

Endothelial Dysfunction and Antioxidants

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Abstract

The vascular endothelium plays a crucial role in the physiology of blood vessels and the pathological processes of atherosclerotic disease and acute coronary syndromes. Endothelial dysfunction is the core problem; it is an impairment of endothelium-dependent vasorelaxation caused by a loss of nitric oxide activity in the vessel wall, which results in impairment in the regulation of vascular homeostasis. Further understanding of its mechanisms of action and possible therapeutic targets will be of great importance. The group of antioxidant vitamins, A, C and E, would seem uniquely situated to reduce cardiovascular events by improving endothelial function by reducing the concentration of reactive oxygen species in the vessel wall and by preventing oxidative modification of low-density lipoprotein. Unfortunately, despite extensive studies in both observational and randomized trials, the weight of evidence points to little or no benefit from antioxidant therapy.

Key Words: Endothelium dysfunction, endothelium, antioxidants, vitamins.

Introduction

FAR FROM BEING AN INERT LINING, the endothelium is actively involved in a wide variety of physiological and pathological processes. Endothelial dysfunction is an impairment of endothelium-dependent vasorelaxation caused by a loss of nitric oxide (NO) activity in the vessel wall, which results in impairment in the regulation of vascular homeostasis. This impairment can lead to the development of atherosclerosis and the aggravation of myocardial ischemia in the setting of pre-existing coronary disease. One of the proposed mechanisms for endothelial dysfunction involves

accelerated NO degradation by reactive oxygen species (ROS) that are created as a byproduct of cellular metabolism. Reducing the concentration of ROS through the use of antioxidant therapy is a potential mechanism for treating endothelial dysfunction. The group of antioxidant vitamins, A, C, and E, are therefore uniquely situated to potentially reduce cardiovascular events by improving endothelial function and also by preventing oxidative modification of low-density lipoprotein (LDL) into the atherogenic oxidized-LDL.

Endothelial Dysfunction

Until recently, the vascular endothelium was considered an inert lining of the blood vessel separating the vascular space from tissues. However, it is now known that the endothelium is actively involved in a wide variety of physiological and pathological processes that mediate inflammation within the vascular wall, plaque stability, thrombus formation, and vasomotor tone (1). The early experiments by Furchgott and Zawadzki demonstrated the essential role of the endothelium in reg-

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ulating vasodilatation via the endothelial-derived relaxing factor, which was later identified to be NO (2). The endothelium is a single-celled lining covering the internal surface of blood vessels, giving it a strategic location to sense changes in hemodynamic forces and blood-borne signals and respond to them by releasing vasoactive substances. The endothelium thereby plays a key role in maintaining vascular homeostasis by balancing endothelial-derived relaxing and contracting factors.

The term “endothelial dysfunction” has been used to refer to a number of pathological conditions involving the vascular endothelium, for example, altered anticoagulant, antithrombotic and anti-inflammatory properties of the endothelium; impaired modulation of vascular growth; and dysregulation of vascular remodeling (3). Much of the current literature now uses this term to refer to an impairment of endothelium-dependent vasorelaxation caused by a loss of NO activity in the vessel wall. Clinical studies have shown that traditional risk factors for atherosclerosis predispose to endothelial dysfunction (4) and that impaired endothelium-dependent vasodilatation in the coronary circulation predicts adverse cardiovascular events and adverse long-term outcome (5). This decline in NO bioavailability may be caused by a number of different mechanisms: decreased expression of the endothelial cell NO synthase (eNOS), a lack of substrate or co-factors for eNOS,

alterations of cellular signaling such that eNOS is not appropriately activated, and accelerated NO degradation by ROS (3).

NO is the key endothelial-derived relaxing factor that plays a pivotal role in vascular tone and reactivity (6). NO is generated in the endothelial cell by nitric oxide synthase, which converts the amino acid L-arginine to nitric oxide and L-citrulline (Fig. 1). NO diffuses from the endothelial cell to the vascular smooth muscle and increases cyclic guanosine monophosphate, thereby causing relaxation of smooth muscle and dilation of the artery. Luminal diffusion of NO and its uptake into circulating platelets inhibits platelet adhesion and, to a lesser extent, suppresses platelet aggregation. NO can also influence gene transcription, leading to differential expression of cell surface adhesion molecules that influence leukocyte activation, adhesion, and migration.

