Annu. Rev. Nutr. 2005. 25:151–74
doi: 10.1146/annurev.nutr.24.012003.132446

VITAMIN E, OXIDATIVE STRESS, AND INFLAMMATION

U. Singh, S. Devaraj, and I. Jialal
Laboratory for Atherosclerosis and Metabolic Research, University of California Davis Medical Center, Sacramento, California 95817;
email: ishwarlal.jialal@ucdmc.ucdavis.edu, sridevi.devaraj@ucdmc.ucdavis.edu, usingh@ucdavis.edu

Key Words α-tocopherol, oxidation, antioxidant, CRP, atherosclerosis

■ **Abstract** Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in the Western world. Its incidence has also been increasing lately in developing countries. Several lines of evidence support a role for oxidative stress and inflammation in atherogenesis. Oxidation of lipoproteins is a hallmark in atherosclerosis. Oxidized low-density lipoprotein induces inflammation as it induces adhesion and influx of monocytes and influences cytokine release by monocytes. A number of proinflammatory cytokines such as interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α) modulate monocyte adhesion to endothelium. C-reactive protein (CRP), a prototypic marker of inflammation, is a risk marker for CVD and it could contribute to atherosclerosis. Hence, dietary micronutrients having anti-inflammatory and antioxidant properties may have a potential beneficial effect with regard to cardiovascular disease. Vitamin E is a potent antioxidant with anti-inflammatory properties. Several lines of evidence suggest that among different forms of vitamin E, α-tocopherol (AT) has potential beneficial effects with regard to cardiovascular disease. AT supplementation in human subjects and animal models has been shown to decrease lipid peroxidation, superoxide (O_2^-) production by impairing the assembly of nicotinamide adenine dinucleotide phosphate (reduced form) oxidase as well as by decreasing the expression of scavenger receptors (SR-A and CD36), particularly important in the formation of foam cells. AT therapy, especially at high doses, has been shown to decrease the release of proinflammatory cytokines, the chemokine IL-8 and plasminogen activator inhibitor-1 (PAI-1) levels as well as decrease adhesion of monocytes to endothelium. In addition, AT has been shown to decrease CRP levels, in patients with CVD and in those with risk factors for CVD. The mechanisms that account for nonantioxidant effects of AT include the inhibition of protein kinase C, 5-lipoxygenase, tyrosine–kinase as well as cyclooxygenase-2. Based on its antioxidant and anti-inflammatory activities, AT (at the appropriate dose and form) could have beneficial effects on cardiovascular disease in a high-risk population.

CONTENTS

INTRODUCTION

Atherosclerosis is the leading cause of mortality in the Western world. Oxidative stress and inflammation are important in the pathogenesis of atherogenesis. Epidemiological studies suggest an association between increased antioxidant intake, especially vitamin E, and reduced morbidity and mortality from coronary artery disease. The focus in this review is on α-tocopherol (AT), the major and most bioavailable form of vitamin E.

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 (Figure 1). 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 and proliferation of monocytes into macrophages. The initial interest in a role for lipid oxidation in the development of atherosclerotic lesions was in its ability to modify LDL sufficiently to promote its uptake by macrophages. The extensively modified LDL (oxidized LDL, or ox-LDL) is not recognized by the LDL receptor but is taken up avidly by the scavenger receptor pathway in macrophages, leading to appreciable cholesterol ester accumulation and foam cell formation (131).

Ox-LDL has several biological consequences (52, 56, 107, 134); it is proinflammatory, it causes inhibition of endothelial nitric oxide synthase (eNOS), it promotes vasoconstriction and monocyte adhesion, it stimulates cytokines such

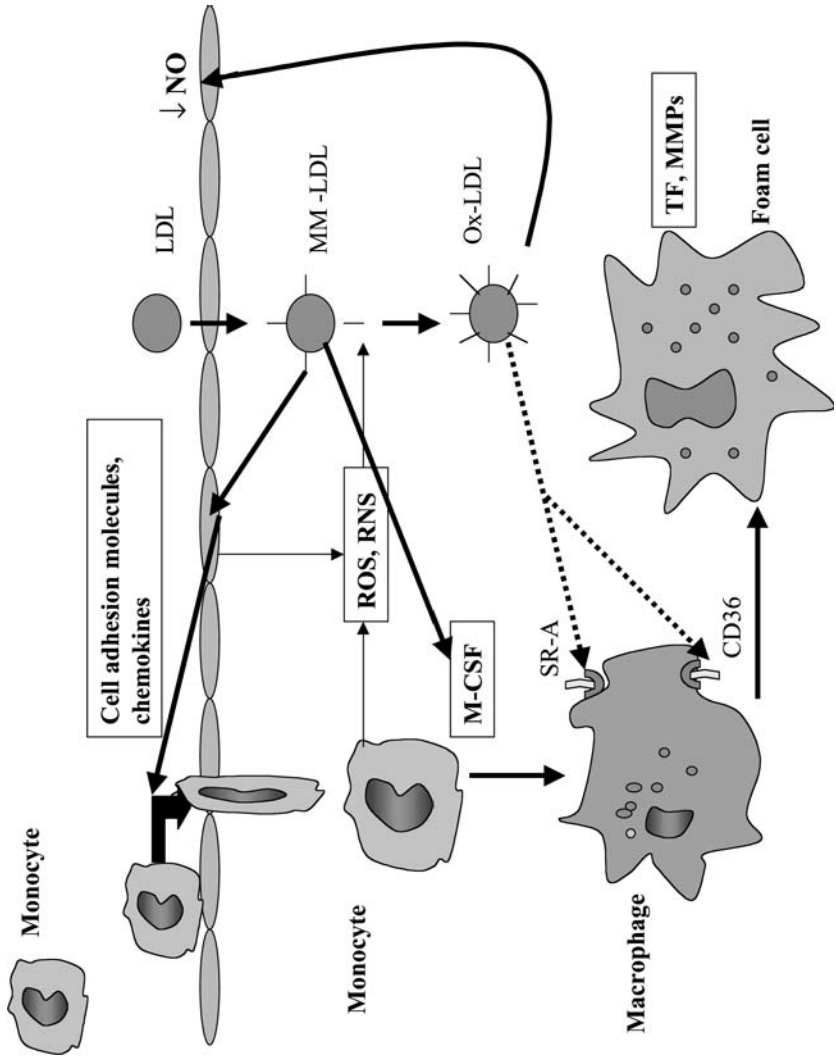

Figure 1 A schema depicting the role of oxidized LDL in atherosclerosis.

as IL-1, and it increases platelet aggregation. Ox-LDL also has been shown to upregulate vascular endothelial growth factor (VEGF) expression in macrophages as well as endothelial cells (ECs) through activation of peroxisome proliferator-activated receptor-γ (PPAR-γ) (44). Ox-LDL-derived products are cytotoxic and can induce apoptosis. Ox-LDL can adversely affect coagulation by stimulating tissue factor (TF) and plasminogen activator inhibitor-1 (PAI-1) synthesis (47). Another atherogenic property of ox-LDL is its immunogenicity and its ability to promote retention of macrophages in the arterial wall by inhibiting macrophage motility (47). Several factors may influence the susceptibility of LDL to oxidation, including its site and composition and the presence of endogenous antioxidant compounds (e.g., AT).

The more direct evidence for the role of oxidative stress in atherosclerosis comes from studies with apoE−/− mice that spontaneously develop atherosclerosis similar to that found in humans. F_2-isoprostanes, prostaglandin-like products of the free radical–catalyzed peroxidation of arachidonic acid and an established biomarker of in vivo lipid peroxidation (18, 84, 92), have been found to localize in foam cells in atherosclerotic lesions of humans as well as of animals and are significantly increased in the tissue, plasma, and urine of apoE knockout mice (38, 88). In addition to serving as biomarkers of in vivo oxidative stress, F_2-isoprostanes, including 8-epi$PGF_{2\alpha}$, exert (patho)physiological effects such as vasoconstriction (92). Both reactive oxygen species [superoxide anion (O_2^-), hydroxyl radical ($OH^\cdot$), and hydrogen peroxide (H_2O_2)] and reactive nitrogen species [nitric oxide (NO) and peroxynitrite ($ONOO^\cdot$)] have been implicated in atherogenesis (107). Although the phagocytic NAD(P)H oxidase is the major source of reactive oxygen species (ROS) in the circulatory system, vascular ECs, smooth muscle cells (SMCs), and fibroblasts also express functional leukocyte-type NAD(P)H oxidases (54). Five major components comprise the endothelial NAD(P)H oxidase: $gp91^{phox}$ (and/or its homologues) and $p22^{phox}$ in the membrane, and $p47^{phox}$, $p67^{phox}$, and Rac in the cytosol, and it has been suggested to be the major source of ROS in these cells (107). $p47^{phox}$ in ECs has been shown to play an essential role in activation of NAD(P)H oxidase and in the production of O_2^- (107). Nitric oxide is released from ECs and has many beneficial effects against atherosclerosis through inhibition of platelet aggregation, SMC proliferation, leukocyte recruitment, and stimulation of vasodilation. Excess O_2^- reacts with $NO^\cdot$ to form $ONOO^\cdot$ within the vessel, leading to vascular dysfunction. The decreased bioactivity of NO in the vascular wall provides evidence that the generation of $ONOO^\cdot$ may be involved in the development of atherosclerosis (107). Furthermore, treatment of hypercholesterolemic rabbits with liposomal or polyethylene glycol-conjugated superoxide dismutase, but not with native superoxide dismutase, improves vascular responses (78). This confirms a role for oxidative stress in vascular dysfunction (73).

