MINIREVIEW

α-Tocopherol and Atherosclerosis

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Cardiovascular disease is the leading cause of morbidity and mortality in the Western world. There is compelling evidence incriminating oxidative stress in the pathogenesis of the atherosclerotic lesion. Several lines of evidence suggest that antioxidants, especially α-tocopherol, have potential beneficial effects with regard to cardiovascular disease. *In vitro*, α-tocopherol has been shown to inhibit platelet adhesion and aggregation and smooth muscle cell proliferation, exert anti-inflammatory effects on monocytes, and improve endothelial function. Also, supplementation with α-tocopherol has been shown to decrease lipid peroxidation, platelet aggregation, and pro-inflammatory activity of monocytes. However, clinical trials with α-tocopherol supplementation to date have been equivocal. Thus, although mounting *in vitro* evidence and animal models provide a sound scientific basis for α-tocopherol supplementation, further clinical trials are required before a definitive recommendation can be made with respect to the primary and secondary prevention of heart disease.

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Atherosclerosis is the leading cause of death in the Western world. Considerable evidence suggests that oxidative stress and inflammation are central to atherogenesis. Epidemiologic studies suggest an association between increased antioxidant intake, especially vitamin E, and reduced morbidity and mortality from coronary artery disease. In this review, we will discuss the current literature with respect to α-tocopherol and cardiovascular disease and the potential mechanisms involved.

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Oxidative Stress and Atherosclerosis

Clinical and epidemiological studies show that increased levels of low-density lipoprotein (LDL) cholesterol promote premature atherosclerosis. According to the oxidative modification hypothesis, the most plausible and biologically relevant modification of LDL is oxidation. LDL can be oxidatively modified by all major cells of the arterial wall (1, 2). In the early phase, mild oxidation of LDL results in the formation of minimally modified LDL (MM-LDL) in the subendothelial space. MM-LDL stimulates production of monocyte chemotactic protein-1 (MCP-1) that promotes monocyte chemotaxis. These molecular events result in monocyte binding to the endothelium and its subsequent migration into the subendothelial space where MM-LDL also stimulates production of monocyte colony stimulating factor (M-CSF). M-CSF promotes the differentiation of monocytes into macrophages. The macrophages can then further oxidize MM-LDL to Ox-LDL, which is not recognized by the LDL receptor but taken up avidly by the scavenger receptor pathway leading to appreciable cholesterol ester accumulation and foam cell formation.

Oxidized LDL has several biological consequences (1, 3); it promotes vasoconstriction, promotes adhesion, stimulates cytokines such as interleukin-1 (IL-1), increases platelet aggregation, inhibits nitric oxide tissue factor secretion, and stimulates plasminogen activator inhibitor-1 synthesis. Several lines of evidence support the *in vivo* existence of oxidized LDL (4–7): (i) antibodies against epitopes on Ox-LDL recognize material from atherosclerotic lesions, but not normal arteries; (ii) LDL extracted from lesions resembles LDL oxidatively modified *in vitro*; (iii) the presence of autoantibodies to Ox-LDL has been positively correlated to the progression of atherosclerosis, as manifested by carotid artery stenosis (8); and (iv) plasma concentrations of immunoreactive oxidized LDL are higher in patients with unstable angina, carotid atherosclerosis, and acute myocardial infarction than in normal subjects (9). However, the most persuasive evidence comes from animal

studies in which antioxidant supplementation decreases lesion formation.

Recent direct evidence for the role of oxidative stress in atherosclerosis comes from studies with apoE-/-mice, which are good models of oxidative stress and spontaneously develop atherosclerosis similar to that found in humans. F_2-isoprostanes (10–13), considered direct measures of oxidative stress, have been found to localize in foam cells in atherosclerotic lesions and are significantly increased in the tissue, plasma, and urine of apoE knockout mice (12, 13).

Inflammation and Atherosclerosis. A growing body of evidence implicates inflammatory reactions playing a major role in the development of atherosclerosis, thus, clearly supporting atherosclerosis as an inflammatory disease (2, 14). Major cellular participants in atherosclerosis include monocytes, macrophages, active vascular endothelium, T lymphocytes, platelets, and smooth muscle cells.