The endothelium also performs a number of additional functions (7). It further controls vascular tone by secreting prostacyclin, another potent vasodilator, and also secretes the potent vasoconstrictor peptide ET-1 (endothelin-1). It affects coagulation through the secretion of tissue-type plasminogen activator. And it regulates endothelial permeability to lipoproteins and other plasma constituents.

The traditional risk factors for coronary artery disease (CAD), hyperlipidemia, smoking, diabetes and hypertension, are also associated with coronary endothelium dysfunction (8). In fact, the total number of coronary risk factors is related to the reduction in endothelium-dependent vasodilator function (8). It is hypothesized that endothelial dysfunction may initiate the formation of atherosclerotic plaques (9). Atherosclerotic plaques typically form at arterial bifurcations and other sites of disturbed blood flow, and the endothelium is uniquely situated to sense these flow disturbances at the interface of the arterial wall and circulating blood. If dysfunctional, the endothelium potentiates flow disturbances due to improper vasoreactivity, and even initiates the inflammatory response by secreting proinflammatory cytokines and chemokines. These in turn encourage leukocyte activation, promote leukocyte adhesion molecule expression, and facilitate entry of activated leukocytes and LDL into the subintimal space (9). Furthermore, endothelial dysfunction also permits smooth muscle proliferation to proceed unchecked in the media and subintima. These processes are accelerated by the presence of risk factors, and are central to the more advanced stages of atherosclerosis, which are characterized by inflammation and a vulnerable plaque. Finally,

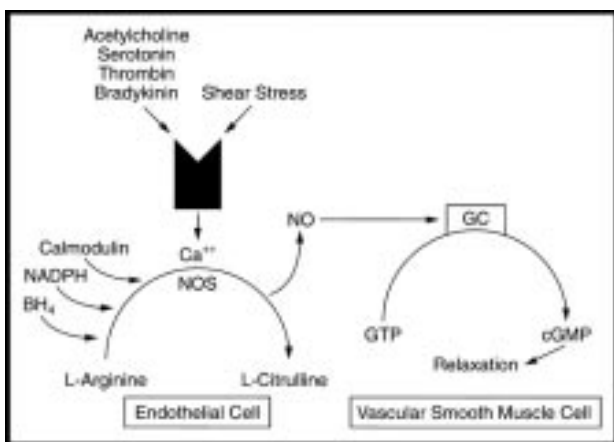


Fig. 1. The nitric oxide pathway. Reproduced with permission from Kinlay S, Ganz P. Role of endothelial dysfunction in coronary artery disease and implications for therapy. *Am J Cardiol* 1997; 80(9A):111–161 (1).

BH₄ = tetrahydrobiopterin, Ca⁺⁺ = calcium ion; cGMP = cyclic guanosine monophosphate, GC = guanylate cyclase, GTP = guanosine triphosphate, NADPH = nicotinamide-adenine dinucleotide phosphate, NO = nitric oxide, NOS = nitric oxide synthase.

impaired endothelium-dependent vasodilatation in coronary arteries with established atherosclerosis results in paradoxical vasoconstriction, which in turn may result in worsened myocardial ischemia during acute coronary syndromes.

Endothelial dysfunction occurs early in the development of atherosclerosis, even before the formation of plaque (10). Measuring and identifying this dysfunction could possibly lead to an early diagnosis of CAD. Endothelium-dependent vasodilatation can be assessed in the coronary and peripheral circulation and be measured both invasively and noninvasively. Fortunately, measures of endothelial dysfunction assessed by peripheral techniques correlate with measures of coronary endothelial dysfunction (11).