Inflammation and Atherosclerosis

Much evidence supports a pivotal role for inflammation in all phases of atherosclerosis, from the initiation of the fatty streak to the culmination in acute coronary

syndromes (plaque rupture) (68, 93). Various noxious insults, including hypertension, diabetes, smoking, dyslipidemia, and hyperhomocysteinemia, can result in EC dysfunction. Major cellular participants in atherosclerosis include monocytes, macrophages, activated vascular endothelium, T lymphocytes, platelets, and SMCs.

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 (Figure 1). Monocytes promote the peroxidation of lipids such as LDL through the generation of ROS. Monocytes and macrophages secrete several proinflammatory, proatherogenic cytokines, such as IL-1β, TNF-α, and IL-6, which have been shown to be present in the atherosclerotic lesions and are known to augment monocyte-endothelial adhesion. IL-1β has been shown to stimulate procoagulant activity, promote cholesterol esterification in macrophages, and stimulate SMC proliferation via platelet-derived growth factor (PDGF). Supportive evidence for the central role played by IL-1 in the development of atherosclerosis has been recently documented by Kirii et al. (60), who demonstrated the decreased severity of atherosclerosis in apoE$-/-$ mice deficient for IL-1β. TNF-α has been shown to promote monocyte adhesion to endothelium and contribute to the necrotic core by promoting apoptosis of macrophages and SMCs (50). Activated macrophages also release matrix metalloproteinases (MMPs) that cause a rent in the endothelium and tissue factor that promotes thrombus formation.

Atherosclerosis is associated with endothelial dysfunction, and these changes induce adhesion and transendothelial migration of monocytes (50). 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. Chemotaxis and entry of monocytes into the subendothelial space is promoted by monocyte chemoattractant protein-1 (MCP-1), interleukin-8 (IL-8), and fractalkine. Several studies have shown a strong association between levels of soluble CAMs (which are shed from activated cells such as ECs) and coronary as well as carotid atherosclerosis (100).

Many stimuli (e.g., angiotensin II and PDGF) are released in response to inflammation, growth, and chemotactic factors from neighboring ECs, monocytes, macrophages, and platelets. These induce SMC migration and subsequent proliferation, thereby resulting in the formation of the fibrous cap (50). The earliest event following plaque fissure is the adhesion and aggregation of platelets leading to thrombus formation. Increased platelet aggregation contributes to acute coronary syndrome such as myocardial infarction. Specific subtypes of T lymphocytes also mediate the inflammatory response of atherosclerosis 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.

Several large population studies have indicated that biomarkers of inflammation predict an increased risk for CVD (50, 68, 93). The prototypic marker of inflammation is C-reactive protein (CRP), a member of the pentraxin family

(48, 50). Its synthesis in the liver is triggered by various proinflammatory cytokines derived from numerous sources, including monocytes, macrophages, and adipose tissue. The proinflammatory response includes an increased secretion of IL-1β and TNF-α, which then results in the release of the principal messenger cytokine, IL-6 from macrophages. IL-6, after engagement of its receptor on the liver, results in the secretion and release of CRP. Recent evidence points to the role of vascular cells, such as ECs (125) and SMCs (135), in the production of CRP. CRP mRNA and protein have been shown to be expressed in the cells of the lesion in an order of magnitude higher than that observed in plasma (61, 135).

Numerous prospective studies from populations throughout the world have shown that elevated levels of CRP confer a greater risk of CVD (for a recent review, see Reference 50). In addition to being a risk marker, a large body of evidence points to a proatherogenic role of CRP in vascular SMCs, monocyte-macrophages, and ECs. In monocytes, CRP induces the production of inflammatory cytokines and TF expression as well as promotes uptake of ox-LDL. CRP upregulates endothelin-1, PAI-1, and chemokines such as MCP-1 and IL-8, as well as increases expression of CAMs. CRP downregulates synthesis and bioactivity of eNOS (50) and decreases another potent vasodilator and inhibitor of platelet aggregation, prostacyclin (PGI_2) release from aortic ECs via increased ONOO formation, resulting in nitration of PGI_2 synthase, which renders the enzyme inactive (123). In addition to the numerous reported proatherogenic properties of CRP in in vitro studies, there is direct evidence for a proatherosclerotic and prothrombotic effect of CRP in vivo. The exposure to CRP (200 ug/ml) resulted in an increase in SMC migration and proliferation, collagen and elastin content, and AT_1-R expression, as well as an increase in neointimal formation in a rat carotid angioplasty model (128). CRP also has been shown to promote arterial thrombosis following femoral injury in transgenic mice that express the human CRP gene (15). Further, human CRP Tg mice in apoE−/− background also have been shown to have increased CRP levels and a modest increase in aortic atherosclerosis in male mice only (83). Inflammation (as manifested by an increase in CRP) is not only increased in CVD but also in diseases with increased cardiovascular risk, e.g., end-stage renal disease (ESRD) (42), metabolic syndrome (91), and diabetes (87).

Vitamin E

CHEMICAL FORM AND ABSORPTION The term "vitamin E" covers eight different forms of the vitamin that are produced by plants alone and have similar chromanol structures: trimethyl (α-), dimethyl (β- or γ-) and monomethyl (δ-) tocopherol, and the corresponding tocotrienols (T3) (116). T3 have an unsaturated side chain, whereas tocopherols contain a saturated phytyl tail with three chiral centers that naturally occur in the *RRR* configuration (110). Commercially available vitamin E consists of either a mixture of naturally occurring tocopherols and tocotrienols; *RRR*-AT (formerly called *d*-AT); synthetic AT (*all rac*-AT, formerly called *dl*-AT), which consists of the eight possible stereoisomers in equal amounts; or their

esters. The ester form prevents the oxidation of vitamin E and prolongs its shelf life. Except for individuals with malabsorption syndromes, these esters are readily hydrolyzed in the gut and are absorbed in the unesterified form (116). The natural vitamin E sources are vegetable oils: Safflower seed oil contains almost exclusively AT (59.3 mg/1 g oil), soy oil is rich in γ-, δ-, and AT (62.4, 20.4, 11.0 mg/1 g of oil, respectively), and palm oil contains T3 (17.2 mg/1 g of oil) in addition to AT (18.3 mg/1 g oil) (79).

The bioavailability of the different forms of vitamin E is highly differential. For example, although the amount of γ-tocopherol in the diet is higher than that of AT, the plasma γ-tocopherol concentration is only 10% of that of AT, which is the most abundant form in plasma (36). Once ingested, all forms of vitamin E are taken up by intestinal cells and released into the circulation in chylomicrons. The vitamins reach the liver via chylomicron remnants. In the liver, a specific protein, α-tocopherol transfer protein (α-TTP), selectively targets RRR-AT for incorporation into very-low-density lipoprotein. Other forms are much less well retained and are excreted via the bile, the urine (as carboxyethyl hydroxychromans), or unknown routes. Relative affinities of tocopherol analogs for α-TTP, calculated from the degree of competition for the α form, are as follows: α-tocopherol, 100%; β-tocopherol, 38%; γ-tocopherol, 9%; δ-tocopherol, 2% (5). The significance of α-TTP and AT is evident from a recent report showing increased basal oxidative stress and inflammatory status in α-TTP-null mice (Ttpa$-/-$) (97) as well as increased severity of atherosclerotic lesions in these mice in apoE$-/-$ background (113).

In vitro studies demonstrate superior antioxidant properties of AT in the prevention of LDL lipid peroxidation due to its lipid solubility and preferential incorporation into lipoproteins (115). Overall, AT is the principal and most potent lipid-soluble antioxidant in plasma and LDL. AT is present in LDL particle in quantities (5–9 molecules/LDL particle) that can be easily modified by dietary intake or oral supplementation (46). Several lines of evidence support a relationship between low AT levels and the development of atherosclerosis (for a review, see Reference 56). Hence, dietary micronutrients, especially with anti-inflammatory and antioxidant properties (e.g., AT) have potential beneficial effects with regard to cardiovascular disease.

Animal Studies

Animal studies generate useful, and often otherwise unattainable, information on the content of arterial lipids, antioxidants, and lipid oxidation in vivo. Verlangieri & Buxh (127) reported 35% inhibition of atherosclerotic lesion formation in cholesterol-fed macaques with AT supplementation as assessed by carotid Doppler studies over a three-year period. Reduced restenosis after angioplasty in rabbits with established experimental atherosclerosis was seen following AT supplementation (66). Dietary AT led to a hypocholesterolemic and antioxidative response in rabbits (98, 130) as well as to less aortic intimal thickening in chickens

(102). Furthermore, 2000 IU/kg chow of AT supplementation significantly reduced isoprostanes generation and aortic lesion without affecting plasma cholesterol levels in apoE−/− mice. The effect of AT (400 mg/kg atherogenic diet) has been shown not only in reducing aortic lesions in 4- to 10-week-old apoE−/− mice, when fatty streaks are absent or very sparse (86), but also after the establishment of the initial aortic lesion (85), which suggests that this vitamin has a protective effect during the establishment of fatty streaks as well.

