 4 weeks resulted in a two-fold increase in plasma α T and reduced CRP-values by ~50% (Upritchard *et al.* 2000). However, soluble ICAM-1 and VCAM-1 concentrations did not change in this study. In another study carried out by Murphy *et al.*, a significant reduction of plasma CRP levels compared to placebo was observed in smokers with acute coronary syndrome after 6 months of supplementation with 400 IU/d of α T (Murphy *et al.* 2004). CRP levels also decreased in the placebo group, however, and were not any different to those in the α T-supplemented patients for the first few months. Plasma levels of soluble ICAM-1, VCAM-1, E-selectin and P-selectin did not significantly change during the study. In another study, whole blood from either smokers, diabetes type 2 patients, or healthy controls, before and after supplementation with α T (600 IU/d for 4 weeks) was stimulated with LPS and analyzed for the production of TNF- α , IL-1 β , and interleukin-1-receptor antagonist (IL-1RA), which counteracts the pro-inflammatory effects of IL-1 β . α T supplementation significantly inhibited LPS-stimulated TNF- α and IL-1RA production in whole blood from smokers, but not in whole blood from controls or diabetic patients (Mol *et al.* 1997), suggesting that α T may be particularly beneficial to smokers.

Based on the observation that α T supplementation (800 IU/d for ~2 years) reduces composite cardiovascular disease and myocardial infarction in hemodialysis patients (Boaz *et al.* 2000), Smith *et al.* investigated whether α T supplementation with 400 IU/d for up to 2 months had an effect on plasma markers of inflammation in end-stage renal patients (Smith *et al.* 2003). However, α T supplementation had no significant effect on plasma levels of IL-6, CRP, and TNF α , and concentrations of free F2-isoprostanes, a marker of non-enzymatic lipid peroxidation. The only significant effect observed was a nearly 2-fold increase in plasma α T that was associated with a 10-fold increase of its metabolite α -carboxyethyl hydroxychromane (α -CEHC, 2,5,7,8-tetramethyl-2-[2'-carboxyethyl]-6-hydroxychroman) and a significant decrease in γ T. In another small clinical trial in dialysis patients with end-stage renal disease,

the effect of α T was compared to that of a tocopherol mixture containing 60% *RRR*- γ T, 28% *RRR*- δ T and 10% *RRR*- α T. Either 300 mg/d of *RRR*- α T or the tocopherol-mixture were administered to the patients or healthy subjects for 14 days (Himmelfarb *et al.* 2003). Serum concentrations of α - and γ T, their metabolites α - and γ -CEHC, IL-6 and CRP were determined as study endpoints. At the onset of the study, α T levels were similar in the two groups, while serum γ T levels were significantly higher in hemodialysis patients than in healthy subjects. While α T supplementation neither significantly increased α T in healthy subjects nor in hemodialysis patients (but significantly increased the levels of its degradation product α -CEHC), it lowered γ T levels in both groups. In contrast, supplementation with the γ T-enriched vitamin E preparation caused a significant increase of γ T and its degradation product γ -CEHC in both the normal healthy people and in the hemodialysis patients. While supplementation with α T had no effect on CRP concentration in hemodialysis patients, they were significantly lowered by the γ T-enriched vitamin E. Interestingly supplementation with α T (but not with the γ T-enriched preparation) increased the serum levels of IL-6. In another study, Liu *et al.* showed that supplementation of normal healthy people with γ T-enriched vitamin E (100 mg γ T, 40 mg, δ T, and 20 mg α T) inhibited ADP-induced aggregation of isolated platelets more potently than supplementation with pure α T (Liu *et al.* 2003). More efficient inhibition of platelet aggregation by the γ T-enriched vitamin E was associated with increased constitutive nitric oxide synthase (NOS) activation in platelets. These results suggest that a mixture of tocopherols enriched with γ T may be more efficient in inhibiting inflammation-associated disease than α T alone.

2.2. Animal studies

Immune function also declines in animals with age. Similar to the humans studies, LPS-stimulated formation of PGE₂ is elevated in macrophages isolated from old mice (24 months of age) compared to young mice (6 months of age) (Wu *et al.* 1998). Supplementing old mice with 500 ppm of α T for 4 weeks diminished LPS-induced PGE₂ concentrations to levels produced by macrophages from young mice. In contrast, α T supplementation of young mice did not alter LPS-induced PGE₂ production compared to unsupplemented controls. α T appears to decrease PGE₂ production in macrophages from old mice by inhibiting the elevated cyclooxygenase (COX) activity in these cells (Wu *et al.* 1998). The inhibition of the age-related increase of PGE₂ may stem from inhibition of lipid peroxidation, which is thought to contribute to the enhanced COX-2 expression with age. In a subsequent study, the same authors showed that γ T and δ T inhibited PGE₂ in macrophages isolated from old mice equally well or even more potently than α T, whereas β T had no effect (Wu *et al.* 2000). In contrast, splenocyte IL-2 production was not affected by either α T or β T, but was increased by γ T and δ T. These results indicate that the different forms of tocopherol have different effects on immune function.