A commonly used invasive method employs quantitative coronary angiography to examine the change in diameter in response to intracoronary doses of acetylcholine (12). In healthy arteries, acetylcholine stimulates the release of NO from the endothelium, causing vasodilatation. However, in patients with endothelial dysfunction, a paradoxical vasoconstriction occurs due to the effect of acetylcholine on vascular smooth muscle. Endothelial function can therefore be assessed by comparing the coronary response to acetylcholine with the coronary response to nitroglycerin. Intracoronary Doppler techniques can also be used to measure coronary blood flow in response to pharmacological or physiological stimuli.

The major noninvasive technique involves brachial artery ultrasound to measure the change in diameter of the vessel in response to an increase in shear stress (13). Upper arm occlusion for 5 minutes results in reactive hyperemia after the cuff is released, causing an endothelium-dependent, flow-mediated vasodilatation. This response is compared to the response achieved from sublingual nitroglycerin to distinguish endothelium-dependent vasodilatation. Doppler echocardiography, positron emission tomography, magnetic resonance imaging, and impedance plethysmography have all been used, although less commonly, to evaluate endothelial function noninvasively (14).

Currently there are several modalities that have been proven to reduce endothelial dysfunction and increase endothelial NO availability (Table).

Some of the most convincing evidence of our ability to treat endothelial dysfunction comes from the substantial data supporting the notion that LDL cholesterol reduction augments endothelial-dependent vasodilatation (15). Most of the relevant studies have observed improvement

TABLE
Treatment Associated with Improvement of Endothelial Dysfunction

Acute	Chronic
LDL lowering with pheresis	LDL lowering with statins, resins
ACE inhibition	ACE inhibition
Antioxidants (vitamin C and E)	Antioxidants (probucoI with lovastatin)
Estrogen	Estrogen
L-arginine, D-arginine	Estrogen + progesterone
Tetrahydrobiopterin	L-arginine
Deferoxamine	Exercise
Glutathione	
Calcium channel blockers	

LDL = low-density lipoproteins, ACE = angiotensin-converting enzyme.

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in acetylcholine-mediated constriction of epicardial coronary arteries and in microvascular dilation. Although a number of modalities that lower cholesterol have been demonstrated to improve endothelial function, much recent attention has focused on the statin class. There is now convincing evidence that statins exert direct, cholesterol-independent (pleotropic) effects to improve endothelial function (16). Such actions may underlie the early and profound effects of these drugs on cardiovascular morbidity and mortality. Trials have shown that treatment and lipid lowering with statins reduce episodes of myocardial ischemia occurring over a shorter period of time than required to obtain regression of atherosclerosis (17, 18). These improvements are, therefore, likely to represent improvement in endothelial-dependent vasomotor function with resultant improved myocardial perfusion. However, studies with other medications and modalities to lower cholesterol have shown improved endothelial function as well, indicating that cholesterol-lowering alone also has significant effect on endothelial function (19–21). In addition to the benefit for endothelial function, large-scale statin studies such as 4S, CARE, LIPID, and HPS have documented this benefit for reductions in clinical cardiovascular events.

The other class of medications with proven significant effects on endothelial function is the

angiotensin-converting enzyme inhibitors (ACE-I). Experimental data suggest that ACE-I's may improve endothelial function by decreasing levels of angiotensin II, by reducing superoxide generation, and by increasing levels of bradykinin and NO (14). The beneficial effects of ACE-I's have been demonstrated in the coronary and peripheral circulation; this becomes apparent after short-term treatment (22). There are potential differences in tissue specificity and the importance of the bradykinin effect among ACE-I's, which have been evaluated in several studies comparing ACE-I's. In a comparison of quinapril, enalapril, losartan, and amlodipine, only the quinapril group showed early improvement in endothelial function (23), while two other studies of enalapril and lisinopril found no benefit (24, 25). The HOPE study generalizes this benefit of improved endothelial function of ACE-I's to hard clinical end points of reduced major cardiac events.