Furthermore, in addition to some in vitro studies showing the influence of AT supplementation on the production of MCP-1 (132), there also exists in vivo evidence for the increased expression of MCP-1 in aortic lesions of apoE−/− mice (86). Thus, one of the favorable effects of AT is changing the expression of some important chemotactic molecules such as MCP-1; this suggests the efficacy of vitamin E in reducing fatty streak formation by reducing cell migration to the lesion site (85).

α-Tocopherol Supplementation in Humans

Several groups have also shown that AT supplementation decreases LDL oxidation initiated by copper in vitro (52, 90) or by cells in culture (103). Esterbauer et al. (32) 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 (21, 53). The minimum dose of AT required to obtain a beneficial effect on LDL was found to be 400 IU/d (21, 32, 51).

In human subjects, AT supplementation (100–600 mg/d for two weeks) has been shown to lower urinary F_2-isoprostanes by 34%–36% in hypercholesterolemic subjects and in diabetic individuals (16, 17). Our group has shown that supplementation of healthy adults with 400 IU/d *RRR*-AT for eight weeks resulted in lower levels of urinary F_2-isoprostanes (70). However, other studies (129) have suggested there is a pro-oxidant effect of vitamin E (400 IU *dl*-AT acetate) in cigarette smokers consuming a high (20%) polyunsaturated fat (PUFA) diet. Although the supplementation of vitamin E prolonged mean LDL oxidation lag time, it paradoxically increased F_2-isoprostanes as well as $PGF_{2\alpha}$. These data suggest that vitamin E may function as a pro-oxidant in cigarette smokers consuming a high-PUFA diet that is far in excess of the normal American diet. In another study carried out by same group (96), the PUFA diet consumption for three weeks increased the mean HDL2 lag time ∼1.8-fold with no change in oxidation rate. Supplementation of vitamin E (800 IU/d for three weeks as *dl*-AT acetate) further increased the HDL2 lag time ∼1.3-fold and decreased the HDL2 oxidation rate ∼1.3-fold. Hence, vitamin E supplementation reduces the oxidation susceptibility of HDL2, which suggests that vitamin E could influence HDL function in vivo (2). The timing of antioxidant intake has been suggested to be a variable factor on postprandial (e.g., a McDonald's Big Mac meal) markers of inflammation and fibrinolysis (12). The measurement of CRP, IL-6, PAI-1, malondialdehyde, and total radical antioxidant parameter four hours before and after the test meal revealed

that there was a significant rise in CRP and PAI-1 after the test supper compared with no meal. Either presupper or prebreakfast vitamins E (800 IU *RRR*-AT) and C (1 g ascorbic acid) significantly prevented the meal-induced rise in CRP, although presupper vitamins were more effective. In contrast, only prebreakfast vitamins significantly prevented the meal-induced rise in PAI-1. However, no significant meal-related changes were found in the concentrations of IL-6, malondialdehyde, or total radical antioxidant parameter.

The supplementation of *all-rac* AT (600 IU/d for four weeks) in diabetic patients and smokers decreased oxidative stress (measured as plasma thiobarbituric acid-reacting substances and lag time of oxidation) in both groups; however, supplementation decreased inflammatory status (as reflected by circulating levels of IL-1, TNF, and IL-1 RA in whole blood) in smokers but not in diabetic patients (75). A subsequent study by Heitzer et al. (40) showed that the long-term supplementation of vitamin E (544 IU/d as *dl*-AT acetate for four months) improved endothelium-dependent relaxation in forearm vessels as well as significantly decreased autoantibodies to ox-LDL in hypercholesterolemic smokers but not in patients with either hypercholesterolemia or chronic smoking. These findings suggested that the beneficial effect of vitamin E might be confined to subjects with increased exposure to oxidized LDL. In this regard, Hodis et al. (43) reported that AT supplementation reduces LDL oxidation without affecting atherosclerosis in healthy individuals. Recently, AT supplementation (400 IU/d of *RRR*-AT acetate for six months) in smokers with acute coronary syndrome (characterized by sustained inflammatory upregulation in terms of the release of proinflammatory cytokines and elevated levels of CRP) has been shown to decrease CRP without affecting other inflammatory biomarkers such as IL-6 or sCAMs (81). Importantly, this was the first report showing an association of AT with a reduction in inflammatory markers in patients with acute coronary syndromes. This finding warrants a larger clinical trial assessing the impact of *RRR*-AT on outcome in this patient population with a sustained elevation in inflammatory markers and a high short-term clinical event rate. The anti-inflammatory activity (measured as decrease in CRP) and antioxidant function (in terms of lag time in oxidation of LDL) has also been reported by Upritchard et al. (118) in patients with diabetes after supplementation of *RRR*-AT (800 IU/d for six weeks).

Interestingly, Van Tits et al. (121), in a clinical trial of *RRR*-AT administration (600 IU/d for six weeks) in primary hypertriglyceridemic ($n = 12$) and normolipidemic subjects ($n = 8$), measured the release of cytokines (TNFα, IL-1β) and the chemokine (IL-8) from peripheral blood mononuclear cells before and after intervention. Following AT supplementation, in vitro cytokine production and IL-8 in response to LPS decreased significantly in both groups. Thus, it is suggested that AT may influence the inflammatory response of immune cells infiltrating subendothelial spaces and hence AT's therapeutic implications become relevant in chronic inflammatory processes such as atherogenesis. This study confirms in vitro reports in the literature that AT inhibited PMA-induced IL-1β expression in human monocyte leukemic cell line THP-1 (1).

In another small clinical trial of patients with ESRD undergoing hemodialysis, oxidative stress (plasma concentrations of vitamin E metabolites, F_2 isoprostanes) as well as inflammatory biomarkers (TNF-α, IL-6, and CRP) was assessed in blood samples obtained from a group of patients (n = 11). Assessments were made before and after dialysis at two occasions prior to, and at one and two months of, daily vitamin E supplementation (400 IU *RRR*-AT) (101). AT supplementation significantly increased plasma AT and decreased γ-tocopherol concentrations. Circulating vitamin E metabolites increased up to tenfold in ESRD patients without affecting plasma IL-6, CRP, TNF, and free F_2-isoprostanes concentration. These findings suggest a complex interrelationship between inflammation and oxidative stress that is not mitigated by short-term vitamin E supplementation. In support of this study, Islam et al. (45) also failed to show any significant effect on any of the parameters studied (autoantibodies to ox-LDL, Mo-EC adhesion, sICAM, and VCAM) by supplementation of *all-rac* AT (800 IU/d for 12 weeks), however, results showed significant enrichment with AT in LDL and increased lag phase of oxidation in chronic renal failure patients.

We have tested the effect of *RRR*-AT supplementation on monocyte function and inflammatory markers. Our group (26, 27) has shown that supplementation with 1200 IU/d AT in normal volunteers (n = 21) as well as in type 2 diabetic patients (T2DM) with and without macrovascular disease (n = 25/group) significantly influenced monocyte function by decreasing lipid oxidation (release of O_2^- and H_2O_2), decreasing release of the proatherogenic cytokine IL-1β, and decreasing monocyte-EC adhesion, clearly documenting that supplementation with *RRR*-AT is anti-inflammatory. In a subsequent report, we documented increased IL-6 release from monocytes and increased serum CRP levels in these diabetic patients (25). Both high-sensitivity CRP and monocyte IL-6 were significantly decreased with AT therapy. This finding was confirmed by another group (118). They showed that *RRR*-AT supplementation (800 IU/d, n = 13) in T2DM compared with placebo (n = 12) for a duration of four weeks resulted in a significant decrease in plasma CRP. AT therapy also decreased serum P-selectin and PAI-1 levels in T2DM patients (22). These findings were suggested to be relevant to strategies aimed at reducing risk of CVD in patients with diabetes.

Furthermore, as discussed above, one of the earliest events in atherosclerosis is endothelial dysfunction. Much of the data obtained from animal studies show that AT supplementation (1000 IU/kg diet of *RRR*-AT) preserved endothelium-dependent vasorelaxation in cholesterol-fed rabbits by a mechanism independent of its antioxidant effect (57, 106). Vascular incorporation of AT has been shown to prevent endothelial dysfunction due to oxidized LDL by inhibiting protein kinase C (PKC) stimulation (59). However, in humans, the data on AT and endothelial dysfunction are conflicting. In hypercholesterolemic subjects, *RRR*-AT (1000 IU for four weeks) significantly increased acetylcholine-mediated vasodilation expressed as changes in absolute forearm blood flow, forearm vascular resistance, or forearm blood flow ratios (39). However, Elliott et al. (30) failed to show any improvement in endothelial function after three months of therapy with vitamin E

(800 IU/d of *RRR*-AT). Another study by Simons et al. (99) also failed to show any improvement in endothelial function in older persons receiving vitamin E (1000 IU/d of *RRR*-AT compared with placebo). In patients with chronic spastic angina, treatment with vitamin E (AT acetate as 300 mg/d compared with placebo) significantly restored flow-dependent vasodilation, and this improvement was associated with the decreases in plasma thiobarbituric acid-reacting substances levels and anginal attacks (76). Also, in hypercholesterolemic smokers, Heitzer et al. (40) showed that vitamin E significantly augmented endothelium-dependent relaxation. AT supplementation (1600 IU/d *RRR*-AT) in T2DM patients also has been shown to improve endothelial function (37). In addition, Paolisso et al. (82) have shown in 40 T2DM patients that supplementation with AT (600 mg/d of *all-rac* AT for eight weeks) was associated with a significant improvement in brachial artery reactivity compared with placebo, along with an improvement in oxidative stress indices. Kinlay et al. (59) reported a positive correlation between measurement of plasma AT and preservation of endothelium-dependent vasomotor function in patients with coronary atherosclerosis.