To see whether the immunostimulatory effect of α T has any impact on the course of a viral infection, both 4 and 22 month-old C57BL/6NIA mice were infected with influenza A/Port Chalmers/1/73 (H₃N₂) virus after feeding them for 6 weeks with a diet containing either 500 ppm or 30 ppm α T acetate. Viral titers were determined at 2, 5, and 7 days post-infection. In young mice, supplementation with high α T levels had no significant effect on pulmonary virus titers compared to the animals fed the low α T diet (*i.e.*, controls), except for a slight reduction on day 5. However, in old mice, pulmonary viral titers were up to 25-fold lower in animals fed with the high α T diet during the course of infection, with titers being in the same range as in young animals (Hayek *et al.* 1997). Subsequently, Han *et al.* investigated whether the inhibitory effect of α T on viral titers in old mice was due to a change in the production of the Th1 cytokines IFN- γ , IL-2, IL-6, and TNF- α (Han *et al.* 2000). Indeed, α T supplementation enhanced IFN- γ levels in old mice on day 5 and 7 post infection to the same high levels as in young mice. Overall, higher IFN- γ levels correlated with lower pulmonary viral titers. IL-2 concentrations were partially restored by α T supplementation. In contrast, α T administration had no effect on

IL-2 and IFN- γ in young mice. There was no significant overall effect of age, α T supplementation, or infection on splenocyte IL-4 or IL-6 production. Macrophages isolated from old mice produced significantly higher levels of PGE₂ in response to LPS, which was diminished by supplementation with α T to levels produced by macrophages from young mice. These results strongly suggest that α T decrease pulmonary influenza virus titers in old mice by inhibiting the elevated production of PGE₂, and by at least partially restoring the attenuated Th1 cytokine response in these animals.

IgE antibodies are involved in mediating the type 1 hypersensitivity response in allergic reactions. In a murine nasal allergy model, Zheng *et al.* showed that supplementation with a diet containing 535 mg α T per kg for 4 weeks significantly reduced quantifiable nasal symptoms compared with a diet containing normal levels of α T (Zheng *et al.* 1999). In contrast to the human studies discussed previously, α T supplementation also led to a significant decrease in lymphocyte proliferation, serum IgE levels and production of the Th2 cytokines IL-4 and IL-5 in these animals. In another study, Bando *et al.* tested the effect of vitamin E treatment in an allergic mouse model (Bando *et al.* 2003). Ovalbumin-specific IgE antibodies were analyzed by passive cutaneous anaphylaxis reaction. Different *all rac*- α T concentrations (0.5, 5, 10 and 50 mg/100 g diet) were used to supplement 6 week old BALB/c mice for a total of 6 weeks. 21 and 35 days after the onset of supplementation, mice were immunized with ovalbumin and bled 1 week after the second immunization. The isolated sera were subcutaneously injected at different concentrations into rats, and 2 days post sensitization, ovalbumin and Evans blue injected and the blue reaction spots evaluated. Sera from control-fed animals (*i.e.*, 5 mg/100 g diet) elicited the strongest immune response, while those from animals fed with 10 and 50 mg α T/100 g diet, showed positive spots only at the highest dilution and were smaller in appearance. A decrease in reactive IgE antibodies was also observed at 0.5 mg/100 g diet, which was ascribed to a generally decreased immune response due to vitamin E deficiency.

Jiang and Ames have tested the relative anti-inflammatory efficacy of γ T and α T in the air-pouch inflammation model, which is thought to mimic human joint disease. Inflammation is induced by a single injection of the long-chain sulfated polysaccharide carrageenan into the intrascapular area of male Wistar rats. γ T (33–100 mg/kg body weight) or γ -CEHC (2 mg in pouch), but not α T (33 mg/kg), significantly inhibited carrageenan-induced PGE₂ accumulation at the site of inflammation (Jiang and Ames 2003). γ T administration also significantly inhibited formation of LTB₄, a potent leukocyte chemoattractant, suggesting that γ T may inhibit 5-lipoxygenase activity. In addition to LTB₄ and other pro-inflammatory eicosanoids, γ T supplementation also led to a reduction of TNF- α at the site of inflammation. γ T administration also attenuated inflammation-mediated oxidative damage as indicated by a significant reduction of 8-isoprostane (an F2 isoprostane), and lactate dehydrogenase, as a marker of tissue damage (Jiang and Ames 2003). γ T also significantly attenuated the partial loss of food consumption caused by the strong inflammatory reaction (Jiang and Ames 2003).

In a study in rats, in which peritonitis was induced by *i.p.* injection of zymosan, we found that moderate γ T supplementation (90 mg/kg diet) attenuated inflammation-induced protein nitration and ascorbate oxidation, as indicated by a significant reduction of protein-bound 3-nitrotyrosine and dehydroascorbate formation in the kidney, compared to animals that received a normal α T-containing diet (Jiang *et al.* 2002). Supplementation significantly attenuated inflammation-induced loss of vitamin C in the plasma and kidney. Interestingly, in untreated control animals, γ T supplementation lowered basal levels of 3-nitrotyrosine in the kidney and buffered the starvation-induced changes in vitamin C in the examined tissues. Similarly, Takahashi *et al.* recently showed that γ T, but not α T, potently inhibited neointimal formation induced by vascular injury in the insulin-resistant rats (Takahashi *et al.* 2006). Along with

vascular protection, γ T, but not α T markedly reduced the increased presence of 3-nitrotyrosine in neointimal tissue in insulin-resistant rats. In another study, Milatovic *et al.* found that *i.p.* administration of γ T (100 mg/kg body weight) significantly inhibited LPS-induced cerebral PGE₂ generation in rats (Milatovic *et al.* 2003). However, supplementation did not significantly attenuate LPS-induced neuronal oxidative damage or dendritic degeneration in the brain. The relatively moderate inhibition of PGE₂ and lack of protection may be due to the relatively low bioavailability of γ T in the brain after *i.p.* administration, which was not assessed, however.