A great deal of evidence indicates that increased vascular free oxygen levels are the prime underlying mechanism for endothelial dysfunction, and as such the use of antioxidants to improve endothelial function would seem to be a logical inference. Unfortunately, there is no corresponding amount of clinical study data demonstrating the efficacy of antioxidants in this setting. Vitamin C supplementation has been shown to improve endothelium-dependent responses in the forearm circulation of patients with various risk factors of CAD (14). However, in other studies, supplementation with Vitamin E or a combination of antioxidant vitamins did not demonstrate any improvement (26, 27). While superoxide dismutase did not show any benefit, the other novel antioxidants probucol, glutathione and N-acetylcysteine have been shown to improve endothelial function in clinical studies (22).

Estrogen is another compound that has been shown to improve endothelium-dependent vasorelaxation via both NO-dependent and independent mechanisms. Administration of estrogen has been shown to improve coronary and peripheral responses to acetylcholine, which occur as soon as 10 minutes after intravascular administration (14, 22). In addition, longer-term benefits may be mediated by estrogen's modification of the lipid profile by increasing high-density lipoprotein (HDL) and decreasing LDL concentrations. Unfortunately, the long-term cardiovascular benefits of hormone replacement therapy have not been demonstrated in clinical trials. On the contrary, the vast majority of randomized studies have shown a trend towards cardiovascular harm with

hormone replacement, making the link from endothelial dysfunction to clinical events less apparent.

Antioxidants

Atherosclerosis is a complex process that involves the deposition of plasma lipoproteins, the proliferation of cellular elements, and an inflammatory response in the artery wall. Atherosclerosis advances through a series of stages, beginning with fatty streak lesions composed of lipid-engorged macrophage foam cells and ultimately ending in a complex plaque consisting of a core of lipid and necrotic debris covered by a fibrous plaque (28). Considerable evidence has been gathered to support the hypothesis that oxidative modification of LDL by free radicals is an important step in the development and progression of atherosclerosis (29). At the center of this hypothesis is LDL cholesterol, which undergoes multiple changes upon oxidation that are thought to be proatherogenic (Fig. 2; 30). This theory is termed the "oxidative modification hypothesis." It is be-

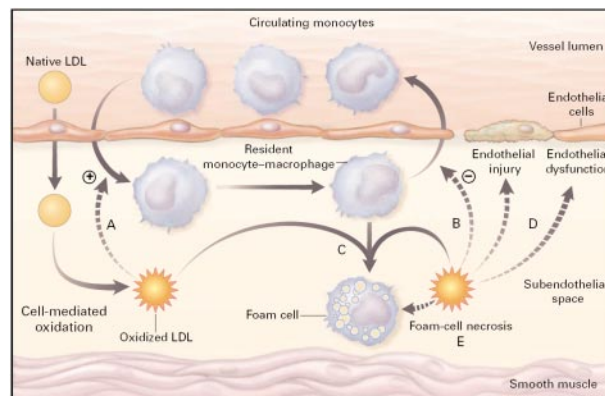


Fig. 2. Early events in atherogenesis. Native LDL becomes trapped in the subendothelial space, where it can be oxidized by resident vascular cells such as smooth-muscle cells, endothelial cells, and macrophages. Oxidized LDL stimulates (plus sign) monocyte chemotaxis (A) and inhibits (minus sign) monocyte egress from the vascular wall (B). Monocytes differentiate into macrophages that internalize oxidized LDL, leading to foam-cell formation (C). Oxidized LDL also causes endothelial dysfunction and injury (D), as well as foam-cell necrosis (E), resulting in the release of lysosomal enzymes and necrotic debris. Broken arrows indicate adverse effects of oxidized LDL. Adapted with permission from Quinn MT, Parthasarathy S, Fong LG, Steinberg D. Oxidatively modified low density lipoproteins: a potential role in recruitment and retention of monocyte/macrophages during atherogenesis. *Proc Natl Acad Sci U S A* 1987; 84(9):2995–2998 (30).

