Dietary modulation of endothelial function: implications for cardiovascular disease[1–3]

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ABSTRACT   The vascular endothelium is the primary site of dysfunction in many diseases, particularly cardiovascular disease. A variety of risk factors, including smoking, hypercholesterolemia, hyperhomocysteinemia, hypertension, and diabetes mellitus, adversely affect endothelial function. Emerging evidence suggests an important role of dietary factors in modulating endothelial function. In particular, n−3 fatty acids, antioxidant vitamins (especially vitamins E and C), folic acid, and L-arginine appear to have beneficial effects on vascular endothelial function, either by decreasing endothelial activation or by improving endothelium-dependent vasodilation in patients at high risk of cardiovascular disease as well as in healthy subjects. These effects may serve as one potential mechanism through which these nutrients reduce the risk of cardiovascular disease, as observed in epidemiologic studies and several clinical trials. This article reviews clinical and experimental evidence regarding the role of these nutrients in modulating endothelial function and their potential to prevent cardiovascular disease.   *Am J Clin Nutr* 2001;73:673–86.

KEY WORDS   Cardiovascular disease, endothelial function, folic acid, antioxidants, vitamin E, n−3 fatty acids, L-arginine, diabetes

INTRODUCTION

Historically, the vascular endothelium was thought of as a static monolayer of cells within the body, acting as a semipermeable barrier between blood and tissue (1). Over the past few decades, experimental and clinical evidence has proven that the endothelium is an active and dynamic tissue involved in maintaining homeostasis in both healthy and diseased states (2). The main functions of the endothelium are to maintain blood circulation and fluidity, regulate vascular tone, and modulate leukocyte and platelet adhesion and leukocyte transmigration.

The endothelium is essential to the hemostatic processes of cell adhesion and migration, thrombosis, and fibrinolysis. Endothelial cells express adhesion molecules such as P-selectin, E-selectin, intercellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion molecule 1 (VCAM-1) on the cell surface that are involved in leukocyte recruitment and platelet adhesion during thrombosis and inflammation (3). In addition, endothelial cells synthesize plasma proteins such as von Willebrand factor (vWF) for platelet adhesion in thrombosis and soluble molecules such as E-selectin and thrombomodulin (TM) (2). When the vascular endothelium encounters inflammatory stimuli, it undergoes several changes, including the up-regulation of surface and soluble cell adhesion molecules and the release of cytokines. This process, termed endothelial activation, can be triggered by a variety of inflammatory stimuli encountered in the blood, including oxidized LDL, free radical species, lipopolysaccharide (LPS), and cytokines, such as tumor necrosis factor α. Under normal physiologic conditions, endothelial activation is transient, and its duration depends on the presence of the inflammatory stimulus.

The activated endothelium plays an integral role in the development of atherosclerosis. Circulating monocytes are attracted to the endothelium by chemokines, bind to the adhesion molecules, adhere, and transmigrate to the subendothelial space, where they become macrophages. Within the subendothelium, macrophages scavenge oxidized LDL, become foam cells, and contribute to the development of the fatty streak in the early stage of atherosclerosis (4).

The endothelial monolayer is also important for smooth muscle cell function, vascular remodeling, and the maintenance of vascular tone through both vasoconstriction and vasodilation (1). The endothelium synthesizes several molecules that are crucial for its vasomotor function and that can be released in response to local mechanical stimuli (eg, flow and shear stress), metabolic conditions (eg, hypoxia), and receptor-mediated agonists (eg, acetylcholine) (5). The major endothelial products that function as vasoconstrictors are thromboxane A_2, prostaglandin H_2, and endothelin 1 (6). The endothelium-derived vasodilators include nitric oxide, endothelium-derived hyperpolarizing factor, and prostacyclin. Vascular tone is determined by the balance between vasoconstricting and vasodilating agents in the environment surrounding the endothelium (1). Nitric oxide or endothelium-derived relaxing factor, a molecule synthesized from L-arginine by nitric-oxide synthase, is the primary compound responsible for vasodilation in arteries (7). Nitric oxide inhibits

platelet aggregation (7), modulates leukocyte-endothelium interactions by altering cell adhesion molecule expression and reducing monocyte adherence (8), and inhibits the proliferation of smooth muscle cells.

ENDOTHELIAL DYSFUNCTION

Although the vascular endothelium functions properly under normal conditions, certain mechanical and physiologic conditions can disturb its function. The 2 primary clinical measurements of endothelial dysfunction are enhanced and maintained endothelial activation and impaired endothelium-dependent vasodilation. Endothelial activation is detected by increased plasma concentrations of soluble adhesion molecules such as ICAM-1, VCAM-1, and E- and P-selectin, which are shed and released into plasma from the activated endothelium and macrophage. Higher concentrations of these soluble molecules have been found in patients with diabetes and hyperlipidemia and in persons with inflammatory conditions (9). Defects in endothelium-dependent vasodilation can be detected in coronary vessels by coronary angiography after infusion of acetylcholine to measure coronary artery diameter and blood flow. However, this method is invasive and time-consuming. A noninvasive ultrasound technique has been developed to measure the vasodilator responses of conduit vessels (eg, brachial artery) after infusion of agonists such as acetylcholine or serotonin or, more commonly, in response to increased flow induced by reactive hyperemia (5). This method, which has excellent reproducibility (10), is now used widely in clinical experimental studies. Anderson et al (11) showed that brachial vasodilator response to reactive hyperemia assessed by an ultrasound technique is closely related to the coronary vasodilator response to acetylcholine.