Monocytes and macrophages are critical cells present at all stages of atherogenesis and, when stimulated, can produce biologically active mediators that have a profound influence on the progression of atherosclerosis. Monocytes promote the peroxidation of lipids such as LDL through the generation of reactive oxygen species. Monocytes and macrophages secrete several proinflammatory, pro-atherogenic cytokines, such as IL-1β and TNF-α, which have been shown to be present in the atherosclerotic lesion and are known to augment monocyte-endothelial adhesion. Although IL-1β has been shown to stimulate procoagulant activity, promote cholesterol esterification in macrophages, and stimulate smooth muscle proliferation *via* platelet-derived growth factor, TNF-α has been shown to contribute to necrotic core by promoting apoptosis of macrophages and smooth muscle cells (15–17). Macrophages also release tissue factor, the major initiator of the blood coagulation cascade (15).

Atherosclerosis is associated with impaired endothelial cell (EC) function, and these changes attract and activate transendothelial migration of monocytes (18). Both IL-1β and TNF-α stimulate expression of adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1), intercellular cell adhesion molecule-1 (ICAM-1), and E-selectin (19, 20). Several studies have shown a strong association between levels of soluble CAMs and coronary as well as carotid atherosclerosis (21–24).

Many stimuli in response to inflammation, growth, and chemotactic factors from neighboring endothelial cells, monocytes, macrophages, and platelets induce smooth muscle cell (SMC) migration and subsequent proliferation, thereby resulting in narrowing of the lumen (20). SMC proliferation represents a significant central event in the fibrous plaque formation. The earliest event following plaque fissure is the adhesion and aggregation of platelets leading to thrombus formation. Increased platelet aggregation contributes to the development of atherosclerosis and increases the risk of myocardial infarction (MI). The inflammatory response of atherosclerosis is mediated by specific subtypes of T lymphocytes at every stage. Thus, there is a complex interaction of a wide variety of cells, and their activation leads to release of hydrolytic enzymes, cytokines, chemokines, and growth factors that can result in further injury.

α-Tocopherol

Dietary micronutrients with antioxidant properties may play an important role in the prevention of atherosclerosis. Vitamin E is the collective name for molecules that exhibit the biological activity of α-tocopherol. Both naturally occurring and synthetic forms of vitamin E are present. Naturally occurring forms of vitamin E include four tocopherols and four tocotrienols (α, β,γ, and δ). Tocotrienols differ from tocopherol in that they have an unsaturated side chain and are potent cholesterol-lowering agents and antioxidants in animal bioassay systems; however, only α-tocotrienol has been shown to possess antioxidant activity, and the purified tocotrienols, in a single human study, do not affect cholesterol levels (25). Synthetic vitamin E consists of eight sterioisomers. Since Trolox is a synthetic water-soluble tocopherol analog, and in humans lipid-soluble AT is carried in lipoprotein and delivered to tissues, studies with Trolox should be interpreted with caution.

α-Tocopherol (AT), a chain-breaking antioxidant that traps peroxyl free radicals, is the principal and most potent lipid-soluble antioxidant in plasma and LDL. AT has proven to be effective in preventing lipid peroxidation and other radical-driven oxidative events (26). Several lines of evidence support a relationship between low AT levels and the development of atherosclerosis (27–30).

Animal Studies. Though the earliest animal experiments yielded equivocal results with AT, most of the later studies have supported slow progression and prevention of atherosclerosis with AT. Verlangieri *et al.* (31) reported 35% inhibition of atherosclerotic lesion formation in cholesterol-fed macaques with AT supplementation as assessed by carotid Doppler studies over a 3-year period, whereas in another study of cholesterol-fed rabbits, Prasad *et al.* (32) reported 70% inhibition of atherosclerotic lesion formation with 40 mg/kg/day AT. Reduced restenosis after angioplasty in rabbits with established experimental atherosclerosis was seen following AT supplementation (33). In this study after 3 weeks following angioplasty, minimum luminal diameter was seen to decrease and the cross-sectional area of the intima-media was greater in the untreated group than in the group receiving AT 19 days before angioplasty ($P < 0.001$). In another study (34), dietary AT brought about a hypocholesterolemic response and conferred on LDL significant protection against oxidative modification in modified Watanabe rabbits. Either or both of these factors contributed to the inhibition of early aortic lesion development. In addition, Pratico *et al.* (35) reported that apoE knockout mice had increased atherogenesis and increased F_2-isoprostanes in urine, plasma, and vascular tissue; supplementation with AT (2000 IU/kg chow) significantly reduced

isoprostanes generation and also aortic lesion but had no effect on plasma cholesterol levels. This is indicative of apoE-deficient mice having increased oxidative stress, which can be suppressed by AT. Also, chickens fed high doses of AT had reduced concentrations of plasma peroxides and less aortic intimal thickening compared with controls (36). The most persuasive data in animal models, of atherosclerosis showing a significant decrease in the degree of LDL oxidation and the extent of atherosclerotic lesions, comes from studies with antioxidants like AT, probucol, N,N-diphenyl phenylenediamine (DPPD), and butylated hydroxytoluene (BHT). However, the side effects of probucol and the toxicity of DPPD and BHT limit their use (37). Thus, the focus has been on AT.