LDL = low-density lipoproteins

lieved that unoxidized LDL is not very harmful and is processed by routine lipid metabolism mechanisms without permanent deposition in vessel walls. However, oxidation of LDL leads to the production of a diverse array of biologically active compounds, including some that influence the functional integrity of vascular cells (Fig. 2; 30, 31). Among the most well-characterized effects of LDL oxidation are increases in the expression of endothelial cell surface adhesion molecules that facilitate the mobilization and uptake of circulating inflammatory cells, and alterations in the chemotactic properties of monocytes and macrophages in a manner expected to increase their residency within the artery wall (32). Once the LDL apolipoprotein B proteins are altered by oxidation, they are not recognized by the LDL receptors, but are instead taken up by scavenger receptors. The scavenger receptors are not saturable, and cells with these receptors, namely macrophages, become overloaded with LDL and become foam cells. In addition, oxidative processes are proposed to play a role in lesion maturation and the precipitation of clinical events. This may involve effects on intimal proliferation, fibrosis, calcification, endothelial function and vasoreactivity, plaque rupture, and thrombosis (30).

Oxidants are a chemically diverse group, which are products of normal aerobic metabolism and the inflammatory response. The identification of specific oxidants responsible for disease processes is made difficult by the multitude of pathophysiological events linked to oxidation and the difficulty in measuring the correspondingly short-lived species within the vessel wall. While oxidant formation is inevitable, oxidant-mediated disease is proposed to occur only under circumstances in which these compounds overwhelm antioxidant defenses. Antioxidants also constitute a diverse group of compounds with different properties and mechanisms of action. They can operate by inhibiting oxidant formation, intercepting oxidants once they have formed, or by repairing oxidant-induced injury. With regard to atherosclerotic disease, inhibition of LDL oxidation is the best-characterized effect and includes both reductions in the concentration and reactivity of oxidants, and improved resistance of LDL to these oxidants (30). Steinberg has hypothesized that, unlike agents that lower cholesterol or blood pressure, antioxidants may have to be used for more than five years to have a demonstrable benefit, since the primary mechanism of these agents may be the prevention of new lesions (33). The antioxidants that have re-

ceived the most attention include vitamin C (ascorbic acid), vitamin E (α -tocopherol), and β -carotene (provitamin A) (32).

Vitamin E

Vitamin E exists as at least 8 naturally occurring compounds, including α -, β -, γ - and δ -tocopherol and α -, β -, γ - and δ -tocotrienol, with α -tocopherol being the most active component in natural vitamin E. *In vitro*, vitamin E at high doses blocks the oxidative modification of LDL cholesterol, decreases LDL deposition in arterial walls, reduces monocyte adhesion to endothelium, and inhibits platelet activation (31). These findings, which are in line with the oxidative modification hypothesis, have fueled numerous epidemiological studies and clinical trials on the role of vitamin E in the prevention and treatment of CAD. Unfortunately, although observational trials of vitamin E have been associated with a 20–40% reduction in the risk of coronary disease, randomized trials have not borne out this benefit.

Observational Studies: The US Nurses' Health Study involved approximately 87,000 participants who were followed up for periods of up to 8 years (34). A dietary questionnaire was used to estimate vitamin E intake. Levels of intake were divided into quintiles in women aged 34–59 years who were free of diagnosed CAD, and they were followed for the primary end point of nonfatal myocardial infarction (MI) or coronary death. The study found an adjusted coronary disease relative risk (RR) of 0.66 (95% confidence interval [CI], 0.50–0.87, $p < 0.001$) for women consuming the highest vs. lowest amount of vitamin E, after adjustment for age and smoking status. Subsequent analyses revealed a 43% lower risk for vitamin E supplement users vs. nonusers and found that the benefit of vitamin E was only evident after more than 2 years of consumption.

The Health Professions Follow-up Study followed almost 40,000 men aged 40–75 without CAD for up to 4 years, for the occurrence of nonfatal MI, CAD-related death and coronary revascularization (35). Again, the highest quintile of vitamin E consumption was associated with an adjusted RR of 0.64 (95% CI, 0.49–0.83, $p < 0.003$). Both studies found that the benefit was primarily evident for those subjects who consumed supplemental sources of vitamin E, and found that cardiovascular protection was maximized at 100 IU/day.