Furthermore, arterial compliance or elasticity is a potential index of arterial function that has been shown to be dependent upon endothelial function (67, 89). Short-term AT supplementation (400 IU/d for four and eight weeks) recently has been reported to improve arterial compliance in middle-aged men and women (77). Beckman et al. (6) recently showed that administration of vitamin C (1000 mg) and vitamin E (800 IU AT) daily compared to placebo for six months significantly increased endothelium-dependent vasodilation in type 1 but not in T2DM subjects. A trial by Engler et al. (31) examined the effect of supplementation of antioxidant vitamins C (500 mg/d) and E (400 IU/d) for six weeks and the National Cholesterol Education Program Step II diet for six months on endothelium-dependent flow-mediated dilation of the brachial artery in 15 children with familial hypercholesterolemia or the phenotype of familial combined hyperlipidemia. Antioxidant vitamin therapy significantly improved flow-mediated dilation of the brachial artery compared with baseline. Furthermore, Ulker et al. (117) recently reported the efficacy of supplementing vitamin E along with C in reversing endothelial dysfunction via regulation of eNOS and nicotinamide adenine dinucleotide phosphate (reduced form) (NADPH) oxidase activities. Recently, an open-label pilot interventional study (95) using 800 IU of vitamin E was undertaken in eight stable outpatients with nondiabetic chronic kidney disease and six healthy controls, with the objective of measuring plasma asymmetrical dimethylarginine levels at baseline and after eight weeks of treatment. After treatment with vitamin E, plasma asymmetrical dimethylarginine significantly decreased in six of eight patients, a finding that implies increased NO availability (95). Thus, although the results from studies on EC dysfunction with AT are equivocal, it appears that the combination of vitamins E and C is more effective in ameliorating EC dysfunction.

In addition, very recently (74) vitamin E (AT acetate 400 mg/d for one month) has been shown to improve fibrinolytic activity (measured as PAI-1 activity) as

well as decrease oxidative stress (thioredoxin levels) in patients with coronary spastic angina as compared with baseline levels, whereas placebo had no effect on these variables. PAI activity as well as thioredoxin levels were significantly high in patients with coronary spastic angina as compared with control subjects ($n = 17$) at day 0.

The antioxidant and anti-inflammatory activity of vitamin E was also explored to improve the biocompatibility of materials such as cellulosic membranes for hemodialysis (9). This group of authors enrolled two group of patients on regular hemodialysis [one group had cellulosic dialyzers whereas the other group had vitamin E–modified dialyzers (CLE)] without clinical atherosclerotic cardiovascular disease, and compared plasma levels of autoantibodies for ox-LDL, von Willebrand factor, and thromomodulin as markers of endothelial damage. In the CLE group, ox-LDL-Ab and von Willebrand factor, but not thrombomodulin levels, decreased significantly and vitamin E increased up to two fold, which indicates efficacy of CLE versus cellulosic in lowering the indices of damage to LDL and ECs.

A recent clinical trial by Micheletta et al. (72) enrolled 16 patients who were candidates for carotid endartectomy and 32 age- and sex-matched controls. Patients were randomly allocated to standard treatment with or without AT (*all-rac*-AT as 450 mg/d for six weeks). At the end of treatment, the different variables (plasma levels of 7-beta-hydroxycholesterol, 7-ketocholesterol, cholesterol, and vitamin E) were measured in plasma and plaques. Patients who were given vitamin E supplementation showed a significant increase of plasma vitamin E with concomitant decrease of 7-beta-hydroxycholesterol. However, no treatment effect was observed in oxysterol or vitamin E content of plaques. This study formed the basis for facilitation of vitamin E transport within atherosclerotic plaque representing an important target for treatment of early-stage atherosclerotic progression. However, this study has been contradicted and brought into question in a recently published editorial (62)—based on previously documented reports (108, 119)—that states vitamin E is not deficient in human atherosclerotic plaques.

Finally, a re-evaluation report on the relative potency of synthetic and natural AT based on experimental and clinical observations concluded that both of these are not equivalent in any dosage ratio (7). The relative bioavailability of *all-rac*-AT and *RRR*-AT varies between tissues as well as with dose, time after dosing, and duration of dosing, which suggests that a fixed dosage ratio of *all-rac*- and *RRR*-AT cannot produce a fixed ratio of effects on all processes after all dosages. In this regard, it is important to mention that most of the reported anti-inflammatory effects of AT have been due to *RRR*-AT, probably because of its higher bioavailability and decreased degradation as compared to *all-rac*-AT. In this regard, we have shown that both 400 IU (55) and 800 IU (122) of *all-rac*-AT, containing the eight isomers, failed to have any significant anti-inflammatory effects in normal subjects.

Molecular and Cellular Effects of α-Tocopherol

Advances have been made in understanding the molecular effects of AT beyond that of preventing LDL oxidation. The understanding of various regulatory,

nonoxidative responses to AT by crucial cells involved in the pathogenesis of atherosclerosis is very important. Such responses include inhibition of SMC proliferation, preservation of endothelial function, inhibition of monocyte-endothelial adhesion, inhibition of monocyte ROS and cytokine release, and inhibition of platelet adhesion and aggregation (3). These cellular responses to AT are associated with transcriptional and post-transcriptional events. Activation of diacylglycerol (DAG) kinase and protein phosphatase 2A, and the inhibition of PKC, COX, lipoxygenase, tyrosine kinase phosphorylation, and cytokine release by AT are all examples of post-transcriptional regulation.

α-Tocopherol and Endothelial Cells

AT enrichment of monocytes or ECs decreases adhesion of monocytes to human ECs in vitro and depends on the expression of various adhesion molecules (14, 34) that correlated with a decrease in cell-surface expression of E-selectin, ICAM-1, and VCAM-1. Martin et al. (71) further showed that in vitro enrichment of human aortic ECs with AT significantly inhibited LDL-induced adhesion of monocytes to EC in a dose-dependent manner with a concomitant reduction in levels of ICAM. Recently, Fan et al. (33) reported the inhibition of ox-LDL mediated ICAM-1 expression in human umbilical vein ECs by different forms of tocopherols. The mixed tocopherols (α and γ) were more potent than AT or γ-tocopherol alone. The inhibitory effect of tocopherols was not seen on recombinant human CRP-mediated adhesion of monocytes to endothelium.

Data from our laboratory have shown that pretreatment of monocytic cells with AT resulted in a decrease in monocyte-EC adhesion mediated by decreased expression of CD11b and VLA-4, via inhibition of NF-kB activity (for a review, see Reference 56). AT acetate and succinate also have been shown to inhibit TNF-α-induced NF-kB activation in vitro (109). Thus, AT has been shown to have beneficial effects in inhibiting monocyte-endothelial adhesion when incubated with either EC or monocytes; it is very likely that following supplementation it partitions into both monocytes and EC and its ability to reduce monocyte-EC adhesion is greater.

Van Aalst et al. (120) recently reported that AT but not probucol or BHT resulted in preservation of EC migration in the presence of ox-LDL. The lack of effect of other antioxidants suggested that the effect of AT is via nonantioxidant action and is probably the result of membrane stabilizing properties. Exploration of the latter revealed that pretreatment with AT inhibited the increase in membrane fluidity of ECs incubated in the presence of physiologically relevant monocyte/macrophage-oxidized LDL. This action of AT might prove to be of clinical significance for improving the healing of endothelial injury.

In recent pioneering work, Dhanasekaran et al. (29) explored the strategy of supplementation of ECs with mitochondrial targeted antioxidants and reported their better efficacy for inhibition of peroxide-induced mitochondrial iron uptake, oxidative damage, and apoptosis. The mitochondria-targeted drugs mitoquinone and

mitovitamin E are a new class of antioxidants containing the triphenylphosphonium cation moiety that facilitates drug accumulation in mitochondria. Pretreatment of bovine aortic ECs with mitoquinone (1 μM) and mitovitamin E (1 μM), but not untargeted antioxidants (e.g., vitamin E), significantly abrogated H_2O_2 and lipid peroxide–induced 2′,7′-dichlorofluorescein fluorescence and protein oxidation.

α-Tocopherol and Platelets

AT inhibits aggregation of platelets both in vitro and in vivo and delays intra-arterial thrombus formation (94). Higashi & Kikuchi (41) were the first to demonstrate the inhibitory effect of AT on platelet aggregation in vitro. Steiner (105) has shown that AT in doses of 200 IU/d decreases platelet adhesion. In hypercholesterolemic subjects, two weeks of supplementation with AT (600 mg/d) reduced elevated plasma concentrations of the platelet-derived adhesion molecule P-selectin by 40% (19). Furthermore, Steiner (104) has shown that at doses of 1200 IU/d, AT produced only a mild inhibition of collagen-induced platelet aggregation, whereas platelet adhesion to collagen was markedly inhibited in its presence. In another study (111), platelet adhesion was significantly reduced in 100 patients with transient ischemic attacks who were given 400 IU/d of AT. A double-blind, randomized, placebo-controlled study was performed on 40 healthy volunteers (20–50 years of age) supplemented daily with vitamin E [*dl*-AT acetate (300 mg/d)], vitamin C (250 mg), or β-carotene (15 mg) for eight weeks (11). 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 prostaglandin E_1 (PGE_1), the decreased plasma α-thromboglobulin concentration, and the decreased ATP secretion. Freedman et al. (35) have shown that supplementation with 400 IU of *RRR*-AT inhibits platelet aggregation through a PKC-dependent mechanism. In another published study, Mabile et al. (69) showed that *RRR*-AT uptake by platelets is optimal at 75 IU/d, and this correlates with the maximal influence on platelet aggregation and platelet responsiveness to inhibition by PGE_1. Increased supplemental levels (200, 400 IU/d) failed to exert greater effects. Thus, the majority of studies support an antiplatelet effect of AT.