Epidemiological Studies. In 1991 the MONICA study, a cross-sectional study of 16 European populations, showed a significant correlation between AT concentrations and mortality in coronary artery disease ($P < 0.002$) (38). Three large prospective, epidemiologic studies that included the Nurses Health Study, which investigated 87,245 nurses, the Health Professionals Follow-Up Study, which investigated 39,910 male health professionals, and an older U.S. population study composed of 11,178 elderly individuals all found that AT supplementation reduced the risk of coronary artery disease. Both the Nurses and the Health Professionals studies found that subjects in the highest quintile of AT intake had about 40% reduction in cardiovascular disease (CVD) (28, 39). In the elderly, AT supplement use was associated with a 41% reduction in coronary artery disease (CAD) mortality and a 37% reduction in total mortality (30). Recently Kushi (40) reported a risk reduction of coronary mortality in 21,809 women from food-derived AT intake (>9.64 IU/day) and not from supplements. However, the number of women on supplements was small. Two other studies lend support to these earlier observations. One longitudinal study involving 5133 Finnish men and women reported an inverse association between dietary AT intake and coronary mortality (32% risk reduction) (41). A Canadian study of 2226 men also reported a significant risk reduction for subjects using AT (42). Retrospective evaluation of clinical trials like the Cholesterol-Lowering Atherosclerosis Study (CLAS), a randomized placebo–controlled study, provided additional support that coronary artery lesion progression was lowered with AT >100 IU/day (43). Azen (44) studying the progression of carotid atherosclerosis using ultrasound showed an effect in the placebo group opposite from results obtained in the coronary arteries by Hodis *et al.* (43) in the same CLAS study.

Intervention Studies. The ATBC trial was the first double-blind randomized intervention trial in which AT (50 mg/day) and β-carotene (20 mg/d) alone and in combination were given to reduce the incidence of lung cancer in a high-risk group of male smokers in Finland. Although AT had no effect on the primary end point (lung cancer), AT supplementation significantly reduced cerebral infarction and onset of angina but increased the risk of subarachnoid hemorrhages (45, 46). Further, AT supplementation (400 or 800 IU/day) compared with placebo, was shown to reduce nonfatal myocardial infarction by 77% in 2002 patients with angiographically proven coronary artery disease in the Cambridge Heart Antioxidant Study (CHAOS) (47). Also, Steiner *et al.* (48) have shown in a double-blind randomized study in 100 patients with transient ischemic attacks, that the group receiving AT (400 IU/day) in addition to aspirin had significantly decreased platelet adhesion and lower incidence of recurrent transient ischemic attacks and ischemic strokes than patients receiving aspirin alone. In a placebo controlled trial (49) DeMaio showed a decrease in restenosis rate with 1200 IU/day of AT ($P < 0.06$). Another Japanese study over a period of 6 years showed no abnormalities in ECG ($P < 0.02$) in the high AT intake group (50). Furthermore, the GISSI trial showed that in 2830 patients who had prior myocardial infarction and were on a Mediterranean diet (which is enriched with antioxidants), supplementation with all rac-AT (272 IU/day for 3.5 years) resulted in the following significant effects when the more appropriate 4-way analysis was undertaken: 20% reduction in cardiovascular deaths, 23% reduction in cardiac death, 25% reduction in coronary death, 35% reduction in sudden death despite the primary end point not being statistically significant (51). The Heart Outcomes Prevention Evaluation (HOPE) Study was recently published in which 2545 women and 6696 women 55 years or older who were at high risk for CAD events since they had CAD or diabetes in addition to one other risk factor were enrolled and randomized to receive 400 IU of AT from natural sources or placebo and either an angiotension converting enzyme (ACE) inhibitor or matching placebo for a mean of 4.5 years (52). Primary outcome was a composite of myocardial infarction, stroke, and CAD death. There were no significant differences in primary or secondary outcome variables in the subjects taking AT. However, this study was undertaken in many countries; dietary intakes especially of antioxidants were not reported, and no objective measures of supplementation (e.g., plasma levels of AT).