Some early evidence suggesting enhanced adhesion molecule expression in patients with cardiovascular disease (CVD) was documented in autopsy studies, which found increased cell surface expression of ICAM-1, VCAM-1, and E-selectin in coronary arteries and abdominal aortas (12–14). Subsequent studies showed that plasma concentrations of soluble cell adhesion molecules are elevated in patients with CVD. In a nested case-control study, Hwang et al (15) found significantly higher circulating ICAM-1 in patients with coronary heart disease (CHD) or carotid artery atherosclerosis than in control subjects. Similarly, Morisaki et al (16) found elevated serum concentrations of ICAM-1 in patients with ischemic heart disease. In addition, Peter et al (17) found that VCAM-1 concentrations correlated well with the extent of atherosclerosis. Ridker et al (18) reported that higher serum concentrations of ICAM-1 predicted future risk of myocardial infarction (MI) in the Physicians' Health Study. Several studies also showed elevated concentrations of E-selectin, VCAM-1, and ICAM-1 in subjects with diabetes (19–21).

Impaired endothelium-dependent vasodilation was first noted in patients with CVD when Ludmer et al (22) injected acetylcholine into the coronary arteries and recorded paradoxical vasoconstriction instead of the normal relaxation and vasodilation response. Subsequently, numerous studies confirmed impaired endothelium-dependent vasodilation in both patients with CVD (10, 23, 24) and patients with type 2 diabetes (25).

Major CVD risk factors, such as smoking, hypercholesterolemia, hypertension, diabetes, and hyperhomocysteinemia, have all been found to cause endothelial dysfunction. Smoking reduces nitric-oxide production and possibly increases nitric-

oxide degradation as a result of increased oxidative stress (26). In addition, smokers tend to have higher concentrations of ICAM-1 and P-selectin than do nonsmokers (27, 28). Hypercholesterolemia decreases nitric-oxide production in human endothelial cells in vitro (29). Oxidized LDL leads to an increased expression of adhesion molecules on the endothelial cell surface, allowing for and potentiating monocyte infiltration to the subendothelium (4). Conversely, HDL provides an atheroprotective effect by inhibiting cytokine-induced endothelial cell adhesion molecule expression (30) and by enhancing agonist-induced vasodilation in coronary arteries (31). Diabetes mellitus is associated with physiologic changes that potentiate endothelial dysfunction, such as hypertriglyceridemia, small LDL size, low HDL, hypertension, and hyperglycemia (32).

Several pharmacologic and nonpharmacologic strategies have been proposed to improve endothelial function. Pharmacologic strategies include angiotensin-converting enzyme inhibitors, which prevent the inactivation of bradykinin, a potent vasodilator, and calcium-channel blockers, which work by reducing cardiac and smooth muscle contractility (1). The cholesterol-lowering agents collectively known as statins were found to decrease markers of inflammation, such as C-reactive protein (33), and to improve endothelium-dependent vasomotion (34). Hormone replacement therapy for women was shown to improve endothelial function (34), although it has been associated with elevated concentrations of C-reactive protein (35). Increasing physical activity improves endothelial function in patients with coronary artery disease (36).

DIETARY FACTORS THAT INFLUENCE ENDOTHELIAL FUNCTION

There has been growing interest in the role of nutritional factors in modulating endothelial function. n−3 Fatty acids, antioxidant vitamins, folic acid, and L-arginine have been the primary focus of investigation. In this article, we summarize the epidemiologic and clinical trial data on these nutrients and cardiovascular endpoints and review in detail experimental evidence regarding the effects of these nutrients on endothelial function. We searched the MEDLINE database for clinical investigations of the effects of these nutrients on endothelial function. We also carefully examined the references in the articles identified to locate additional relevant studies. The studies can be broadly classified into 2 categories: 1) those that assessed adhesive properties of the endothelium by measuring plasma concentrations of soluble adhesion molecules or circulating concentrations of homeostatic factors such as vWF and TM and 2) those that assessed the endothelium-dependent dilation of conduit vessels after reactive hyperemia by noninvasive ultrasound technique.

We excluded studies involving single acute doses of the nutrients and those that examined the effects of pharmacologic antioxidants such as probucol, because our main interest was in the role of dietary intake. We also did not review studies on resistance vessel endothelial dysfunction because endothelial-dependent vasodilation of resistance vessels is not solely mediated by nitric-oxide and there is little evidence that endothelial function of resistance vessels correlates with that of coronary arteries (37). In contrast, conduit vessel endothelial function as measured by flow-mediated vasodilation of the brachial artery by ultrasound is closely related to the response of coronary arteries to acetylcholine (11).

TABLE 1
In vitro studies of n−3 fatty acids and endothelial cell adhesion properties[1]

Reference	Year	Fatty acid and concentration	Methods	Outcome
De Caterina et al (57)	1994	DHA, EPA, oleate, and AA: 10 μmol/L	Adhesion molecule surface and mRNA expression in cytokine-stimulated HSVEC	DHA reduced VCAM-1 cell surface and mRNA expression
			Monocyte adhesion assays	DHA decreased monocyte adhesion
Weber et al (58)	1995	DHA, EPA, and AA: 20 μmol/L	Adhesion molecule surface and mRNA expression in cytokine-stimulated HUVEC	DHA reduced VCAM-1 cell surface and mRNA expression
			Monocyte adhesion assays	DHA reduced monocyte adhesion
Khalfoun et al (59)	1996	DHA, EPA, and AA: 100 mg/L	Adhesion molecule surface expression in cytokine-stimulated HUVEC	DHA and EPA decreased VCAM-1 cell surface expression
			PBL adhesion assays	DHA and EPA reduced PBL adhesion
De Caterina et al (60)	1998	DHA and ricinoleic, oleic, palmitoleic stearic, and palmitic acids: 25 μmol/L	Adhesion molecule surface and mRNA expression in cytokine-stimulated HSVEC	DHA reduced VCAM-1 cell surface and mRNA expression

[1] DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid; HSVEC, human saphenous vein endothelial cells; HUVEC, human umbilical vein endothelial cells; PBL, peripheral blood lymphocytes; VCAM-1, vascular cell adhesion molecule 1; mRNA, messenger RNA.