A long-term Finnish study followed 5,000 men and women aged 30–69, free from CAD, for 14

years, for death from CAD (36). An RR of 0.68 (95% CI, 0.42–1.11) for men and 0.35 (95% CI, 0.14–0.88) for women were seen in the tertiles of greatest vs. lowest vitamin E consumption. The tertile boundaries in this study were relatively close, representing only a 25% difference, making the results in this study difficult to interpret.

The Iowa Women's Study, of almost 35,000 postmenopausal women without CAD who filled out questionnaires, was conducted for 7 years and observed for the end point of death from CAD (37). Compared to the lowest quintile of intake, women in the highest quintile had an RR of 0.38 (95% CI, 0.18–0.80, $p=0.004$). In contrast to both the US Nurses' Health Study and the Health Professions Follow-up Study, this study found benefit only for those subjects who consumed vitamin E in their diet, not in those who used vitamin E supplementation.

The Established Populations for Epidemiologic Studies of the Elderly (EPESE) observed more than 11,000 men and women for 6 years and found that vitamin E supplement use resulted in an RR of 0.53 (95% CI, 0.34–0.84) (38).

There were, however, two negative observational studies for vitamin E. The Scottish Heart Health Study followed almost 12,000 patients for 8 years for the occurrence of new CAD and all-cause mortality (39). No significant benefit was found on the primary end point with higher levels of vitamin E consumption. The Rotterdam Study followed almost 5,000 subjects for 4 years for the occurrence of MI and also found no effect of vitamin E (40).

Randomized, Prospective Studies: The Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study randomized more than 29,000 male smokers to 50 IU/day of vitamin E, 20 mg of β -carotene, both, or placebo for 5–8 years (41). While the major end point was lung cancer, major coronary events were a secondary end point. The study found no overall effect on major coronary events for the population as a whole with vitamin E. In the subgroup initially free of CAD, a very modest decrease in the incidence of angina was noted in those taking vitamin E (RR 0.91, 95% CI, 0.83–0.99, $p=0.04$), while there was no effect on fatal or nonfatal CAD (42, 43). In patients with a history of angina and in those with a history of MI, vitamin E had no effect on any major coronary events (44, 45). One possible confounding factor was that the dose of vitamin E used in this study was lower than the optimal dose of 100 IU/day used in observational studies.

The Cambridge Heart Antioxidant Study (CHAOS) tested the effects of high doses (400 or

800 IU/day) of vitamin E on subsequent cardiovascular events in 2,002 patients with angiographically proven CAD (46). The relative risk of all cardiovascular events was 0.53 (95% CI, 0.34–0.83) for patients who had been taking vitamin E for a mean of 1.4 years. The beneficial effects of supplementation appeared to occur after 200 days of therapy.

The Heart Outcomes Prevention Evaluation (HOPE) Study enrolled 9,541 men and women aged 55 or older who were at high risk of cardiovascular events because they had cardiovascular disease or diabetes with another CAD risk factor (47). Patients were randomized to 400 IU/day of vitamin E or placebo, and followed for a mean of 4.5 years for the primary end point of MI, stroke, or cardiovascular death. The study found no significant differences in the composite primary end point (RR 1.05, 95% CI, 0.95–1.16, $p=0.33$), in cardiovascular deaths, in MIs, in strokes, or in any of the secondary cardiovascular outcomes.

The GISSI-Prevenzione study randomized more than 11,000 patients surviving a recent MI to either vitamin E (300 mg = 378 IU daily), n-3 polyunsaturated fatty acids, both, or placebo and followed them for 3.5 years for the composite outcome of death, MI, or stroke (48). While the n-3 polyunsaturated fatty acid group showed some benefit, no significant benefit was seen with the vitamin E.