Although large cohort studies widely support the role of AT as an antioxidant against the proatherogenic and prothombotic effects of LDL oxidation, controlled trials on the other hand produced variable results. It is important to note that in CHAOS, GISSI, or HOPE that used higher doses of AT than in the ATBC trial, there was no increase in hemorrhagic strokes.

AT and Antioxidant Function

Oxidation. Several groups have also shown that α-tocopherol inhibits LDL oxidation initiated by copper *in vitro* (53, 54) or by cells in culture (55). Esterbauer *et al.* (56) have shown that increasing LDL AT *in vitro* can prolong the lag phase of oxidation. Human studies have demonstrated that AT supplementation can reduce the susceptibility of LDL to oxidation (56–58). The minimum dose of

AT required to obtain a beneficial effect on LDL was found to be 400 IU/day (56, 58).

AT may have additional benefits for cardiovascular disease. α-Tocopherol supplementation may slow the progression of atherosclerosis by reducing oxidative stress, thereby decreasing lipid peroxidation and LDL oxidative susceptibility. In human subjects AT supplementation (100–600 mg/day for 2 weeks) has been shown to lower urinary F_2-isoprostanes by 34%–36% in hypercholesterolemic subjects and in diabetic individuals (59, 60). Also, in a recent report, our group has shown that supplementation of healthy adults with 400 IU/day RRR-α-tocopherol for 8 weeks resulted in lower levels of urinary F_2-isoprostanes (61).

AT-Molecular and Cellular Effects

AT has antiatherogenic effects on cells crucial in atherogenesis (62) as outlined below.

AT and EC. Adhesion of monocytes to the endothelium stimulates the expression of endothelium-derived adhesion molecules like endothelial leukocyte adhesion molecule (ELAM), ICAM-1 and VCAM-1, which aid in monocyte adhesion and their subsequent migration into the intima where monocytes differentiate into macrophages (9). Faruqi *et al.* (63) observed that when endothelial cells were cultured in the media containing AT, there was less agonist-induced adhesion of monocytes to EC which correlated with a decrease in message and cell surface expression of E-selectin. Cominacini *et al.* (64) have shown that pretreatment of endothelial cells (HUVEC) with AT significantly reduced the expression of the adhesion molecules ICAM-1 and VCAM-1 on HUVEC induced by oxidized LDL. Martin *et al.* (65) showed that *in vitro* enrichment of human aortic endothelial cells with AT significantly inhibited LDL-induced adhesion of monocytes to EC in a dose-dependent manner with a concomitant reduction in levels of ICAM.

A mechanism that may link inflammation and oxidative stress in atherosclerosis is the transcription factor NF-κ B, which is directly involved in the activation of genes responsible for inflammation. The importance of activated NF-κB in atherosclerosis has been demonstrated in smooth muscle cells, macrophages, and endothelial cells of human atherosclerotic lesion tissue, but not in normal vessels (66, 67). A variety of genes included in atherosclerotic lesion, are regulated by NF-κB, such as genes coding for ICAM-1 and VCAM-1 and cytokines. Schreck *et al.* (68) have suggested a novel signal transduction pathway for NF-κB ctivation involving reactive oxygen species as second messengers, thus linking oxidative stress, inflammation, and atherosclerosis. Data from our laboratory has shown that pretreatment of monocytic cells with AT resulted in a decrease in monocyte-endothelial cell adhesion mediated by decreased expression of CD11b and VLA-4, possibly by inhibiting the activation of NF-κB (69). α-Tocopherol acetate and succinate have also been shown to inhibit TNF-α–induced NF-κB activation *in-vitro* (70). Thus AT has been shown to have beneficial effects in inhibiting monocyte-endothelial adhesion when incubated with either EC or monocytes, and it is very likely that following supplementation it partitions into both monocytes and EC, and its ability to reduce monocyte-EC adhesion is greater.