Long-chain n−3 fatty acids

Intake of n−3 fatty acids and risk of cardiovascular disease

CVD is rare in populations with a very high intake of fish, such as Alaskan Native Americans (38, 39), Greenland Eskimos (40, 41), and Japanese of fishing villages (42, 43), which raises the possibility that fish oil is protective against atherosclerosis. Subsequent prospective cohort studies evaluated the association between fish consumption and the risk of CVD in several different populations. Kromhout et al (44) showed in the Dutch component of the Seven Countries Study, with 20 y of follow-up, that men who consumed 30 g fish/d had a 50% lower mortality from CHD than did men who did not eat fish. In the Western Electric Study, Daviglus et al (45) found that men who consumed ≥35 g fish/d had a relative risk (RR) of death from CHD of 0.62 (95% CI: 0.40, 0.94) compared with those who rarely ate fish. In the US Physicians' Health Study, Albert et al (46) found that weekly fish consumption was associated with an RR of 0.48 (95% CI: 0.24, 0.96) for sudden cardiac death, although in the same cohort, no significant association was observed between fish consumption and overall cardiovascular endpoints (47). In the Health Professionals Follow-up Study, Ascherio et al (48) found no overall association between dietary intake of n−3 fatty acids or fish intake and the risk of CHD, but there was a nonsignificant trend for a reduction in risk of fatal CHD with increasing fish consumption. The results of these studies suggest that fish intake is probably more protective against fatal CHD than against nonfatal MI.

Two interventional studies, the Diet and Reinfarction Trial (DART) (49) and the GISSI-Prevenzione trial (50), evaluated whether fish consumption or fish-oil supplementation reduces coronary mortality in MI patients. DART showed that men who received advice to increase fish consumption had a significant reduction in total mortality of 29% after 2 y. In the more recent GISSI-Prevenzione trial, MI patients were randomly assigned to 4 different daily supplementation treatments: n−3 fatty acids [850–882 mg eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (1:2)], vitamin E (330 mg α-tocopherol), both n−3 and vitamin E, and neither supplement (control). Daily supplementation with n−3 fatty acids reduced cardiovascular deaths by 10–15%; combined treatment with vitamin E and n−3 fatty acids resulted in a similar reduction. The results of both studies support the notion that increased consumption of n−3 fatty acids reduces mortality in high-risk patients (51).

Effects of n−3 fatty acids on endothelial function

The exact mechanism by which n−3 fatty acids exert an atheroprotective effect is unclear. However, n−3 fatty acids can influence many aspects of the pathogenesis of CHD, including lipid concentrations, the size and oxidizability of lipids (52), platelet aggregation (53), and arrhythmia (54). There is growing evidence regarding the effect of fish oil on endothelial function (55, 56). Below, we summarize experimental evidence regarding the role of n−3 fatty acids in modulating endothelial function.

The in vitro studies that examined the effects of n−3 fatty acids on cell adhesion molecule surface and mRNA expression, as well as leukocyte and endothelium interactions (57–60), are summarized in **Table 1**. Overall, these studies showed that n−3 fatty acids, particularly DHA, decrease expression of VCAM-1 on the vascular endothelium and decrease leukocyte rolling and adhesion to the endothelium. One additional study looked at the effects of n−3 fatty acids on nitric-oxide production. Okuda et al (61) used human umbilical vein endothelial cells to assess nitric-oxide production after incubation with EPA. They found an increase in nitric-oxide production 3, 10, and 30 min after addition of 0.3 mmol EPA/L. The study also illustrated that the increased nitric-oxide production was dependent on a Ca^{2+}/calmodulin pathway. In an interesting ex vivo study (62), hypercholesterolemic patients and control subjects received either 10-g fish-oil capsules or placebo for 3 mo. Before and after the 3 mo of supplementation, a sample of skin and gluteal fat was biopsied, small arterial segments were removed, and vasodilation was assessed in response to acetylcholine (endothelium-dependent) and nitroprusside (endothelium-independent). Peripheral small arteries from the hypercholesterolemic patients showed significant improvement in endothelium-dependent relaxation after fish-oil supplementation compared with those from the control subjects.

Although the results of these in vitro studies are consistent in supporting a favorable effect of n−3 fatty acids, DHA in particular, on endothelial function, results from in vivo studies are

TABLE 2
Clinical studies of n−3 fatty acids and endothelial function[1]

Reference	Year	Study design	Subjects	Daily dosage	Duration	Methods	Outcome
Fleischhauer et al (63)	1993	p, r, p-c	Heart transplant patients (n = 14)	5 g n−3 fatty acids	3 wk	ACH-mediated dilation of coronary arteries	Improved vasodilation
Abe et al (64)	1998	p, r, p-c	Hypertriglyceridemic subjects (n = 39)	4 g n−3 fatty acids (Omacor)	6 wk, ≥7 mo	ICAM-1, VCAM-1, E-selectin	Decreased ICAM-1 and E-selectin after ≥7 mo
Seljeflot et al (65)	1998	p, r, p-c	Smokers with hyperlipidemia (n = 41)	4.8 g n−3 fatty acids	6 wk	TM, vWF, P-selectin, E-selectin, VCAM-1	Decreased TM and vWF; increased VCAM-1 and E-selectin
Johansen et al (66)	1999	p, r	CHD patients (n = 54)	5.1 g n−3 fatty acids	4 wk	TM, vWF, P-selectin, E-selectin, VCAM-1	Decreased TM and vWF; increased VCAM-1 and E-selectin
Goodfellow et al (56)	2000	p, r, p-c	Hypercholesterolemic subjects (n = 30)	4 g n−3 fatty acids	120 d	Flow-mediated dilation of brachial artery	Improved vasodilation