α-Tocopherol and Monocytes/Macrophages

It has been shown that AT decreases monocyte $O_2^{\cdot}$ release and monocyte-mediated lipid oxidation (23), and this appears to be via inhibition of PKC. Furthermore, results from an in vitro study (10) revealed that AT inhibits $O_2^{\cdot}$ production by monocytes by impairing the assembly of NADPH oxidase, the enzyme responsible for generating the respiratory burst. AT inhibits $p47^{phox}$ translocation to the membrane and also impairs phosphorylation of $p47^{phox}$. 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. In addition, data showed that under hyperglycemic conditions, using

antisense to PKCα and PKCβ, AT inhibits the increased $O_2^{\cdot}$ released from monocytes via inhibition of $p47^{phox}$ phosphorylation through inhibition of α-isoform of PKC (126).

With regard to cytokine release, we (24) have shown that AT inhibits IL-1 release from activated human monocytes by inhibiting 5-lipoxygenase at post-transcriptional levels. Also, as discussed above, we have shown that AT enrichment of monocytes inhibits subsequent adhesion to human endothelium via inhibition of counterreceptors CD11b and VLA-4 on monocytes and inhibition of the transcription factor NF-kB (56). Furthermore, using siRNA technology, we recently showed that the increased IL-6 release from monocytes under hyperglycemia is mediated via upregulation of both PKCα and PKCβ, through p38 MAPK and NFκB, resulting in increased mRNA and protein for IL-6. We also showed that cells enriched with AT, which inhibits both PKCα and PKCβ, released significantly decreased amounts of IL-6 under hyperglycemia (28). In addition, AT has been demonstrated to downregulate scavenger receptor activity and CD36 receptor expression in human blood–derived macrophages in vitro, whereas γ-tocopherol showed only a weak suppression of scavenger receptor activity, scavenger receptor class A expression, and AP-1 activity (114). Very recently, we reported that inhibition of CD36 expression in human monocyte–derived macrophages is via inhibition of tyrosine kinase phosphorylation (124). An age-associated increase in PGE_2 synthesis and COX-2 activity in murine macrophages has been shown to be reversed by AT treatment (133). There was no effect on COX mRNA and protein levels, which indicates a post-translational regulation of COX by AT.

α-Tocopherol and Smooth Muscle Cells

The antiproliferative effects of AT have been well demonstrated in rat aortic SMC stimulated with PDGF (4, 8, 13), and the effect has not been related to its antioxidant effect (112). 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. (5). It has been shown that this effect is not related to antioxidant effects of AT because only *RRR*-AT, 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. 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. Compelling data suggests that antioxidant activity alone cannot mediate PKC inhibition (56). Direct inhibition of PKC by AT does not appear likely because several studies showed no effect on DAG or on calcium-stimulated PKCα and PKC β-2 activity (63, 65). An indirect mechanism for AT inhibition of PKC seems more likely, and data to support this come from SMCs (4), where PKCβ-2 is activated by hyperglycemia and AT inhibits this effect by decreasing cellular DAG levels through stimulation of DAG kinase activity. In addition, it has been demonstrated that okadaic acid prevents the antiproliferative effect of AT in SMC proliferation, a finding that clearly indicates 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. (20) reported that supplementation with AT (1200 IU/d for four months) resulted in a trend to decreased restenosis in coronary artery 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 (66). Ox-LDL stimulated DNA synthesis in rabbit vascular SMCs, and AT was found to inhibit this effect. These findings support the hypothesis that oxidized lipids can stimulate hyperplasia, and AT can limit this effect by inhibiting either oxidation or the proliferative effects of oxidants on cells. Recently, the effect of a mixture of AT phosphate and *dl*-AT PO_4 was shown to inhibit the proliferation of rat aortic SMCs at doses lower than the dose of AT alone (80). The higher potency of the former has been attributed to better uptake of this molecule and its intracellular hydrolysis.

Intervention Studies

Although AT has several beneficial effects on oxidation and on different cells that participate in atherogenesis, the results of randomized clinical trials have been equivocal (see References 49 and 64 for recent reviews).

CONCLUSION

Vitamin E, especially AT, exhibits antioxidant as well as anti-inflammatory activity and inhibits several biological events involved in atherogenesis (as summarized in Table 1). Although the studies carried out with cell culture and animal models suggest that AT has promising antiatherosclerotic effects, the results of its supplementation in humans are somewhat controversial, possibly because of inadequate

TABLE 1 Effect of α-tocopherol on biomarkers of oxidative stress and inflammation/thrombosis in atherosclerosis

Oxidative stress	Inflammation/thrombosis
↓ LDL oxidative susceptibility	↓ hs-CRP
↓ Autoantibodies to ox-LDL	↓ Pro-inflammatory cytokines (IL-1 & 6, TNF)
↓ Urinary isoprostanes	↓ Monocyte adhesion to endothelium
↓ ROS (O_2^-) production in monocytes	↓ Soluble cell adhesion molecules ↓ PAI-1 ↓ PGE_2 synthesis ↓ Platelet aggregation

Abbreviations: hs-CRP, high sensitivity C-reactive protein; LDL, low-density lipoprotein; ox-LDL, oxidative low-density lipoprotein; PAI-1, plasminogen activator inhibitor-1; PGE_2, prostaglandin E_2; ROS, reactive oxygen species; TNF, tumor necrosis factor; IL, interleukin.

selection of subjects (by gender, vitamin E status, etc.) or of the dose, timing of intake, and chemical form of tocopherol. Despite some of these limitations, it appears that *RRR*-AT demonstrates a multifaceted effect on vascular homeostasis.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health Grants NIH K24 AT00596 and RO1 AT00005.

The *Annual Review of Nutrition* is online at http://nutr.annualreviews.org

LITERATURE CITED

1. Akeson AL, Woods CW, Mosher LB, Thomas CE, Jackson RL. 1991. Inhibition of IL-1 beta expression in THP-1 cells by probucol and tocopherol. *Atherosclerosis* 86:261–70
2. Arrol S, Mackness MI, Durrington PN. 2000. Vitamin E supplementation increases the resistance of both LDL and HDL to oxidation and increases cholesteryl ester transfer activity. *Atherosclerosis* 150:129–34
3. Azzi A. 2004. The role of alpha-tocopherol in preventing disease. *Eur. J. Nutr.* 43(Suppl. 1):I18–25
4. Azzi A, Breyer I, Feher M, Ricciarelli R, Stocker A, et al. 2001. Nonantioxidant functions of alpha-tocopherol in smooth muscle cells. *J. Nutr.* 131:378S–81S
5. Azzi A, Ricciarelli R, Zingg JM. 2002. Non-antioxidant molecular functions of alpha-tocopherol (vitamin E). *FEBS Lett.* 519:8–10
6. Beckman JA, Goldfine AB, Gordon MB, Garrett LA, Keaney JF Jr, Creager MA. 2003. Oral antioxidant therapy improves endothelial function in Type 1 but not Type 2 diabetes mellitus. *Am. J. Physiol. Heart Circ. Physiol.* 285:H2392–98
7. Blatt DH, Pryor WA, Mata JE, Rodriguez-Proteau R. 2004. Re-evaluation of the relative potency of synthetic and natural alpha-tocopherol: experimental and clinical observations. *J. Nutr. Biochem.* 15:380–95
8. Boscoboinik D, Szewczyk A, Hensey C, Azzi A. 1991. Inhibition of cell proliferation by α-tocopherol, role of PKC. *J. Biol. Chem.* 266:6188–94
9. Bufano G, Usberti M, Mandolfo S, Malberti F, Piroddi M, Galli F. 2004. Von Willebrand factor and autoantibodies against oxidized LDL in hemodialysis patients treated with vitamin E–modified dialyzers. *Int. J. Artif. Organs* 27:214–21
10. Cachia O, Benna JE, Pedruzzi E, Descomps B, Gougerot-Pocidalo MA, Leger CL. 1998. α-Tocopherol inhibits the respiratory burst in human monocytes: attenuation of p47 (phox) membrane translocation and phosphorylation. *J. Biol. Chem.* 273:32801–5
11. Calzada C, Bruckdorfer K, Rice Evans C. 1997. The influence of antioxidant nutrients on platelet function in healthy volunteers. *Atherosclerosis* 128:97–105
12. Carroll MF, Schade DS. 2003. Timing of antioxidant vitamin ingestion alters postprandial proatherogenic serum markers. *Circulation* 108:24–31
13. Chatelain E, Boscoboinik DO, Bartoli GM, Kagan VE, Gey FK, et al. 1993. Inhibition of smooth muscle cell proliferation and protein kinase C activity by tocopherols and tocotrienols. *Biochim. Biophys. Acta* 1176:83–89
14. Cominacini L, Garbin U, Pasini AF, Davoli A, Campagnola M, et al. 1997. Antioxidants inhibit the expression of

intercellular cell adhesion molecule-1 and vascular cell adhesion molecule-1 induced by oxidized LDL on human umbilical vein endothelial cells. *Free Radic. Biol. Med.* 22:117–27