Vitamin A

While vitamin E and vitamin A have been of particular interest because both are carried within LDL particles, β -carotene has less impressive *in-vitro* evidence for preventing oxidative modification of LDL (49, 50). Nonetheless, support for vitamin A has come from many *in-vitro* observational studies that showed cardiovascular benefit in a subgroup of current or former smokers.

Observational Studies: The Health Professions Follow-up Study also accumulated information on β -carotene consumption for the 40,000 men enrolled (35). These men, aged 40–75 and without CAD, were observed for up to 4 years for the occurrence of nonfatal MI, CAD-related death, and coronary revascularization. The highest quintile of vitamin A consumption was associated with an adjusted RR of 0.30 (95% CI, 0.11–0.72) compared to the lowest quintile of consumption. In subgroup analysis, this benefit was only significant for current and former smokers. The Rotterdam Study followed almost 5,000 subjects aged 55–95, without a past history of MI, who filled out dietary

questionnaires (40). After 4 years there was a 0.55 relative risk (95% CI, 0.34–0.83, $p=0.013$) of MI in the highest vs. the lowest tertile of β -carotene consumption. This association was also most evident for current and former smokers.

The Iowa Women's Study followed nearly 35,000 postmenopausal women without CAD who filled out questionnaires on vitamin A consumption (37). After 7 years of observation, there was no significant difference in cardiovascular deaths between the highest and the lowest quintiles of consumption. The Finnish study followed 5,000 men and women without CAD for 14 years, for death from CAD, and no benefit of β -carotene was seen (36). The Scottish Heart Health Study's observation of 12,000 patients for 8 years revealed only a trend towards reducing newly diagnosed CAD and cardiac mortality with higher levels of vitamin A consumption (39).

Randomized, Prospective Studies: The Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study was designed to determine the effects of antioxidants on the incidence of lung cancer (41). The trial randomized more than 29,000 male smokers to 50 IU/d of vitamin E, 20 mg/d of β -carotene, both, or placebo, and followed them for 5–8 years for the primary end point of lung cancer, with major coronary events as secondary end points. There was no reduction in the risk of lung cancer or ischemic heart disease with β -carotene, and there was an unexpected increase in mortality from lung cancer and ischemic heart disease.

The Beta-Carotene and Retinol Efficacy Trial (CARET) tested the effects of combined treatment with β -carotene (30 mg/d) and retinyl palmitate (vitamin A) in 18,000 patients with a history of cigarette smoking or exposure to asbestos (51). This study, like the ATBC, also showed increases in the risk of lung cancer and ischemic heart disease.

The Physicians' Health Study enrolled 22,000 US male physicians and randomized them to β -carotene (50 mg every other day), aspirin (325 mg/d), both, or neither, and followed them for 12 years (52). No effect was seen with β -carotene on the risk of MI, stroke, or cancer. These results are potentially more robust than the other two trials due to the large sample size, the long duration of observation, and the broader study population, which was not limited to smokers or high-risk individuals.

Vitamin C

Vitamin C is also believed to inhibit oxidative modification of LDL. However, the predominance

of evidence does not support the existence of a benefit of vitamin C supplementation.

Observational Studies: The Health Professions Follow-up Study also accumulated information on vitamin C consumption for the 40,000 men enrolled (35). These men without CAD were observed for up to 4 years for the occurrence of nonfatal MI, CAD-related death, and coronary revascularization. There was no effect of vitamin C found on any of the clinical end points. The Iowa Women's Study, of almost 35,000 postmenopausal women without CAD, also found no significant difference in cardiovascular death after 7 years of observation with vitamin C consumption (37). The Established Populations for Epidemiologic Studies of the Elderly (EPESE), which observed more than 11,000 men and women for 6 years, did not find any significant effect on cardiovascular death with vitamin C (38). The Scottish Heart Health Study followed almost 12,000 patients for 8 years for the occurrence of new CAD and all-cause mortality (39). No significant benefit was found, with higher levels of vitamin C consumption, on the primary end point. The Rotterdam Study followed almost 5,000 subjects for 4 years for the occurrence of MI and also found no beneficial effect of vitamin C (40).