[1] p, prospective; r, randomized; p-c, placebo-controlled; TM, soluble thrombomodulin; vWF, von Willebrand factor; ACH, acteylcholine; ICAM-1, intercellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1.

less consistent. Five studies examined n−3 supplementation and measures of endothelial function (56, 63–66) (**Table 2**). Abe et al (64) looked at plasma concentrations of soluble adhesion molecules in hypertriglyceridemic and diabetic patients who were supplemented with 4 g purified n−3 fatty acids daily for 2 periods. At baseline, the hypertriglyceridemic patients had higher concentrations of ICAM-1, VCAM-1, and E-selectin than did the control subjects. The authors found no reduction in soluble adhesion molecules after 6 wk in patients who received n−3 fatty acids but did find a significant reduction in ICAM-1 of 9 ± 3.4% and in E-selectin of 16 ± 3.2% after 7 mo. Interestingly, patients with diabetes expressed the greatest reduction in soluble adhesion molecules after the n−3 supplementation. Seljeflot et al (65) evaluated the effect of supplementation with 4.8 g n−3 fatty acids/d in male smokers with hyperlipidemia (n = 41) for 6 wk on soluble adhesion and hemostatic variables. They found that n−3 supplementation caused a significant reduction in vWF and TM but an increase in VCAM-1 and E-selectin. In a subsequent study Johansen et al (66) examined the effects of n−3 fatty acids on hemostatic and inflammatory markers in 54 patients enrolled in the Coronary Angioplasty Restenosis Trial (CART). These patients were treated by percutaneous transluminal coronary angioplasty followed by 5.1 g n−3 fatty acid/d (group 1) or placebo (corn oil; group 2) for 6 mo. For another 4 wk (study period), both groups received 5.1 g n−3 fatty acids/d. At baseline, group 1 had significantly lower concentrations of vWF and TM and higher concentrations of VCAM-1 and E-selectin than did group 2. After the 4-wk supplementation in both groups, serum concentrations of tissue-type plasminogen activator antigen and TM significantly decreased in group 2, whereas concentrations of VCAM-1 and E-selectin significantly increased. The changes in these markers were greater in group 2 than in group 1.

These results contradict the results of the in vitro studies reviewed above, which clearly showed that n−3 fatty acids reduce expression of cytokine-induced adhesion molecules. Johansen et al (66) attributed the increased adhesion molecules associated with fish oil supplementation to the increased oxidative stress because they observed higher levels of lipid peroxidation, as measured by serum thiobarbituric acid–reactive substances (TBARS), in group 1 than in group 2 at baseline, which also significantly increased in group 2 during the 4-wk

fish-oil supplementation. Interestingly, serum vitamin E concentrations decreased after fish-oil supplementation in both groups, even though each fish oil capsule contained 4 mg vitamin E. It is possible that consumption of antioxidants increased as a result of an increased level of oxidation after n−3 fatty acid supplementation. There is some evidence that highly concentrated n−3 fatty acids in fish oil may be prone to peroxidation (67), which can stimulate expression of endothelial cell adhesion molecules. Thus, Johansen et al (66) suggested that a high dose of fish oil without adequate protection by antioxidant vitamins might induce proinflammatory responses and adversely affect endothelial function.

In contrast, the discrepancies in these in vivo studies may have been due to methodologic differences, such as varying lengths of the studies and different patient populations. Seljeflot et al (65) looked at the effects of supplementation for only 6 wk, and the "study period" in Johansen et al's study (66) lasted for only 4 wk. In Abe et al's study (64), there was no change in the concentrations of soluble adhesion molecules at 6 wk but a significant reduction after 7 mo. The 3 studies also investigated different patient populations. The study by Seljeflot et al (65) included male smokers with hyperlipidemia and the study by Johansen et al (66) included patients with advanced CHD, whereas the study by Abe et al (64) included persons with severe hypertriglyceridemia, ≈30% of them with diabetes. In the study by Abe et al (64), the long-term reduction in E-selectin was more pronounced in subjects with diabetes than in those without diabetes, and the reduction in VCAM-1 concentration was observed only in subjects with diabetes, which suggests that fish oil is particularly important for altering endothelial cell activation among hypertriglyceridemic and diabetic patients.

Nonetheless, both Seljeflot et al (65) and Johansen et al (66) found that n−3 fatty acid supplementation decreased concentrations of vWF, TM, and tissue-type plasminogen activator antigen. All these factors are hemostatic markers associated with impaired endothelial function, and elevated circulating concentrations of these factors were found in patients with CHD (68). The apparent paradoxical effects of n−3 fatty acids on hemostatic and inflammatory markers observed in these studies warrant further investigation.

Two studies examined the effects of n−3 fatty acid supplementation on endothelium-dependent vasomotor function. In one study, Fleischhauer et al (63) assessed the effects of dietary

TABLE 3
Clinical trials of vitamin E supplements and cardiovascular outcomes[1]