15. Danenberg HD, Szalai AJ, Swaminathan RV, Peng L, Chen Z, et al. 2003. Increased thrombosis after arterial injury in human C-reactive protein-transgenic mice. *Circulation* 108:512–15
16. Davi G, Alessandrini P, Mezzetti A, Minotti G, Bucciarelli T, et al. 1997. In vivo formation of 8-epi-prostaglandin-F_2 is increased in hypercholesterolemia. *Arterioscler. Thromb. Vasc. Biol.* 17: 3230–35
17. Davi G, Ciabattoni G, Consoli A, Mezzetti A, Falco A, et al. 1999. In vivo formation of 8-epi-PGF_2- and platelet activation in diabetes mellitus: effect of improved metabolic control and vitamin E supplementation. *Circulation* 99:224–29
18. Davi G, Falco A, Patrono C. 2004. Determinants of F2-isoprostane biosynthesis and inhibition in man. *Chem. Phys. Lipids* 128:149–63
19. Davi G, Romano M, Mezzetti A, Procopio A, Iacobelli S, et al. 1998. Increased levels of soluble P-selectin in hypercholesterolemic patients. *Circulation* 97:953–57
20. DeMaio SJ, King SB III, Lembo NJ, Roubin GS, Hearn JA, et al. 1992. Vitamin E supplementation, plasma lipids, and incidence of restenosis after percutaneous transluminal coronary angioplasty (TCA). *J. Am. Coll. Nutr.* 11:68–73
21. Devaraj S, Adams-Huet B, Fuller CJ, Jialal I. 1997. Dose-response comparison of RRR-AT and all-rac-tocopherol on LDL oxidation. *Arterioscler. Thromb. Vasc. Biol.* 17:2273–79
22. Devaraj S, Chan AV Jr, Jialal I. 2002. Alpha-tocopherol supplementation decreases plasminogen activator inhibitor-1 and P-selectin levels in type 2 diabetic patients. *Diabetes Care* 25:524–29
23. Devaraj S, Jialal I. 1998. The effects of α-tocopherol on critical cells in atherogenesis. *Curr. Opin. Lipidol.* 9:1115–17
24. Devaraj S, Jialal I. 1999. Alpha-tocopherol decreases interleukin-1 beta release from activated human monocytes by inhibition of 5-lipoxygenase. *Arterioscler. Thromb. Vasc. Biol.* 19:1125–33
25. Devaraj S, Jialal I. 2000. Alpha-tocopherol supplementation decreases serum C-reactive protein and monocyte interleukin-6 levels in normal volunteers and type 2 diabetic patients. *Free Radic. Biol. Med.* 29:790–92
26. Devaraj S, Jialal I. 2000. Low-density lipoprotein postsecretory modification, monocyte function, and circulating adhesion molecules in type 2 diabetic patients with and without macrovascular complications: the effect of alpha-tocopherol supplementation. *Circulation* 102:191–96
27. Devaraj S, Li D, Jialal I. 1996. The effects of alpha-tocopherol supplementation on monocyte function. Decreased lipid oxidation, interleukin 1 beta secretion, and monocyte adhesion to endothelium. *J. Clin. Invest.* 98:756–63
28. Devaraj S, Venugopal SK, Singh U, Jialal I. 2005. Hyperglycemia induces monocytic IL-6 via induction of PK-C alpha and beta. *Diabetes* 54:85–91
29. Dhanasekaran A, Kotamraju S, Kalivendi SV, Matsunaga T, Shang T, et al. 2004. Supplementation of endothelial cells with mitochondria-targeted antioxidants inhibit peroxide-induced mitochondrial iron uptake, oxidative damage and apoptosis. *J. Biol. Chem.* 279:37575–87
30. Elliott TG, Barth JD, Mancini GB. 1995. Effects of vitamin E on endothelial function in men after myocardial infarction. *Am. J. Cardiol.* 76:1188–90
31. Engler MM, Engler MB, Malloy MJ, Chiu EY, Schloetter MC, et al. 2003. Antioxidant vitamins C and E improve endothelial function in children with

hyperlipidemia: Endothelial Assessment of Risk from Lipids in Youth (EARLY) Trial. *Circulation* 108:1059–63

32. Esterbauer H, Dieber-Rotheneder M, Striegl G, Waeg G. 1991. Role of vitamin E in preventing the oxidation of low-density lipoprotein. *Am. J. Clin. Nutr.* 53(Suppl. 1):314S–21S
33. Fan Y, Liu ML, Qi YY, Ren ZW. 2004. Effect of different isoforms of tocopherols on expression of intercellular adhesion molecule-1 in human umbilical vein endothelial cells. *Beijing Da Xue Xue Bao* 36:70–74
34. Faruqui R, De La Motte C, Dicorleto P. 1994. α-Tocopherol inhibits agonist-induced monocyte cell adherence to cultured human endothelial cells. *J. Clin. Invest.* 94:592–600
35. Freedman JE, Farhat JH, Loscalzo J, Keaney JF Jr. 1996. α-Tocopherol inhibits aggregation of human platelets by a protein kinase C–dependent mechanism. *Circulation* 94:2434–40
36. Friedrich MJ. 2004. To "E" or not to "E," vitamin E's role in health and disease is the question. *JAMA* 292:671–73
37. Gazis A, White DJ, Page SR, Cockcroft JR. 1999. Effect of oral vitamin E (alpha-tocopherol) supplementation on vascular endothelial function in type 2 diabetes mellitus. *Diabet. Med.* 16:304–11
38. Gniwotta C, Morrow JD, Roberts LJ II, Kuhn H. 1997. Prostaglandin F_2-like compounds, F_2 isoprostanes, are present in increased amounts in human atherosclerotic lesions. *Arterioscler. Thromb. Vasc. Biol.* 17:3236–41
39. Green D, O'Driscoll G, Rankin JM, Maiorana AJ, Taylor RR. 1998. Beneficial effect of vitamin E administration on nitric oxide function in subjects with hypercholesterolaemia. *Clin. Sci. (Lond.)* 95:361–67
40. Heitzer T, Yla Herttuala S, Wild E, Luoma J, Drexler H. 1999. Effect of vitamin E on endothelial vasodilator function in patients with hypercholesterolemia, chronic smoking or both. *J. Am. Coll. Cardiol.* 33:499–505
41. Higashi O, Kikuchi Y. 1974. Effects of vitamin E on the aggregation and lipid peroxidation of platelets exposed to hydrogen peroxide. *Tohoku J. Exp. Med.* 112:271–78
42. Himmelfarb J, Kane J, McMonagle E, Zaltas E, Bobzin S, et al. 2003. Alpha and gamma tocopherol metabolism in healthy subjects and patients with end-stage renal disease. *Kidney Int.* 64:978–91
43. Hodis HN, Mack WJ, LaBree L, Mahrer PR, Sevanian A, et al. 2002. Alpha-tocopherol supplementation in healthy individuals reduces low-density lipoprotein oxidation but not atherosclerosis. *Circulation* 106:1453–59
44. Inoue M, Itoh H, Tanaka T, Chun TH, Doi K, et al. 2001. Oxidized LDL regulates vascular endothelial growth factor expression in human macrophages and endothelial cells through activation of peroxisome proliferator-activated receptor-gamma. *Arterioscler. Thromb. Vasc. Biol.* 21:560–66
45. Islam KN, O'Byrne D, Devaraj S, Palmer B, Grundy SM, Jialal I. 2000. Alpha-tocopherol supplementation decreases the oxidative susceptibility of LDL in renal failure patients on dialysis therapy. *Atherosclerosis* 150:217–24
46. Jessup W, Kritharides L, Stocker R. 2004. Lipid oxidation in atherogenesis: an overview. *Biochem. Soc. Trans.* 32:134–38
47. Jialal I. 1998. Evolving lipoprotein risk factors: lipoprotein(a) and oxidized low-density lipoprotein. *Clin. Chem.* 44:1827–32
48. Jialal I, Devaraj S. 2001. Inflammation and atherosclerosis: the value of the high-sensitivity C-reactive protein assay as a risk marker. *Am. J. Clin. Pathol.* 116(Suppl.):S108–15
49. Jialal I, Devaraj S. 2003. Antioxidants and atherosclerosis: Don't throw out the baby