As with the human studies, Saldeen and his coworkers found that supplementation of Sprague Dawley rats with γ T-enriched tocopherol led to a more potent decrease in platelet aggregation and delay of arterial thrombogenesis compared to supplementation with α T (Saldeen *et al.* 1999). Supplementation with the γ T-enriched tocopherol also resulted in stronger *ex vivo* inhibition of superoxide generation, lipid peroxidation and LDL oxidation. In a follow-up study, they reported that γ T-enriched tocopherol was significantly more potent than α T in enhancing SOD activity in plasma and arterial tissue, as well as increasing arterial protein expression of both MnSOD and Cu/Zn SOD (Li *et al.* 1999). Furthermore, although both tocopherols increased NO generation and endothelial nitric oxide synthase (eNOS) activity, only supplementation with the tocopherol mixture resulted in increased protein expression of eNOS.

Finally, Yoshida *et al.* recently demonstrated that topical application of 5% γ -tocopherol-*N*, *N*-dimethylglycinate hydrochloride, a hydrophilic γ T derivative, either before or after UV irradiation significantly attenuated PGE₂ synthesis and edema formation (Yoshida *et al.* 2006). Although the pre-application of 10% α T acetate had a similar anti-inflammatory effect as that of the γ T derivative, post-treatment of α T was ineffective in comparison. The authors also showed that administration of the γ T derivative more potently inhibited COX-2 activity than α T, while α T caused a more extensive downregulation of COX-2 protein in pre-treated animals.

In summary, the animal studies show that both α - and γ T have significant anti-inflammatory activity, supporting the observations made in the humans studies, but that their relative potencies vary depending on the system tested. An explanation for these differences may be they impact on different molecular targets.

3. Possible molecular mechanisms involved in the anti-inflammatory effects of α - and γ T

As discussed previously, the anti-inflammatory and immunostimulatory properties of α - and γ T do not seem to be related to their antioxidant activity *sensu stricto*. Instead, several distinct molecular targets are thought to be involved in the anti-inflammatory effects mediated by the two tocopherols. This chapter summarizes the current knowledge on the possible molecular pathways involved in the anti-inflammatory effects. A scheme illustrating the possible molecular targets of α - and γ T, and the interactions between these different pathways in relation to their anti-inflammatory effect is depicted in Figure 1.

3.1. Modulation of cell signaling

In seminal work, the group of Azzi *et al.* showed that α T, but not β T, inhibits protein kinase C (PKC) activity in vascular smooth muscle cells induced by the PKC activator phorbol 12-myristate 13-acetate (PMA) (Boscoboinik *et al.* 1991; Tasinato *et al.* 1995), indicating that PKC activity was affected in a non-antioxidant fashion. Further mechanistic studies showed that α T specifically inhibited the α isoform of PKC and that decreased PKC α activity appears to be the result of enhanced protein phosphatase type 2A (PP2A) activity, thus inhibiting

PKC α auto-phosphorylation (Ricciarelli *et al.* 1998). PKC regulates activation and transcription of a large number of proteins involved in the inflammatory process, such as the production of IL-1 β , expression of COX, or the formation of O₂ $\bullet^-$ by NADPH oxidase. PKC has been reported to have higher activity in patients or animals with diabetes compared to normoglycemic controls (Devaraj and Jialal 2000a; Koya and King 1998).

Increased PKC activity has been shown to lead to enhanced phosphorylation of the NADPH oxidase subunit p47^{phox}; which upon phosphorylation, translocates to the cell membrane and thereby activates NADPH oxidase. Cachia *et al.* showed that α - but not β T effectively inhibits O₂ $\bullet^-$ production in PMA-stimulated human monocytes (Cachia *et al.* 1998). This inhibition was associated with a reduction in p47^{phox} phosphorylation and translocation, and a reduction in PKC activity, thus suggesting that α T inhibited the PMA-induced O₂ $\bullet^-$ production in monocytes by inhibiting PKC-dependent activation of the NADPH oxidase. In a more recent study, Venugopal *et al.* showed that inhibition of hyperglycemia-induced O₂ $\bullet^-$ production by α T in human monocytic THP-1 cells is also due to inhibition of PKC, specifically of the α form (Venugopal *et al.* 2002).