Reference	Year	Study design	Subjects	Type and daily dosage	Duration	Outcome
CHAOS, Stephens et al (75)	1996	p, r, p-c	CHD patients (n = 2002)	800 or 400 IU α-Tocopherol or placebo	≈1.5 y	RR: 0.53 (95% CI: 0.34, 0.83) for cardiovascular death and nonfatal MI
ATBC, Virtamo et al (76)	1998	p, r, p-c	Healthy subjects (n = 27271)	50 mg Vitamin E,[2] 20 mg β-carotene, or placebo	≈6.1 y	Fatal CHD: RR: 0.92 (95% CI: 0.81, 1.05) Combined primary outcomes (nonfatal MI and fatal CHD): RR: 0.96 (95% CI: 0.88, 1.04)
GISSI-Prevenzione trial, GISSI-Prevenzione Investigators (50)	1999	p, r	Patients with previous MI (n = 3658)	300 mg Vitamin E	≈3.5 y	Combined cardiovascular outcomes: RR: 0.88 (95% CI: 0.75, 1.04) Cardiovascular death: RR: 0.80 (95% CI: 0.65, 0.99)
HOPE Trial, HOPE Investigators (77)	2000	p, r, p-c	Patients with CVD or diabetes (n = 9541)	400 IU Vitamin E or placebo	≈4.5 y	No effect on cardiovascular outcomes (MI, stroke, and death from cardiovascular disease): RR: 1.05 (95% CI: 0.95, 1.16)

[1] p, prospective; r, randomized; p-c, placebo-controlled; CHAOS, Cambridge Heart Antioxidant Study; ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; HOPE, Heart Outcomes Prevention Evaluation study; RR, relative risk; CHD, coronary heart disease; MI, myocardial infarction.
[2] 1 IU = 0.67 mg.

supplementation of 5 g EPA plus DHA on endothelium-dependent vasodilator responses of coronary arteries to intracoronary acetylcholine infusion in heart transplant recipients. After 3 wk of treatment, patients treated with fish oil improved their vasodilator response to normal levels, whereas control patients showed vasoconstrictor response. In another study, Goodfellow et al (56) randomly assigned 30 hypercholesterolemic subjects to a treatment group with n−3 fatty acids at a dosage of 4 g/d or to a placebo group. At baseline, the hypercholesterolemic patients exhibited impaired endothelium-dependent vasodilation compared with the healthy subjects. After 4 mo of treatment, the patients supplemented with n−3 fatty acids had significantly improved endothelium-dependent vasodilation compared with the control subjects.

The mechanisms by which n−3 fatty acids influence the function of endothelium are still under investigation. It is known that the n−3 fatty acids must be incorporated into the cellular phospholipids to exert their effect (57). This incorporation results in a concomitant reduction of n−6 fatty acids in the phospholipids (55), suggesting that a specific ratio of n−3 to n−6 fatty acids is important in reducing endothelial activation. It also appears that the reduction in cell surface expression of adhesion molecules by n−3 fatty acids may be due to modulation at the transcriptional level, given the finding of a reduced level of cell adhesion molecule messenger RNA after incubation with n−3 fatty acids (60).

Antioxidant vitamins

Intake of antioxidant vitamins and risk of cardiovascular disease

There is a substantial body of epidemiologic evidence linking intake of antioxidant vitamins, particularly vitamin E, with reduced risk of coronary disease. In the Nurses' Health Study (69), the RR of CHD was 0.66 (95% CI: 0.50, 0.87) for women in the highest quintile of vitamin E intake compared with those in the lowest quintile. A similar reduction in risk was observed in the Health Professionals' Follow-up Study (70). Knekt et al (71) reported an inverse association between dietary vitamin E intake and coronary mortality in both healthy men (RR for extreme tertiles: 0.68; 95%: CI, 0.42, 1.11) and women (0.34; 0.14, 0.88).

The Iowa Women's Study found that dietary vitamin E intake, as opposed to supplemental vitamin E, was inversely associated with the risk of death from CHD (72). An additional study by Losonczy et al (73), in an elderly population, showed a reduced risk of all-cause mortality and coronary disease mortality with increased vitamin E intake (including both supplements and diet). These authors also found that supplementation with both vitamin E and vitamin C further reduced the risk of total and coronary mortality, suggesting a synergistic effect of vitamins E and C. Prospective cohort studies on the relation between vitamin C and CVD are limited and inconsistent. Neither the Nurses' Health Study nor the Health Professionals' Follow-Up study found a significant association between vitamin C consumption and risk of CHD. However, the first National Health and Nutrition Examination Survey epidemiologic follow-up study found that higher vitamin C intake was associated with a reduced risk of total mortality in men and of cardiovascular death in men and women (74).

To date, 4 clinical trials have been published regarding the effects of vitamin E supplementation on the risk of CVD: The Cambridge Heart Antioxidant Study (CHAOS; 75), the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC; 76), the GISSI-Prevenzione trial (50), and the Heart Outcomes Prevention Evaluation (HOPE) study (77; **Table 3**). CHAOS enrolled 2002 patients with documented CVD and evaluated daily supplementation with 400 or 800 IU α-tocopherol. The treatment resulted in an RR of 0.53 (95% CI: 0.34, 0.83) for cardiovascular death and nonfatal MI. The decreased risk was due predominantly to the reduction in nonfatal MI in the treatment group. The ATBC trial assessed supplementation with 50 mg (75 IU) vitamin E, 20 mg β-carotene, or both daily on cardiovascular outcomes in 27271 male smokers. The trial reported a nonsignificant, small reduction in the incidence of fatal coronary disease (RR: −8%; 95% CI: –19%, 5%). The GISSI-Prevenzione trial studied 3658 patients with a previous MI and assessed the effect of daily supplementation with 300 mg vitamin E/d on cardiovascular outcomes. Although there was no significant effect of vitamin E on the combined endpoints of cardiovascular death, nonfatal MI, and nonfatal stroke (RR: 0.88; 95% CI: 0.75, 1.04),

TABLE 4
Clinical studies of antioxidant vitamins and endothelial function[1]