with the bath water. *Circulation* 107:926–28
50. Jialal I, Devaraj S, Venugopal. 2004. C-reactive protein: risk marker or mediator in atherothrombosis? *Hypertension* 44:1–6
51. Jialal I, Fuller C, Huet B. 1995. The effect of AT supplementation on LDL oxidation: a dose-response study. *Arterioscler. Thromb. Vasc. Biol.* 15:190–98
52. Jialal I, Grundy SM. 1992. Effect of dietary supplementation with α-tocopherol on the oxidative modification of low-density lipoprotein. *J. Lipid Res.* 33:899–906
53. Jialal I, Grundy SM. 1992. Influence of antioxidant vitamins on LDL oxidation. *Ann. N.Y. Acad. Sci.* 669:237–48
54. Jones SA, O'Donnell VB, Wood JD, Broughton JP, Hughes EJ, et al. 1996. Expression of phagocytic NADPH oxidase components in human endothelial cells. *Am. J. Phys*. 271:H1626–34
55. Kaul N, Devaraj S, Grundy SM, Jialal I. 2001. Failure to demonstrate a major anti-inflammatory effect with alpha tocopherol supplementation (400 IU/day) in normal subjects. *Am. J. Cardiol.* 87:1320–23
56. Kaul N, Devaraj S, Jialal I. 2001. Alpha-tocopherol and atherosclerosis. *Exp. Biol. Med*. (Maywood) 226:5–12
57. Keaney JF Jr, Gaziano JM, Xu A, Frei B, Curran-Celentano J, et al. 1994. Low-dose α-tocopherol improves and high-dose α-tocopherol worsens endothelial vasodilator function in cholesterol-fed rabbits. *Clin. Invest*. 93:844–51
58. Keaney JF Jr, Guo Y, Cunningham D, Shwaery GT, Xu A, Vita JA. 1996. Vascular incorporation of α-tocopherol prevents endothelial dysfunction due to oxidized LDL by inhibiting protein kinase C stimulation. *J. Clin. Invest*. 98:386–94
59. Kinlay S, Fang JC, Hikita H, Ho I, Delagrange DM, et al. 1999. Plasma alpha-tocopherol and coronary endothelium-dependent vasodilator function. *Circulation* 100:219–21
60. Kirii H, Niwa T, Yamada Y, Wada H, Saito K, et al. 2003. Lack of interleukin-1ß decreases the severity of atherosclerosis in apoE-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* 23:656–60
61. Kobayashi S, Inoue N, Ohashi Y, Terashima M, Matsui K, et al. 2003. Interaction of oxidative stress and inflammatory response in coronary plaque instability: important role of C-reactive protein. *Arterioscler. Thromb. Vasc. Biol.* 23:1398–404
62. Kontush A, Chapman MJ, Stocker R. 2004. Vitamin E is not deficient in human atherosclerotic plaques. *Arterioscler. Thromb. Vasc. Biol*. 24:e139–40
63. Koya D, Haneda M, Kikkawa R, King GL. 1998. α- and δ-Tocopherol treatment prevents glomerular dysfunctions in diabetic rats through inhibition of protein-kinase C–diacylglycerol pathway. *Biofactors* 7:69–76
64. Kris-Etherton PM, Lichtenstein AH, Howard BV, Steinberg D, Witztum JL. 2004. Nutrition Committee of the American Heart Association Council on Nutrition, Physical Activity, and Metabolism. Antioxidant vitamin supplements and cardiovascular disease. *Circulation* 110:637–40
65. Kunisaki M, Bursell SE, Umeda F, Nawata H, King GL. 1994. Normalization of DAG-PKC activation by vitamin E in aorta of diabetic rats and cultured rat SMC exposed to elevated glucose levels. *Diabetes* 42:1372–77
66. Lafont AM, Chai YC, Cornhill JF, Whitlow PL, Howe PH, et al. 1995. Effect of α-tocopherol on restenosis after angioplasty in a model of experimental atherosclerosis. *J. Clin. Invest.* 95:1018–25
67. Laursen JB, Boesgaard S, Trautner S, Rubin I, Poulsen HE, Aldershvile J. 2001. Endothelium-dependent vasorelaxation in inhibited by in vivo depletion of vascular thiol levels: role of endothelial nitric oxide synthase. *Free Radic. Res.* 35:387–94

68. Libby P. 2002. Inflammation in atherosclerosis. *Nature* 420:868–74
69. Mabile L, Bruckdorfer K, Rice Evans C. 1999. Moderate supplementation with natural alpha-tocopherol decreases platelet aggregation and low-density lipoprotein oxidation. *Atherosclerosis* 147:177–85
70. Marangon K, Devaraj S, Jialal I. 1999. The effect of α-lipoate and α-tocopherol on plasma, whole body, and LDL oxidation. *Free Radic. Biol. Med.* 27:1114–21
71. Martin A, Foxall T, Blumberg JB, Meydani M. 1997. AT inhibits LDL-induced adhesion of monocytes to human aortic endothelial cells *in vitro*. *Arterioscler. Thromb. Vasc. Biol.* 17:429–36
72. Micheletta F, Natoli S, Misuraca M, Sbarigia E, Diczfalusy U, Iuliano L. 2004. Vitamin E supplementation in patients with carotid atherosclerosis: reversal of altered oxidative stress status in plasma but not in plaque. *Arterioscler. Thromb. Vasc. Biol*. 24:136–40
73. Miller FJ Jr, Gutterman DD, Rios CD, Heistad DD, Davidson BL. 1998. Superoxide production in vascular smooth muscle contributes to oxidative stress and impaired relaxation in atherosclerosis. *Circ. Res*. 82:1298–305
74. Miyamoto S, Kawano H, Takazoe K, Soejima H, Sakamoto T, et al. 2004. Vitamin E improves fibrinolytic activity in patients with coronary spastic angina. *Thromb. Res*. 113:345–51
75. Mol MJ, de Rijke YB, Demacker PN, Stalenhoef AF. 1997. Plasma levels of lipid and cholesterol oxidation products and cytokines in diabetes mellitus and cigarette smoking: effects of vitamin E treatment. *Atherosclerosis* 129:169–76
76. Motoyama T, Kawano H, Kugiyama K, Hirashima O, Ohgushi M, et al. 1998. Vitamin E administration improves impairment of endothelium-dependent vasodilation in patients with coronary spastic angina. *J. Am. Coll. Cardiol*. 32:1672–79
77. Mottrram P, Shige H, Nestel P. 1999. Vitamin E improves arterial compliance in middle-aged men and women. *Atherosclerosis* 145:399–404
78. Mugge A, Elwell JH, Peterson TE, Hofmeyer TG, Heistad DD, Harrison DG. 1991. Chronic treatment with PEG-SOD partially restores endothelial-dependent vascular relaxation in cholesterol-fed rabbits. *Circ. Res.* 69:1293–300
79. Munteanu A, Zingg JM, Azzi A. 2004. Anti-atherosclerotic effects of vitamin E—myth or reality? *J. Cell. Mol. Med*. 8:59–76
80. Munteanu A, Zingg JM, Ogru E, Libinaki R, Gianello R, et al. 2004. Modulation of cell proliferation and gene expression by alpha-tocopheryl phosphates: relevance to atherosclerosis and inflammation. *Biochem. Biophys. Res. Commun.* 318:311–16
81. Murphy RT, Foley JB, Tome MT, Mulvihill NT, Murphy A, et al. 2004. Vitamin E modulation of C-reactive protein in smokers with acute coronary syndromes. *Free Radic. Biol. Med*. 36:959–65
82. Paolisso G, Tagliamonte MR, Barbieri M, Zito GA, Gambardella A, et al. 2000. Chronic vitamin E administration improves brachial reactivity and increases intracellular magnesium concentration in type II diabetic patients. *J. Clin. Endocrinol. Metab*. 85:109–15
83. Paul A, Ko KW, Li L, Yechoor V, McCrory MA, et al. 2004. C-reactive protein accelerates the progression of atherosclerosis in apolipoprotein E–deficient mice. *Circulation* 109:647–55
84. Patrano C, Fitzgerald G. 1997. Isoprostanes: potential markers of oxidant stress in atherothrombotic disease. *Arterioscler. Thromb. Vasc. Biol.* 17:2309–15
85. Peluzio MC, Homem AP, Cesar GC, Azevedo GS, Amorim R, et al. 2001. Influences of alpha-tocopherol on cholesterol metabolism and fatty streak development in apolipoprotein E–deficient mice fed an atherogenic diet. *Braz. J. Med. Biol. Res.* 34:1539–45