The involvement of PP2A in mediating the inhibition of PKC by α T was also indicated by experiments in other types of cells. Thus, similar to human monocytes, α T inhibited LPS- and PMA-induced O₂ $\bullet^-$ production in murine BV-2 microglia cells (Egger *et al.* 2001). Inhibition was associated with a reduction in the translocation of p67^{phox}, another NADPH oxidase subunit necessary for activity. The protein phosphatase inhibitors okadaic acid and calyculin A reversed α T-mediated inhibition of O₂ $\bullet^-$ production, suggesting that either PP1 or PP2A was involved in the inhibitory effect of α T. In a succeeding study, they showed that α T treatment indeed caused a significant increase of PP2A in BV-2 microglia cells and that this upregulation was associated with an attenuation of the LPS-induced upregulation of COX-2 and consequent production of PGE₂ (Egger *et al.* 2003). As with the O₂ $\bullet^-$ production, the α T-mediated inhibition of PGE₂ production was reversed by okadaic acid and calyculin A. The elevation of PP2A levels by α T was associated by a decrease in ERK1/2 and p38 MAPK phosphorylation, and a decrease in NF κ B DNA binding activity. The specific inhibition of PKC or ERK1/2 also lead to inhibition of COX-2 upregulation and PGE₂ production, indicating that the attenuation of COX-2 upregulation by α T is mediated *via* these signaling pathways (Egger *et al.* 2003). In another study, treatment of human aortic endothelial cells with physiological concentrations of α T dose-dependently stimulated the production of prostaglandin I₂ (prostacyclin) and PGE₂ *via* induction of phospholipase A₂ protein synthesis, while it significantly inhibited cellular COX activity (Wu *et al.* 2005). COX-1 and -2 protein levels were unaffected by the α T treatment. These results indicate that α T modulates the formation of prostanoids by exerting opposite effects on the two key enzymes involved in their biosynthesis.

In another series of experiments, Freedman *et al.* investigated the mechanism by which α T inhibits platelet aggregation. Incubation of platelet-rich plasma with 0.5 mM α T, but not with an equivalent concentration of the potent antioxidant butylated hydroxytoluene, inhibited ADP, arachidonic acid, or PMA-induced platelet aggregation, indicating that the effect of α T was independent of its antioxidant activity (Freedman *et al.* 1996). While the effect on ADP- and arachidonic acid-induced platelet aggregation was relatively modest, incorporation of α T into platelets reduced their sensitivity to aggregation by PMA by ~100-fold. Platelets isolated from healthy volunteers orally supplemented with α T for 14 days (400–1200 IU/d) responded similarly. In both cases, inhibition of platelet aggregation was associated with a significant reduction in PKC activity. Subsequently, the authors found that platelets supplemented with α T (both *in vitro* and *in vivo*) release comparatively more nitric oxide (NO) than unsupplemented platelets, and that increased NO release correlates with decreased ADP-induced aggregation. Furthermore, incubation of platelets with the PKC inhibitor chelerythrine

also caused increased NO production (and reduced $O_2\bullet^-$ production), while incubation of platelets with αT inhibited PKC-dependent phosphorylation of eNOS, which was previously shown to reduce its catalytic activity. Thus, αT appears to inhibit platelet aggregation by inhibiting the inactivation of eNOS by PKC.

Tocopherols also target other kinases than PKC. In a recent study, Kempna *et al.* showed that 50 μM of either α - or δT strongly inhibited proliferation of the mastocytoma cell line HMC-1 (Kempna *et al.* 2004). Mast cells are involved in the immediate hypersensitivity response during allergic reactions and in the host defense against certain parasites and bacteria. In contrast to the studies mentioned above, inhibition of HMC-1 proliferation was due to the inhibition of the activation of Akt, and not due to inhibition of PKC. Akt is an important regulator of cell survival by modulating the activity of several anti- and pro- apoptotic factors such as the Bcl-2 family member Bad. Inhibition of Akt phosphorylation by αT was shown to be due to inhibition of the phosphatidylinositol 3-kinase (PI3K)-phosphoinositide-dependent kinase (PDK) pathway. The exact target of αT is still elusive, however. Thus, inhibition of Akt by tocopherols may be another potential mechanism by which tocopherols act anti-inflammatory, either by inhibiting proliferation of inflammatory cells and/or inhibition of Akt-mediated activation of the transcription factor nuclear factor NF κ B.

NF κ B is a master regulator in the upregulation of inflammatory cytokines, adhesion molecules and other inflammatory gene products by inflammatory stimuli, and is subject to redox-control by various mechanisms such as phosphorylation by mitogen- and stress-activated protein kinases (Sury *et al.* 2006). In an early study, Suzuki and Packer showed that TNF- α -induced activation of NF κ B in Jurkat T cells is dose-dependently inhibited by αT acetate (Suzuki *et al.* 1993). Low concentrations of αT succinate (50 μM) have been shown to inhibit LPS-induced NF κ B DNA binding in activated rat Kupffer cells (assessed by electrophoretic mobility shift assay), which was associated with diminished TNF α synthesis (Fox *et al.* 1997). Islam *et al.* studied the effect of αT treatment on monocytic expression of the integrin adhesion molecules CD11a, CD11b, CD11c, VLA-4 and L-selectin, as well as on the adhesion of the monocytic cancer cell line U937 to human umbilical vein endothelial cells. Thus, pre-treatment of U937 with different concentrations of αT (25–100 μM) significantly decreased LPS- or chemotactic peptide formyl-Met-Leu-Phe (fMLP)-induced expression of CD11b and VLA-4, while it had no effect on the expression of CD11a, CD11c and L-selectin (Islam *et al.* 1998). LPS or fMLP-stimulated adhesion of U937 to human umbilical vein endothelial cells was reduced by αT at both 50 and 100 μM . Like in the studies discussed above, inhibition of integrin expression and adhesion to endothelial cells by αT was associated with decreased NF κ B DNA-binding. A study by Sugiyama *et al.* showed that the inhibition of lysophosphatidylcholine-induced activation of NF κ B in human umbilical vein endothelial cells by αT is due to inhibition of PKC (Sugiyama *et al.* 1998). In a contrasting study, Devaraj and Jialal showed that LPS-induced NF κ B DNA binding activity in human monocytes was inhibited by αT and the 5-lipoxygenase inhibitor MK886. While αT and MK886 also inhibited TNF- α release, specific PKC inhibitors had no effect, indicating that PKC was not involved in this paradigm. While most studies have been conducted with αT , the NF κ B pathway is also inhibited by γT (Li *et al.* 1999). However, no comparative data are available on their relative efficacy in inhibiting NF κ B.