Reference	Year	Study design	Subjects	Antioxidant and daily dosage	Duration	Methods	Outcome
Endothelial adhesive properties							
Devaraj et al (84)	1996	p	Healthy subjects (n = 21)	1200 IU α-Tocopherol	8 wk	Monocyte adhesion assay	Decreased monocyte adhesion
Weber et al (85)	1996	p	Smokers (n = 10), nonsmokers (n = 6)	2000 mg Vitamin C[2]	10 d	Monocyte adhesion assay	Decreased monocyte adhesion
Seljeflot et al (65)	1998	p, r, p-c	Smokers with hyperlipidemia (n = 41)	150 mg Vitamin C, 75 mg vitamin E, and 15 mg β-carotene	6 wk	Plasma TM, vWF, P-selectin, E-selectin, VCAM-1	No improvement
Davi et al (86)	1998	p	Subjects with hypercholesterolemia (n = 20)	600 mg 2-epi-1-α-Tocopherol	2 wk	Plasma P-selectin	Reduction in P-selectin
Endothelial-dependent vasodilation							
Hornig et al (87)	1998	p, p-c	Subjects with chronic heart failure (n = 10)	2 g Vitamin C or placebo	4 wk	Flow-mediated dilation of radial artery	Improvement
Motoyama et al (88)	1998	p, r, p-c	Subjects with coronary spastic angina (n = 60)	300 mg α-Tocopherol plus 200 mg diltiazem or diltiazem plus placebo	4 wk	Flow-mediated dilation of brachial artery	Improvement
Kugiyama et al (89)	1999	p, r, p-c	Patients with high remnant lipoprotein (n = 70)	300 IU α-Tocopherol	4 wk	Flow-mediated dilation of brachial artery	Improvement
Gocke et al (90)	1999	p, r, p-c	CAD patients (n = 46)	500 mg Vitamin C	4 wk	Flow-mediated dilation of brachial artery	Improvement
Chambers et al (91)	1999	p, r, p-c	Healthy subjects (n = 17)	1000 mg Vitamin C	1 wk	Flow-mediated dilation of brachial artery	Improvement
Simons et al (92)	1999	p, r, p-c	Healthy subjects (n = 20)	1000 IU Vitamin E	10 wk	Flow-mediated dilation of brachial artery	No improvement
Raitakari et al (93)	2000	p, r, p-c	Smokers (n = 20)	1000 mg Vitamin C	8 wk	Flow-mediated dilation of brachial artery	No improvement
Neunteufl et al (94)	2000	p, r, p-c	Smokers (n = 22)	600 IU Vitamin E	4 wk	Flow-mediated dilation of brachial artery	Improvement
Skyrme-Jones et al (95)	2000	p, r, p-c	Patients with type 1 diabetes (n = 41)	1000 IU Vitamin E	3 mo	Flow-mediated dilation of brachial artery	Improvement

[1] Adapted from Aminbakhsh and Mancini (37). p, prospective; randomized; p-c, placebo-controlled; TM, thrombomodulin; ICAM-1, intercellular adhesion molecule 1; VCAM-1, vascular cell adhesion module 1; CAD coronary artery disease; vWF von Willebrand factor.

[2] 1 IU = 0.67 mg.

there appeared to be a significantly decreased risk of cardiovascular death, including cardiac, coronary, and sudden death (RR: 0.80; 95% CI: 0.65, 0.99). Finally, the HOPE study, which enrolled 2545 women and 6996 men with a high risk of CVD (existing CVD or diabetes), assessed supplementation with 400 IU vitamin E/d on cardiovascular outcomes over a period of 4.5 y. The results of the study indicate that treatment of high-risk cardiovascular patients with vitamin E daily had no effect on cardiovascular outcomes; the RR was 1.05 (95% CI: 0.95, 1.16) for primary outcomes (MI, stroke, and death from cardiovascular causes). The overall results of the clinical trials are somewhat disappointing given the consistent and promising findings from epidemiologic studies. However, more conclusive evidence awaits the results of several ongoing clinical trials.

Effects of antioxidant vitamin supplementation on endothelial function

The major theory regarding the mechanism of antioxidants in CVD is that antioxidants reduce the susceptibility of LDL to oxidation and scavenge free radicals within the body, reducing the overall oxidative status of a person (78). Numerous in vitro and in vivo studies have provided support for oxidation as a possible mechanism of action. Emerging evidence also suggests an important role of antioxidants in modulating endothelial function, which is probably in part mediated by their antioxidant activity. In animal models, α-tocopherol was found to preserve nitric-oxide-mediated vascular relaxation (79, 80). In vitro studies showed a reduction in cell adhesion molecule expression (81), monocyte adhesion to the endothelium (82), and improvement in endothelium-dependent vasodilation (83) after incubation with antioxidants.

In addition to the in vitro evidence, there is growing clinical evidence to support a favorable effect of antioxidants on endothelial function (37). Studies of the effect of antioxidant supplementation on endothelial function (65, 84–95) are delineated in **Table 4**. These studies, ranging in duration from 1 wk to 3 mo, used either vitamin C or vitamin E supplementation and assessed soluble markers of endothelial activation or endothelium-dependent vasodilation in conduit arteries.

Four studies focused on endothelial activation as a primary outcome, and 3 of them showed that high doses of vitamins E and C decreased soluble markers of endothelial activation, such as P-selectin, and reduced monocyte adhesion (65, 84–86). In a study conducted by Weber et al (85), dietary supplementation with vitamin C (2 g/d) for 10 d raised the plasma vitamin C concentrations of smokers and decreased monocyte adhesion to values found in nonsmokers. In contrast, Seljeflot et al (65) found no reduction in concentrations of soluble cell adhesion molecules after treatment with antioxidants for 6 wk in smokers with hyperlipidemia, but this study involved a much lower concentration of antioxidants (150 mg vitamin C/d, 75 mg vitamin E/d, 15 mg β-carotene/d) than did the other studies.