In atherosclerosis Ox-LDL accumulates in the vascular wall where it is cytotoxic and chemotactic for monocytes, leading to the production of oxygen-derived free radicals that can inactivate endothelium-derived nitric oxide. Keaney *et al.* (71) and Stewart-Lee *et al.* (72) showed that AT supplementation preserved endothelium-dependent vasorelaxation in cholesterol-fed rabbits by a mechanism independent of its effect on serum lipoproteins, and this did not correlate with lipoprotein oxidation. Furthermore, Keaney *et al.* (73) showed that AT incorporation in the artery wall prevents endothelial dysfunction caused by Ox-LDL, and this effect is mediated independent of its antioxidant effect. AT supplementation at high doses has been shown to preserve endothelium-dependent vasorelaxation in hypercholesterolemic men and smokers (74, 75). Arterial compliance or elasticity is a potential index of arterial function that has been shown to be dependent upon endothelial function (76). Although structural changes play a role in increased arterial stiffening, the functional component has been shown to be reversible with dietary interventions. Short-term AT supplementation (400 IU/day for 4 and 8 weeks) has been recently reported to improve arterial compliance in middle-aged men and women (77).

AT and Platelets. AT modulates platelet adhesion and aggregation. Higashi and Kikuchi (78) were the first to demonstrate the inhibitory effect of AT on platelet aggregation *in vitro*. The effectiveness of AT as an anti aggregatory agent *in vitro* stands in contrast to its *in vivo* effect. Also Steiner (79) have shown that AT in ≥doses 200 IU/day decreases platelet adhesion. In hypercholesterolemic subjects, 2 weeks of supplementation with AT (600 mg/day) reduced elevated plasma concentrations of the platelet-derived adhesion molecule, P-selectin by 40% (80). Furthermore, Steiner (81) have shown that at doses of 1200 IU/day, AT produced only a mild inhibition of collagen-induced platelet aggregation whereas platelet adhesion to collagen was markedly inhibited in its presence. Also, in another study, the same authors showed that in 100 patients with transient ischemic attacks, there was a significant reduction in platelet adhesion in patients given 400 IU/day of AT (50). A double-blind, randomized, placebo-controlled study was performed on 40 healthy volunteers (20–50 years) supplemented daily with vitamin E (300 mg), vitamin C (250 mg), or β-carotene (15 mg) for 8 weeks (82). Platelet function was significantly decreased by vitamin E as revealed by the decreased platelet aggregation in response to ADP and arachidonic acid, the increased sensitivity to inhibition by PGE_1, the decreased plasma β-thromboglobulin concentration and the decreased ATP secretion (82). Freedman *et al.* (83) have shown that supplementation with 400 IU of AT inhibits platelet aggregation through a PKC-

dependent mechanism. In a recently published study, Mabile *et al.* (84) showed that the AT uptake by platelets is optimal at 75 IU/day, and this correlates with the maximal influence on platelet aggregation and platelet responsiveness to inhibition by PGE_1. Increased supplemental levels (200, 400 IU/day) failed to exert greater effects.

AT and Monocytes/Macrophages. AT has been shown to inhibit macrophage-mediated lipid oxidation *in vitro*. However, there is limited data on the effect of AT on monocyte function. Our group has shown that supplementation with 1200 IU/day of AT significantly influenced monocyte function by decreasing lipid oxidation, release of O_2- and hydrogen peroxide, decreasing release of the pro-atherogenic cytokine, IL-1β, as well as decreasing monocyte-endothelial cell adhesion. (1200 IU/day significantly reduced superoxide anion release by 45%, hydrogen peroxide release by 40%, lipid oxidation by 40%, release of IL-1β by 90% as well as monocyte-endothelial cell adhesion by 35% (85). Also, we have recently shown that monocytes from type II diabetic subjects with and without macrovascular disease are more pro-atherogenic than matched controls as assessed by increased levels of superoxide and interleukin-1β and greater adhesion to endothelium. AT supplementation (1200 IU/day) significantly reduced monocyte-proatherogenic activity. In addition, AT supplementation also resulted in a significant inhibition of levels of soluble adhesion molecules, ICAM, VCAM and E-selectin (86).

One common mechanism to account for AT's effects may be through protein kinase C (PKC) inhibition (87). PKC plays a major role in signal transduction and has been implicated in a variety of events ranging from respiratory burst and platelet aggregation to cellular differentiation. Many oxidant-initiated signaling processes are known to involve PKC. It has been shown previously that O_2- release and lipid oxidation are mediated *via* PKC (88). Our group has shown that AT decreases monocyte superoxide anion release and lipid oxidation, and this appears to be mediated by inhibition of protein kinase C. Furthermore, results from an *in vitro* study by Cachia *et al.* (89) revealed that AT inhibits superoxide production by monocytes by impairing the assembly of NADPH oxidase, the enzyme responsible for generating the respiratory burst. AT inhibits p47phox translocation to the membrane and also impairs phosphorylation of p47phox. This study also suggests that inhibition of PKC activity is not due directly to the antioxidant capacity of AT, but requires AT integration into the cell membrane where it can interact directly with PKC.