Peroxisome proliferator-activated receptors (PPAR) are a family of transcription factors induced by various ligands that have recently been reported to act anti-inflammatory by interfering with inflammatory signaling cascades such as the NF κ B pathway (Moraes *et al.* 2006; Rizzo and Fiorucci 2006). Several research groups have recently begun to investigate the effect of αT on the expression and activity of PPARs. Thus, αT upregulates PPAR γ in primary rat hepatocytes to almost the same degree as equimolar concentrations of the PPAR γ ligand troglitazone, which has structural similarity with αT (Davies *et al.* 2002). Treatment of SW480 human colon cancer cells with either 5 or 10 μM of αT or γT resulted in

increased PPAR γ mRNA levels 24 h post treatment. γ T increased PPAR γ mRNA levels by about 3-fold, whereas α T only by about 1.5 to 2-fold. Higher mRNA levels also resulted in enhanced translation, with γ T causing stronger protein expression (Campbell *et al.* 2003). In the human keratinocytic cell line NCTC 2544, all of the four natural tocopherol homologues increased PPAR γ transcriptional activity, as determined by a luciferase reporter gene with PPAR γ -specific promoter elements. Interestingly, the strongest transcriptional activity was shown with γ T. Protein expression of PPAR γ and transglutaminase-1 (TG-1), whose gene expression is regulated by PPAR γ , were induced more or less equally by the different tocopherol homologues (De Pascale *et al.* 2006). Whether activation of PPARs such as PPAR γ explains some of the anti-inflammatory effects mediated by α - and γ T (*e.g.*, inhibition of NF κ B-dependent gene transcription) clearly warrants further investigation.

3.2. Modulation of eicosanoid biosynthesis

As pointed out earlier, tocopherols and their metabolites act anti-inflammatory also at the post-transcriptional level by inhibiting either COX or 5-LOX activity. The constitutively expressed COX-1 is found in many tissues and performs a 'housekeeping' function of synthesizing prostaglandins, which regulate normal cells' activity. On the other hand, COX-2 is normally expressed in limited tissues, but is induced by endotoxin and cytokines in many immune cells including macrophages, monocytes and epithelial cells (Vane and Botting 1998). Under most inflammatory conditions, COX-2 is up-regulated and is the primary enzyme responsible for the formation of the pro-inflammatory PGE₂ (Vane and Botting 1998). Because of the central roles of PGE₂ and LTB₄ in inflammation, COX-2 and 5-LOX have been recognized as key targets for drug therapy in inflammatory diseases. For example, COX-2 specific inhibitors have been proven to be effective in attenuating inflammatory response and beneficial in the treatment of certain inflammation-associated diseases (Wynne and Campbell 1993).

Jiang *et al.* reported that γ T, and its water-soluble metabolite γ -CEHC reduced PGE₂ synthesis in LPS-stimulated RAW 264.7 macrophages and IL-1 β -treated human lung epithelial cells (A549) at physiologically relevant concentrations (Jiang *et al.* 2000). In contrast, α T was much less potent than γ T in reducing PGE₂ in these cells. The inhibitory effects of γ T and γ -CEHC appear to be rooted in their inhibition of COX-2 activity rather than affecting COX-2 protein expression or substrate availability, and are independent of antioxidant activity (Jiang *et al.* 2000). This study has also suggested that γ T and γ -CEHC may act as competitive inhibitors of COX-2 based on the observation that when assayed in intact cells, their inhibitory potency was diminished by an increase in the concentration of arachidonic acid, the physiological substrate of the enzyme. Although γ T has been shown to be an effective scavenger of reactive nitrogen species (Christen *et al.* 1997; Cooney *et al.* 1993), inhibition of PGE₂ appears to be independent of nitric oxide. This notion is based on the observation that the presence of the nitric oxide synthase inhibitor N^G-monomethyl-L-arginine, which almost completely abolishes nitrite accumulation, does not significantly alter PGE₂ production or the inhibitory potency of γ T (Jiang *et al.* 2000).

In subsequent studies, it was found that vitamin E forms other than γ T also inhibit COX-2-catalyzed formation of PGE₂. Thus, in IL-1 β -activated A549 cells, δ T is a stronger inhibitor than γ T. In contrast, β T did not show a significant effect at concentrations between 20 and 50 μ M (Jiang, unpublished data). Grammas *et al.* reported that in TNF- α -activated murine microglial EOC-20 cells, α -CEHC and γ -CEHC inhibited PGE₂ with an apparent IC₅₀ of 74 and 66 μ M, respectively (Grammas *et al.* 2004). On the other hand, the parental molecules, α T and γ T, had no effect. This study, however, did not provide mechanistic information, *e.g.*, regarding whether α T and γ T affect COX-2 protein expression or substrate availability in this cellular system. The mechanisms underlying the inhibitory effect of tocopherols on COX-2 activity have been investigated further. O'Leary *et al.* showed that γ T, at 10 μ M, inhibits

peroxidase activity of COX-2 by 56%, whereas α T is less potent, which only shows a maximum inhibition of 33% (O'Leary *et al.* 2004).