Single-dose parenteral administration of antioxidant vitamins improved endothelial dysfunction in diverse patient populations, including chronic smokers (96), patients with diabetes (97), patients with hypertension (97), and patients with chronic heart failure (87). Several studies assessed the relatively long-term effect of antioxidant oral supplementation on endothelium-dependent vasodilation (87–95) (Table 4). Hornig et al (87) found that flow-mediated vasodilation was significantly improved after 4 wk of oral supplementation with vitamin C (2 g/d) in patients with heart failure, which was partly mediated by increased availability of nitric oxide. However, flow-mediated vasodilation was not affected by vitamin C in healthy control subjects. The benefit of vitamin C supplementation on endothelium-mediated vasodilation was also shown in patients with existing CHD (90). In a study by Chambers et al (91), pretreatment with vitamin C (1 g/d orally for 1 wk) was effective in ameliorating a reduction in flow-mediated vasodilation induced by acute hyperhomocysteinemia in healthy subjects. In contrast with these studies that showed relatively long-term effects of vitamin C, Raitakari et al (93) found that, although a single dose of vitamin C improved vascular function in the short term in adult smokers with baseline endothelial dysfunction, vitamin C supplementation (1 g/d) for 8 wk had no significant benefit, despite sustained elevated concentrations of plasma vitamin C.

Vitamin E supplementation was also shown to improve endothelium-dependent vasodilation in several studies. Motoyama et al (88) found that vitamin E treatment (300 mg α-tocopherol/d) for 4 wk improved endothelium-dependent vasodilation and decreased plasma TBARS concentrations in 60 patients with documented coronary spastic angina. Kugiyama et al (89) found that treatment with α-tocopherol (300 IU/d) for 4 wk significantly improved the impaired endothelium-dependent vasodilation in patients with high remnant concentrations, and the improvement was associated with decreased plasma concentrations of TBARS. This finding suggests that the beneficial effects of vitamin E on endothelial dysfunction were partly mediated by the reduction in oxidative stress with vitamin E therapy. Neunteufl et al (94) found that vitamin E supplementation (600 IU/d) for 4 wk did not restore endothelial dysfunction caused by smoking in 22 healthy male smokers. However, pretreatment with vitamin E was effective in preventing a further decline in endothelium-dependent vasodilation in the brachial artery induced by acute smoking.

Only 2 studies lasted ≥10 wk. In one study, Simons et al (92) found that supplementation with vitamin E (1000 IU/d) for 10 wk did not affect flow-mediated endothelium-dependent dilation in response to reactive hyperemia in 20 asymptomatic subjects aged 45–70 y. On the contrary, in another study with a slightly longer duration (3 mo), Skyrme-Jones et al (95) showed that 1000 IU vitamin E/d significantly improved flow-mediated vasodilation in the brachial artery and flow responses to intrabrachial acetylcholine in the forearm resistance vessels in subjects with type 1 diabetes.

Taken together, the results of most of the studies support a role for vitamins C and E in preserving endothelium-dependent vasodilation when challenged with cardiovascular risk factors such as hyperlipidemia or in patients with diabetes or established CVD. In several of the studies, the improvement in endothelial function was directly related to a reduction in oxidative stress, supporting the theory that the benefit of these vitamins on endothelial function is at least partly mediated by their antioxidant property.

Folic acid

Intake of folic acid and risk of cardiovascular disease

Folic acid, or folate, is a micronutrient found in many green leafy vegetables, such as spinach, and in some animal products, such as egg yolk. Recently, the recommended daily allowance of folic acid was raised from 200 to 400 μg. Folic acid fortification of enriched grain products has increased the percentage of the US population with adequate folate intakes (98), but a substantial portion of the US population still has a suboptimal intake of folic acid, especially women of childbearing age (99). Folic acid is critical for preventing neural tube defects in newborns, which instigated the policy of fortification of grain products with folic acid (100). Increasing evidence suggests that folate is beneficial in preventing CVD as well because folic acid and other B vitamins are the primary determinants of plasma concentrations of homocysteine, a recognized independent risk factor for CVD (101).

Several epidemiologic studies examined the association between folate intake and risk of CVD. A case-control study of 130 MI patients and 118 control subjects by Verhoef et al (102), found that folate intake was significantly lower in cases than in controls. The odds ratio (OR) comparing extreme quintiles (>682 compared with ≤310 μg/d) of total folate intake (supplement plus food) was 0.38 (95% CI: 0.15, 0.95). The OR comparing extreme quintiles (>467 compared with ≤282 μg/d) of folate from food only was 0.30 (95% CI: 0.11, 0.81). The Nurses' Health Study (103), which included 80 082 women who were followed up for 14 y, found an RR of CHD of 0.69 (95% CI: 0.55, 0.87) for women in the highest quintile of folate intake (median: 696 μg/d) compared with those in the lowest quintile (median: 158 μg/d). Recently, Chee and Stamler (104) reported a significant inverse association between folate intake and mortality from all causes and CVD in 6426 men from the Multiple Risk Factor Intervention Trial (MRFIT) usual care group.