The Finnish cohort of 5,000 men and women without CAD were followed for 14 years, for death from CAD (36). The tertiles of greatest vs. lowest vitamin C consumption showed a benefit attributed to vitamin C with an RR of 0.49 (95% CI, 0.32–0.98). The National Health and Nutrition Examination Survey (NHANES 1) followed more than 11,000 men and women for 10 years and found a reduction in cardiovascular death with vitamin C (53). Men with the most vitamin C consumption had an RR of 0.58 (95% CI, 0.53–0.82) and women 0.75 (95% CI, 0.55–0.99).

Randomized, Prospective Studies: The only randomized trial of vitamin C was the Chinese Cancer Prevention Trial, which examined the effect of dietary supplementation on cardiovascular death in almost 30,000 men and women over 5 years (54). One arm of the study involved subjects treated with 120 mg of vitamin C daily, which resulted in no significant effect on cardiovascular death.

Combination Therapy

The use of combination antioxidant therapy has been attempted in several smaller angiographic trials and several large clinical trials.

In a three-year, double-blind trial, 160 patients with CAD, low HDL, and normal LDL were ran-

domized to receive simvastatin plus niacin, antioxidants, both, or placebo (55). The antioxidants consisted of 800 IU of vitamin E, 1000 mg of vitamin C, 25 mg of β -carotene, and 100 μ g of selenium daily. The primary end point was change in angiographic stenosis and the occurrence of a first cardiovascular event. Simvastatin plus niacin showed a significant reduction in clinical events and a regression of coronary stenosis compared to placebo; antioxidants were found to have no significant benefit over placebo. In fact, antioxidants in combination with simvastatin and niacin actually attenuated the benefit of the lipid-lowering agents. The frequency of clinical events was 24% with placebo, 21% with antioxidants, 3% with simvastatin plus niacin, and 14% with combination therapy. Coronary stenosis followed a similar trend, with 3.9% progression with placebo, 1.8% with antioxidants, 0.7% with combination, and 0.4% regression with simvastatin plus niacin.

The Women's Angiographic Vitamin and Estrogen (WAVE) Trial randomized 423 postmenopausal women to antioxidants or placebo, and to estrogen or placebo in another arm (56). The antioxidant therapy consisted of 800 IU of vitamin E and 1,000 mg of vitamin C daily. After a mean of 2.8 years, there was a non-significant trend towards worsening stenosis in the antioxidant group, with 0.044 mm/year worsening with antioxidants and 0.028 mm/year with placebo.

The Heart Protection Study tested the hypothesis that increased intake of antioxidant vitamins would reduce the risk of vascular disease, cancer, and other adverse outcomes in a UK cohort of almost 21,000 adults 40–80 years old (57). These patients, with CAD, diabetes, or other occlusive arterial disease, were randomized to 600 mg (756 IU) vitamin E, 250 mg vitamin C, and 20 mg β -carotene daily or placebo, and followed for 5 years. At the end of follow-up there was no significant difference in all-cause mortality, vascular death, non-vascular death, MI, stroke, or revascularization.

The Chinese Cancer Prevention Trial examined the effect of dietary supplementation on cardiovascular death in almost 30,000 men and women, with 30 IU of vitamin E plus 15 mg of β -carotene daily (54). Patients were followed for 5 years, and no significant effect of antioxidants was seen on cardiovascular death.

Conclusion

At this time the study of endothelial dysfunction and how it relates to the genesis of athero-

sclerotic disease and the pathogenesis of acute coronary syndrome holds a great deal of promise for improved treatment and prevention of CAD. The vascular endothelium plays a crucial role in the physiology of blood vessels, and further understanding of its mechanisms of action and possible therapeutic targets will be of great importance. Antioxidant vitamin therapy, on the other hand, has already been extensively studied in both large observational and randomized, prospective trials. Unfortunately, the weight of the evidence points to little or no benefit of this therapy in reducing cardiovascular outcomes, and there appears to be no important niche for antioxidant vitamins in cardiovascular therapy.

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