Another protein that appears to be involved in the anti-inflammatory effects mediated by α - and γ T is 5-lipoxygenase (5-LOX). 5-LOX is the rate-limiting enzyme involved in the formation of LTB₄. Studies performed in the early 80's have found an inverse correlation between high plasma levels of α T and the formation of 5-LOX pathway products such as LTB₄ by human leukocytes (Goetzl 1980). A study in weanling rats investigated the effect of α T supplementation on 5-LOX-catalyzed eicosanoid formation in polymorphonuclear neutrophils (PMN). In this study, the rats were fed a semi-purified diet for 17 weeks containing either 0, 30 or 3000 ppm of *RRR*- α T acetate (Chan *et al.* 1989). When isolated PMN were stimulated with A23187, the synthesis of 5-HETE, LTB₄ and 19-hydroxy-LTB₄ was decreased in proportion to increasing dietary tocopherol. However, when exogenous arachidonate was added together with A23187, only the lower dose (30 ppm) but not the higher dose (3000 ppm) suppressed the formation of 5-LOX products. It is not clear, however, how exactly α T suppressed the formation of the 5-LOX products. In a study with 5-LOX isolated from potato tubers, α T and γ T were directly shown to inhibit 5-LOX activity. Interaction experiments with ¹⁴C-labelled α T showed that α T was associated with 5-LOX, suggesting that α T specifically interacts with the enzyme (Reddanna *et al.* 1985). An involvement of the 5-LOX pathway in the anti-inflammatory effect of α T is also supported by several *in vitro* cell culture experiments. Thus, LPS-induced IL-1 β release in human peripheral monocytes, which is inhibited by α T, is unaffected by specific PKC and COX inhibitors, but is reversed by co-treatment with LTB₄ (Devaraj and Jialal 1999). Similar observations were made for LPS-induced TNF- α release (Devaraj and Jialal 2005). In addition to α T, the effect of γ T on 5-LOX catalyzed LTB₄ formation was also evaluated in the carageenan-induced air pouch model described above, where we showed that γ T but not α T significantly inhibited the appearance of LTB₄ at the site of inflammation (Jiang and Ames, 2003). Further studies are needed to compare the relative effects of the two vitamin E forms on 5-LOX and to investigate the mechanisms involved in this effect.

Discussion and Conclusions

Despite the well-documented anti-inflammatory effects of α T, none of the recently conducted large-scale controlled interventional trials found a significant protective effect of α T supplementation on the incidence of CVD, cancer, or neurodegenerative diseases (Guallar *et al.* 2005), in all of which inflammation is thought to play an important role in disease development.

The following reasons might explain these unexpected results. It is possible that beneficial effects are only observed in individuals who are in the stage of α T deficiency, which could be the result of low dietary intake of α T, depletion of α T due to the pathological condition, or malabsorption associated with the disease or excessive alcohol use. In these cases, supplementation of α T is probably likely to be beneficial. Indeed, a recent study by the group of Stocker *et al.* revealed that α T supplementation inhibits atherosclerosis development in ApoE knockout mice only under vitamin E-deficient conditions (caused by deleting the tocopherol transfer protein), while supplementation had no effect in mice that had normal basal levels of α T (Suarna *et al.* 2006). Vitamin E deficiency is common in elderly, smokers, and patients with certain medical conditions. Future studies should distinguish the benefits between low vs. adequate α T status, which would also help to eliminate possible side effects imposed by high dose supplements. Thus, intake of high doses of α T has been associated with increased overall mortality (Miller *et al.* 2005) and, as discussed further above, may exacerbate respiratory tract infections in elderly (Graat *et al.* 2002). The exact reasons for these adverse effects are not clear, but may be related to dysregulation of immune function and/or the

induction of key xenobiotic metabolizing enzymes. It is well known that high levels of α T depress plasma and eventually tissue concentrations of γ T. The potential effect of α T on targets such as xenobiotic metabolizing enzymes and ABC transporters may complicate or in some cases even counteract with the drug functions. For example, Brown *et al.* reported that the protective increase in HDL2 with simvastatin plus niacin was attenuated by concurrent therapy with vitamin E, vitamin C and Se (Brown *et al.* 2001). Mustacich *et al.* recently reported that high dose of α T results in an upregulation of cytochrome P450 proteins, including CYP3A and CYP2B, as well as P-glycoprotein, a protein involved in biliary xenobiotic excretion (Mustacich *et al.* 2006). In contrast, high doses of γ T do not have the same effect on xenobiotic metabolizing enzymes (Traber *et al.* 2005). Increases in xenobiotic metabolism and excretion pathways by α T may lead to alterations in the efficacy of certain pharmaceutical drugs. We propose that it is important to investigate the potential biological activities of the vitamin E metabolites, some of which have been shown to have beneficial effects in experimental systems.