With regard to cytokine release, we have shown that AT inhibits IL1 release from activated human monocytes by inhibiting 5-lipoxygenase at the post-transcriptional levels (90). Also, as discussed previously, we have shown that α-tocopherol enrichment of monocytes inhibits subsequent adhesion to human endothelium *via* inhibition of counter receptors, CD11β and VLA-4 on the monocytes and inhibition of the transcription factor, NF-κB (69).

Also, AT has recently been demonstrated to downregulate scavenger activity (SR) activity in human blood–derived macrophages *in vitro*, whereas γ-tocopherol, a homolog of AT, showed only a weak suppression of SR activity, SR- class A expression, and AP-1 activity (91).

T and SMC. AT has been reported to inhibit SMC proliferation in culture. The antiproliferative effects of AT have been well demonstrated in rat aortic SMC stimulated with platelet derived growth factor (PDGF) (92–96), and the effect has not been related to its antioxidant effect (92–96). These studies collectively suggest that AT inhibits SMC proliferation *in vivo* and thus retards narrowing of the artery lumen. The role of AT in cellular signaling especially in relation to PKC has been delineated by Azzi *et al.* (87). They have shown that this effect is not related to ATs antioxidant effects because only RRR-α-tocopherol, and not β-tocopherol, binds to a receptor resulting in activation of AP-1 leading to the dephosphorylation of PKC even though both have similar antioxidant activity. In fact β-tocopherol has been reported to actually abrogate the α-tocopherol effect. Thus RRR-AT appears to act as a sensor of the oxidation status of the cell and as a transducer capable of informing cells of the oxidation status. There is compelling data suggesting that antioxidant activity alone cannot mediate PKC inhibition (92–96). Direct inhibition of PKC by AT does not appear likely as several studies showed no effect on diacylglycerol or calcium-stimulated PKC α and β-2 activity (97–100). An indirect mechanism for AT inhibition of PKC seems more likely and data to support this come from smooth muscle cells where PKC β2 is activated by hyperglycemia and AT inhibits this effect by decreasing cellular DAG levels (97–100) through stimulation of DAG kinase activity. Also it has been demonstrated that okadaic acid prevents the antiproliferative effect of AT in SMC proliferation clearly indicating that PKC phosphorylation and/or protein phosphatase activity is involved. Evidence for AT inhibition of SMC proliferation is mostly *in vitro,* and there are few data available *in vivo.* De Maio *et al.* (49) reported a trend in patients following percutaneous transluminal coronary angioplasty (PTCA) supplemented with 1200 IU/day for 4 months, indicating that AT supplementation reduces the incidence of coronary artery restenosis after angioplasty. The ability of AT to reduce restenosis after angioplasty was further tested in a rabbit model in which angioplasty was performed on established atherosclerotic lesions (33). Ox-LDL stimulated DNA synthesis in rabbit vascular smooth muscle cells, and AT was found to inhibit this effect. These findings support the hypothesis that oxidized lipids can stimulate hyperplasia, and AT limits

Table I. Effects of α-Tocopherol Supplementation

Antioxidant activity (LDL oxidation, F2-isoprostanes)
Inhibition of platelet aggregation
Anti-inflammatory effects (inhibits monocyte pro-atherogenic activity)
Improvement in EDRF activity

this effect by inhibiting either oxidation or the proliferative effects of oxidants on cells.

Thus, in addition to AT being a potent antioxidant as evidenced by decreased LDL oxidative susceptibility and F_2-isoprostanes, it has additional biological effects such as inhibition of platelet aggregation, decreasing monocyte pro-atherogenic activity, and improving endothelial function.

It is clear that AT demonstrates a multifaceted effect promoting vascular homeostasis. *In vivo* supplementation with AT has clearly shown that it is an antioxidant that inhibits platelet aggregation and is anti-inflammatory (Table I). Though we are beginning to unravel the molecular mechanisms by which AT acts, precise mechanistic understanding still eludes us. The epidemiological evidence from studies with AT, though strong, needs to be confirmed by clinical trials before treatment strategies can be formulated for both primary and secondary prevention.

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