86. Peluzio MC, Miguel E Jr, Drumond TC, Cesar GC, Santiago HC, et al. 2003. Monocyte chemoattractant protein-1 involvement in the alpha-tocopherol-induced reduction of atherosclerotic lesions in apolipoprotein E knockout mice. *Br. J. Nutr*. 90:3–11
87. Pickup JC. 2004. Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. *Diabetes Care* 27:813–23
88. Pratico D, Juliano L, Mauriello A, Spagnoli L, Lawson JA, et al. 1998. Localization of distinct F_2-isoprostanes in human atherosclerotic lesions. *J. Clin. Invest.* 100:915–24
89. Ramsey MW, Goodfellow J, Jones CJ, Luddington LA, Lewis MJ, Henderson AH. 1995. Endothelial control of arterial distensibility is impaired in chronic heart failure. *Circulation* 92:3212–19
90. Reaven P, Witztum J. 1993. Comparison of supplementation with RRR-α-tocopherol and all-rac-tocopherol in humans. *Arterioscler. Thromb. Vasc. Biol.* 13:601–8
91. Ridker PM. 2003. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. *Circulation* 168:363–69
92. Roberts LJ 2nd, Morrow JD. 2002. Products of the isoprostane pathway: unique bioactive compounds and markers of lipid peroxidation. *Cell. Mol. Life Sci.* 59:808–20
93. Ross R. 1999. Atherosclerosis: an inflammatory disease. *N. Engl. J. Med*. 340:115–26
94. Saldeen T, Li D, Mehta JL. 1999. Differential effects of alpha- and gamma-tocopherol on low-density lipoprotein oxidation, superoxide activity, platelet aggregation and arterial thrombogenesis. *J. Am. Coll. Cardiol*. 34:1208–15
95. Saran R, Novak JE, Desai A, Abdulhayoglu E, Warren JS, et al. 2003. Impact of vitamin E on plasma asymmetric dimethylarginine (ADMA) in chronic kidney disease (CKD): a pilot study. *Nephrol. Dial. Transplant*. 18:2415–20
96. Schnell JW, Anderson RA, Stegner JE, Schindler SP, Weinberg RB. 2001. Effects of a high polyunsaturated fat diet and vitamin E supplementation on high-density lipoprotein oxidation in humans. *Atherosclerosis* 159:459–66
97. Schock BC, Van der Vliet A, Corbacho AM, Leonard SW, Finkelstein E, et al. 2004. Enhanced inflammatory responses in alpha-tocopherol transfer protein null mice. *Arch. Biochem. Biophys.* 423:162–69
98. Schwenke DC, Rudel LL, Sorci-Thomas MG, Thomas MJ. 2002. Alpha-tocopherol protects against diet-induced atherosclerosis in New Zealand white rabbits. *J. Lipid Res*. 43:1927–38
99. Simons LA, von Konigsmark M, Simons J, Stocker R, Celermajer DS. 1999. Vitamin E ingestion does not improve arterial endothelial dysfunction in older adults. *Atherosclerosis* 143:193–99
100. Singh U, Jialal I. 2004. Anti-inflammatory effects of alpha-tocopherol. *Ann. N.Y. Acad. Sci.* 1031:195–203
101. Smith KS, Lee CL, Ridlington JW, Leonard SW, Devaraj S, Traber MG. 2003. Vitamin E supplementation increases circulating vitamin E metabolites tenfold in end-stage renal disease patients. *Lipids* 38:813–19
102. Smith T, Kummerow F. 1989. Effect of dietary vitamin E on plasma lipids and atherogenesis in restricted ovulator chicken. *Atherosclerosis* 75:105–9
103. Steinbrecher U. 1984. Modification of low-density lipoprotein by endothelial cells involves lipid peroxidation and degradation of low-density lipoprotein phospholipids. *Proc. Natl. Acad. Sci. USA* 81:3883–87
104. Steiner M. 1983. Effect of alpha-tocopherol administration on platelet function in man. *Thromb. Haemost.* 49:73–77
105. Steiner M. 1991. Influence of vitamin E on

platelet function in humans. *J. Am. Coll. Nutr.* 10:466–73

106. Stewart-Lee AL, Forster LA, Nourooz-Zadeh J, Ferns GA, Anggard EE. 1994. Vitamin E protects against impairment of endothelium-mediated relaxations in cholesterol-fed rabbits. *Arterioscler. Thromb. Vasc. Biol*. 14:494–99
107. Stocker R, Keaney JF Jr. 2004. Role of oxidative modifications in atherosclerosis. *Physiol. Rev*. 84:1381–478
108. Suarna C, Dean RT, May J, Stocker R. 1995. Human atherosclerotic plaque contains both oxidized lipids and relatively large amounts of alpha-tocopherol and ascorbate. *Arterioscler. Thromb. Vasc. Biol.* 15:1616–24
109. Suzuki YJ, Packer L. 1993. Inhibition of NF-κB DNA binding activity by AT succinate. *Biochem. Mol. Biol. Int.* 31:693–700
110. Suzuki YJ, Tsuchiya M, Wassall SR, Choo YM, Govil G, et al. 1993. Structural and dynamic properties of AT and T3: implication to the molecular mechanism of their antioxidant potency. *Biochemistry* 32:10692–99
111. Takamatsu S, Takamatsu M, Satoh K. 1995. Effects on health of dietary supplementation with 100 mg α- and δ-tocopheryl acetate, daily for 6 years. *J. Int. Med. Res.* 23:342–57
112. Tasinato A, Boscoboinik D, Bartoli GM, Maroni P, Azzi A. 1995. d-α-Tocopherol inhibition of vascular smooth muscle cell proliferation occurs at physiological concentrations, correlates with protein kinase C inhibition, and is independent of its antioxidant properties. *Proc. Natl. Acad. Sci.* USA 92:12190–94
113. Terasawa Y, Ladha Z, Leonard SW, Morrow JD, Newland D, et al. 2000. Increased atherosclerosis in hyperlipidemic mice deficient in alpha-tocopherol transfer protein and vitamin E. *Proc. Natl. Acad. Sci. USA* 97:13830–34
114. Teupser D, Thiery J, Seidel D. 1999. AT downregulates SR activity in macrophages. *Atherosclerosis* 144:109–15
115. Thomas SR, Stocker R. 2000. Molecular action of vitamin E in lipoprotein oxidation: implications for atherosclerosis. *Free Radic. Biol. Med*. 28(12):1795–805
116. Traber MG, Arai H. 1999. Molecular mechanism of vitamin E transport. *Annu. Rev. Nutr.* 19:343–55
117. Ulker S, McKeown PP, Bayraktutan U. 2003. Vitamins reverse endothelial dysfunction through regulation of eNOS and NAD(P)H oxidase activities. *Hypertension* 41:534–39
118. Upritchard JE, Sutherland WHE, Mann JI. 2000. Effect of supplementation with tomato juice, vitamin E, and vitamin C on LDL oxidation and products of inflammatory activity in type 2 diabetes. *Diabetes Care* 23:733–38
119. Upston JM, Terentis AC, Morris K, Keaney Jr JF, Stocker R. 2002. Oxidized lipid accumulates in the presence of alpha-tocopherol in atherosclerosis. *Biochem. J*. 363:753–60
120. van Aalst JA, Burmeister W, Fox PL, Graham LM. 2004. α-Tocopherol preserves endothelial cell migration in the presence of cell-oxidized low-density lipoprotein by inhibiting changes in cell membrane fluidity. *J. Vasc. Surg*. 39:229–37
121. Van Tits LJ, Demacker PN, de Graaf J, Hak-Lemmers HL, Stalenhoef AF. 2000. α-Tocopherol supplementation decreases production of superoxide and cytokines by leukocytes ex vivo in both normolipidemic and hypertriglyceridemic individuals. *Am. J. Clin. Nutr*. 71:458–64
122. Vega-Lopez S, Kaul N, Devaraj S, Cai RY, German B, Jialal I. 2004. Supplementation with ω3 polyunsaturated fatty acids and all-rac AT alone and in combination failed to exert an anti-inflammatory effect in human volunteers. *Metabolism* 53:236–40
123. Venugopal SK, Devaraj S, Jialal I. 2003. CRP decreases prostacyclin release from

human aortic endothelial cells. *Circulation* 108:1676–78

124. Venugopal SK, Devaraj S, Jialal I. 2004. RRR-AT decreases the expression of the major SR, CD36, in human macrophages via inhibition of tyrosine kinase (Tyk2). *Atherosclerosis* 175:213–20
125. Venugopal SK, Devaraj S, Jialal I. 2005. Macrophage conditioned medium induces the expression of CRP in human aortic endothelial cells: potential for paracrine/autocrine effects. *Am J. Pathol.* 166:1265–71
126. Venugopal SK, Devaraj S, Yang T, Jialal I. 2002. AT decreases superoxide anion release in human monocytes under hyperglycemic conditions via inhibition of PKC-α. *Diabetes* 51:3049–54
127. Verlangieri A, Buxh M. 1992. Effects of AT supplementation on experimentally induced primate atherosclerosis. *J. Am. Coll. Nutr.* 11:131–38
128. Wang CH, Li SH, Weisel RD, Fedak PW, Dumont AS, et al. 2003. C-reactive protein upregulates angiotensin type 1 receptors in vascular smooth muscle. *Circulation* 107:1783–90
129. Weinberg RB, VanderWerken BS, Anderson RA, Stegner JE, Thomas MJ. 2001. Pro-oxidant effect of vitamin E in cigarette smokers consuming a high polyunsaturated fat diet. *Arterioscler. Thromb. Vasc. Biol*. 21:1029–33
130. Williams RJ, Motteram JM, Sharp CH, Gallagher PJ. 1992. Dietary vitamin E and attenuation of early lesion development in modified Watanabe rabbits. *Atherosclerosis* 94:153–59
131. Witzum J, Steinberg D. 1991. Role of oxidized low-density lipoprotein in atherogenesis. *J. Clin. Invest.* 88:1785–92
132. Wu D, Koga T, Martin KR, Meydani M. 1999. Effect of vitamin E on human aortic endothelial cell production of chemokines and adhesion to monocytes. *Atherosclerosis* 147:297–307
133. Wu D, Mura C, Beharka AA, Han SN, Paulson KE, et al. 1998. Age-associated increase in PGE2 synthesis and COX activity in murine macrophages is reversed by vitamin E. *Am. J. Physiol*. 275:C661–68
134. Yang T, Devaraj S, Jialal I. 2001. Oxidative stress and atherosclerosis. *J. Clin. Ligand Assay* 24:13–24
135. Yasojima K, Schwab C, McGeer EG, McGeer PL. 2001. Generation of CRP and complement components in atherosclerotic plaques. *Am. J. Pathol*. 158:1039–51

Annual Review of Nutrition
Volume 25, 2005

CONTENTS