The inhibition of PKC by α T is well-documented. This is an important signaling pathway for the regulation of inflammation and other processes. However, most studies only showed an effect in cultured cells or under *ex vivo* conditions. Many, if not most cell culture studies are done under conditions of vitamin E deficiency. This might partially explain the inconsistency observed between cell culture studies and studies performed in animals or humans. As pointed out in this review, more and more evidence indicates that γ T and other vitamin E forms than α T have unique bioactivities that may be important for maintaining and improving human health (Dietrich *et al.* 2006; Jiang *et al.* 2001). For example, γ T is a stronger inhibitor of cyclooxygenase and possibly lipoxygenase than α T. Furthermore, γ T traps reactive nitrogen species more efficiently than α T. Some of these *in vitro* effects are slowly being confirmed *in vivo*, but more studies are needed here. In addition, γ T but not α T exhibits anti-proliferative and pro-apoptotic effects on cancer but not normal epithelial cells (Jiang *et al.* 2004). This anti-cancer effect may be due to modulation of sphingolipid metabolism and action. Thus, despite the undisputed anti-inflammatory effects of α - and γ T, the recent large-scale interventional studies aimed at reducing diseases associated with chronic inflammation have been disappointing, but may be explained by the complex interaction of the different vitamin E forms with inflammatory signaling, xenobiotic transformation, and as yet undefined pathways. To be able to provide the public with optimal health recommendations, further studies into the effects of the different vitamin E forms are needed.

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Abbreviations

αT	α -tocopherol
CEHC	carboxyethyl hydroxychroman
Con A	concanavalin A
COX	cyclooxygenase
CRP	C-reactive protein
CVD	cardiovascular disease
DTH	delayed type hypersensitivity
δT	δ -tocopherol
γT	γ -tocopherol
H₂O₂	hydrogen peroxide
ICAM-1	intercellular adhesion molecule 1
IL-1β	interleukin 1 β

IL-2	interleukin 2
IL-6	interleukin 6
IFN-γ	interferon γ
LOX	lipoxygenase
LTB₄	leukotriene B ₄
NFκB	nuclear factor κ B
O₂$\bullet^-$	superoxide
PGE₂	prostaglandin E ₂
PHA	phytohemagglutinin
PKC	protein kinase C
PMA	phorbol 12-myristate 13-acetate
PPAR	peroxisome proliferator-activated receptor
TNF-α	tumor necrosis factor α
VCAM-1	vascular cell adhesion molecule 1

Figure 1. Possible molecular mechanisms of the anti-inflammatory effects mediated by α - and γ T

The two tocopherols can act anti-inflammatory either by affecting transcription of inflammatory genes by modulating the signaling pathways involved in upregulation of these genes, or by inhibiting the activity of enzymes involved in eicosanoid biosynthesis. α T acts primarily through inhibition of cell signaling, while γ T (and its water-soluble metabolite γ -CEHC) potentially inhibits COX-2-mediated biosynthesis of PGE₂. Akt, protein kinase B; p38^{MAPK}, p38 mitogen-activated protein kinase; PDK, phosphoinositide-dependent kinase; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; PLC, phospholipase C; PP2A, protein phosphatase 2A;

Table 1

Effect of vitamin E supplementation on immune response and inflammation in humans

Subjects	Daily dose and duration	Effect	References
Elderly people (≥ 60 y)	800 mg <i>all rac</i> - α T acetate for 30 d	Restoration of DTH response, elevated IL-2 production in Con A-stimulated lymphocytes, diminished PGE ₂ synthesis in peripheral blood mononuclear cells, decreased plasma lipid peroxides	Meydani <i>et al.</i> 1990
	60 mg, 200 mg or 800 mg <i>all rac</i> - α T for 120 d (DTH response) and 235 d (vaccination efficacy)	Restoration of DTH response in elderly, increased antibody titers to hepatitis B vaccine with 200 and 800 mg (to tetanus vaccine at 200 mg), antibody titers to diphtheria and pneumococcal vaccination did not change	Meydani <i>et al.</i> 1997
	100 mg and 50 mg <i>all rac</i> - α T acetate for 24 wk ^a , 100 mg <i>all rac</i> - α T acetate for 3 mo ^b	Restoration of DTH response, elevated IL-2 production in Con A-stimulated lymphocytes, no significant effect on IL-2, IFN- γ and IL-4 production in PHA-stimulated lymphocytes. ^a No effect on Con A or PHA-induced lymphocyte proliferation. ^b	^a Pallast <i>et al.</i> 1999 ^b De Waart <i>et al.</i> 1997
	50 mg/d α T for 4 yr; 200 IU <i>all rac</i> - α T for 1 yr; 200 mg α -T acetate for 441 d	Slightly lower incidence of common colds in elderly smokers (subgroup from ATBC cancer prevention study); Less participants with 1 or more upper respiratory tract infections, lower incidence of common colds; Increased duration and severity of acute respiratory infection.	Hemila <i>et al.</i> 2002; Meydani <i>et al.</i> 2004; Graat <i>et al.</i> 2002
Seasonal allergic rhinitis patients	800 mg α T for 2.5 mo ^c , 400 IU for 4 wk ^d	No significant effect on overall symptoms and specific IgE serum levels, only modest effect on certain nasal symptoms	^c Montano Velazquez <i>et al.</i> 2006 ^d Shahar <i>et al.</i> 2004
Diabetic patients	1200 IU <i>RRR</i> - α T for 3 mo ^{c,f} , 800 IU <i>RRR</i> - α T for 4 wk ^g	Decreased TNF- α and IL-1 β ^e , decreased IL-6 ^f , decreased O ₂ ^{•-} production in monocytes ^e , decreased soluble ICAM, VCAM, and E-selectin ^e , decreased CRP ^{f,g} , decreased endothelial cell adhesion ^e	^e Devaraj and Jialal 2000a ^f Devaraj and Jialal 2000b ^g Upritchard <i>et al.</i> 2000
Hemodialysis patients with end stage renal disease	300 mg <i>RRR</i> - α T or a mixture of 60% <i>RRR</i> - γ T, 28% <i>RRR</i> - δ T, and 10% <i>RRR</i> - α T for 14 d ^h , 400 IU <i>RRR</i> - α T for 1 or 2 mo ⁱ	Decreased CRP levels with tocopherol mixture ^h , no effect with α T ^{h,i} , no effect on TNF- α ⁱ , decreased IL-6 levels with α T, no effect with T mixture ^h , no effect on IL-6 ⁱ	^h Himmelfarb <i>et al.</i> 2003 ⁱ Smith <i>et al.</i> 2003
Patients with acute coronary syndrome	400 IU <i>RRR</i> - α T acetate for 6 mo	Decreased CRP levels in smokers with acute coronary heart syndrome	Murphy <i>et al.</i> 2004
Healthy normal people (25–74 y)	400 IU <i>all rac</i> - α T for 28 d 1200 IU <i>RRR</i> - α T acetate for 2 mo ^j , 1200 IU <i>RRR</i> - α T for 3 mo ^{k,l}	Enhanced CD4 T-helper lymphocyte proliferation, higher CD4/CD8 ratios, less H ₂ O ₂ production in lymphocytes Decreased TNF α ^k , decreased IL-1 β ^{j,k} , decreased IL-6 ⁱ , decreased O ₂ ^{•-} and H ₂ O ₂ production in monocytes ^{j,k} , decreased soluble ICAM-1, VCAM-1, and E-selectin ^k , decreased monocyte-endothelial cell adhesion ^j	Lee and Man-Fan Wan 2000 ^j Devaraj <i>et al.</i> 1996 ^k Devaraj and Jialal 2000a ^l Devaraj and Jialal 2000b
	100 mg <i>all-rac</i> α T or mixed tocopherol (100 mg γ T, 20 mg δ T and 20 mg α T) for 8 wk	Decrease of ADP-induced platelet aggregation with mixed tocopherol, no effect with α T. Increased superoxide dismutase (SOD) activity in plasma and arterial tissue, increased protein expression of endothelial NOS, manganese SOD and copper/zinc SOD in arteria with α T and mixed tocopherol	Liu <i>et al.</i> 2003

Table 2
Comparative effect of different tocopherol forms on inflammatory reactions^a

Effect	Dose	α T	β T	γ T	δ T	Mixed T ^b	References
Inhibition of ADP-induced platelet aggregation, delayed arterial thrombogenesis, inhibition of superoxide generation and lipid peroxidation, increased endogenous SOD and constitutive NOS activity in rats	100 mg/kg diet/d for 7 to 10 d	+				++	Li <i>et al.</i> 1999 Saldeen <i>et al.</i> 1999
Inhibition of ADP-induced platelet aggregation, eNOS activation and NO release in human platelets	160 mg/d mixed T, 100 mg/d <i>all-rac</i> α T acetate for 8 wk	-(+)				++	Liu <i>et al.</i> 2003
Increased Cu/Zn SOD protein content, inhibition of platelet PKC activation		++				++	
Decrease of CRP levels in chronic hemodialysis patients	300 mg/d for 14 d	-				++	Himmelfarb <i>et al.</i> 2003
Inhibition of MPTP-induced dopamine loss in heterozygous α T transfer protein knockout mice	1'000 mg/kg diet/d for 3 wk	+		++			Itoh <i>et al.</i> 2006
Inhibition of LPS-induced neuronal oxidative damage (F4-neuroprostane formation) and dendritic degeneration in mice	100 mg/kg <i>i.p.</i> for 3 d	++		(+)			Milatovic <i>et al.</i> 2003
Inhibition of PKC activation	50 μ M	++	-	+	++		Ricciardielli <i>et al.</i> 1998
Inhibition of Akt activation	5 – 50 μ M	+	(+)	+	++		Tassinato <i>et al.</i> 1995
Inhibition of PGE ₂ production in splenocytes from old mice	1.25 – 25 μ M	++	-				Kempna <i>et al.</i> 2004
Inhibition of COX activity		++	++	++	+		Wu <i>et al.</i> 2000
Inhibition of cytokine-induced PGE ₂ production in macrophages and epithelial cells, inhibition of COX-2 activity	5 – 50 μ M (1 – 10 μ M for purified enzyme)	(+)	-	++			Jiang <i>et al.</i> 2000
Inhibition of 5-LOX-dependent TNF- α -release from LPS-stimulated human monocytes	100 μ M	+					O'Leary <i>et al.</i> 2004
Upregulation of PPAR γ mRNA and protein	5 – 10 μ M	+		++			Devaraj and Jialal 2005
Increased PPAR γ transcriptional activity	50 μ M	+		++			Campbell <i>et al.</i> 2003
				++	+		De Pascale <i>et al.</i> 2006

^a All natural *RRR*-form, unless indicated.

^b γ T-enriched (60–63% γ T, 25–28% δ T, 10–12% α T).