Although numerous experimental studies showed the homocysteine-lowering effects of folic acid supplementation (101, 105), few clinical trial data are currently available regarding the effect of folate on CVD outcomes. In a randomized, placebo-controlled trial, Vermeulen et al (106) investigated the effect of folate on subclinical CVD in 158 healthy siblings of patients with existing CVD who received either 5 mg folic acid and 250 mg vitamin B-6 or placebo for 2 y. These authors found that folic acid and vitamin B-6 treatment resulted in a decrease in fasting homocysteine concentrations and a decreased rate of subclinical heart disease as assessed by abnormal exercise electrocardiography tests (OR: 0.40; 95% CI: 0.17, 0.93). In 2 noncontrolled studies (107, 108), supplementation with folic acid and vitamin B-6 reduced the risk

TABLE 5
Clinical studies of folic acid supplementation and endothelial function[1]

Reference	Year	Study design	Subjects	Daily dosage	Duration	Methods	Outcome
Van den Berg et al (120)	1995	p	Mildly hyperhomocysteinemic subjects (n = 18)	Folic acid (5 mg) + pyridoxine (250 mg)	12 wk	Measurement of plasma vWF, TM	Decreased vWF, TM
Woo et al (121)	1999	p, r, p-c	Healthy subjects (n = 17)	10 mg Folic acid or placebo	8 wk	Measurement of flow-mediated vasodilation of brachial artery	Improved endothelium-dependent vasodilation
Verhaar et al (122)	1999	p, r, p-c	Familial hypercholesterolemic (n = 40)	5 mg Folic acid or placebo	4 wk	Serotonin-induced forearm blood flow measurement	Restored endothelium-dependent vasodilation
Bellamy et al (123)	1999	p, r, p-c	Hyperhomocysteinemic patients (n = 18)	5 mg Folic acid or placebo	6 wk	Measurement of flow-mediated vasodilation of brachial artery	Improved endothelium-dependent vasodilation
Constans et al (124)	1999	p	Hyperhomocysteinemic patients and control subjects (n = 44)	5 mg Folic acid and 250 mg vitamin B6	3 mo	Plasma TM, vWF measurement	Decreased TM
Wilmink et al (110)	2000	p, r, p-c	Healthy subjects postprandially (n = 20)	10 mg Folic acid or placebo	2 wk	Measurement of flow-mediated vasodilation of brachial artery	Prevention of vasodilation impairment after fat-load
Thambyrajah et al (125)	2000	p, r, p-c	Patients with predialysis renal failure (n = 100)	5 mg Folic acid or placebo	12 wk	Measurement of flow-mediated vasodilation of brachial artery, vWF	No change

[1] TM, thrombomodulin; vWF, von Willebrand factor; p, prospective; r, randomized; p-c, placebo-controlled.

of CVD events (coronary, peripheral, or cerebral) in hyperhomocysteinemic patients with existing CVD to the level of normohomocysteinemic patients with existing CVD.

Several large, ongoing clinical trials using CVD endpoints (MI and stroke), such as the Norwegian study of homocysteine lowering with B vitamins in MI (NORVIT) and the Women's Antioxidants and Cardiovascular Disease Study (WACS) will help to further clarify the beneficial role of folic acid in reducing clinical cardiovascular events.

Effects of folic acid on endothelial function

The primary mechanism proposed for the effect of folic acid on CVD is a reduction in plasma homocysteine concentrations by remethylation of homocysteine back to methionine (109). There is increasing evidence that folic acid can have a beneficial effect on the vascular endothelium by reducing plasma homocysteine concentrations or through other mechanisms (eg, reduction of oxidative stress) (110).

Homocysteine harms the vascular endothelium in a variety of ways, impairing the ability of the endothelium to maintain homeostasis. Homocysteine increases platelet aggregation and thrombosis through enhanced thromboxane synthesis and inactivation of anticoagulant substances (109). Homocysteine increases oxidative stress by increasing superoxide production (111). It also increases leukocyte-endothelium interactions and was found to be toxic at high concentrations (112). In addition, homocysteine down-regulates nitric-oxide production (113) and acts as a mitogen to increase vascular smooth muscle proliferation (114). Impaired endothelium-dependent, flow-mediated vasodilation was documented in hyperhomocysteinemic subjects (115, 116) and in healthy subjects with oral methionine load–induced hyperhomocysteinemia (91, 117, 118).

Acute administration of folic acid can restore impaired endothelial function induced by acute hyperhomocysteinemia (119). Several relatively long-term studies (2–12 wk) evaluated the effects of folic acid supplementation on endothelial function in (110, 120–125) (**Table 5**). In a study of 18 mildly hyperhomocysteinemic patients with peripheral arterial occlusive disease, Van den Berg et al (120) found that daily treatment with pyridoxine (250 mg) plus folic acid (5 mg) significantly decreased concentrations of vWF and TM after 12 wk of treatment. Woo et al (121) found that supplementation with 10 mg folic acid/d for 8 wk significantly improved flow-mediated endothelium-dependent vasodilation in 17 healthy subjects with relative hyperhomocystinemia (mean homocysteine concentration: 9.8 mmol/L). Verhaar et al (122) assessed supplementation with 5 mg folic acid for 4 wk on serotonin-induced blood flow in patients with familial hypercholesterolemia. Endothelium-dependent vasodilation in these patients was restored to normal control levels after folic acid supplementation. Bellamy et al (123) assessed endothelium-dependent vasodilation in hyperhomocysteinemic patients after supplementation with 5 mg folic acid/d for 6 wk. There was a significant improvement in flow-mediated vasodilation after folic acid supplementation compared with placebo.

Constans et al (124) evaluated soluble markers of vascular function after folic acid (5 mg/d) and vitamin B-6 (250 mg/d) supplementation for 3 mo in hyperhomocysteinemic patients and found a significant reduction in TM after supplementation. Wilmink et al (110) evaluated the effect of pretreatment with 10 mg folic acid/d or placebo for 2 wk on postprandial endothelial dysfunction after an oral fat load. The oral fat load resulted in an increase in triacylglycerol concentrations in both the treated group and the placebo group and impaired flow-mediated vasodilation in the control group. However, flow-mediated

TABLE 6
Clinical studies of L-arginine supplementation and endothelial function[1]

Reference	Year	Study design	Subjects	Daily dosage	Duration	Methods	Outcome
Adams et al (129)	1997	p, r, p-c	CHD patients ($n = 10$)	21 g L-Arginine or placebo	3 d	Flow-mediated dilation brachial artery	Improvement
						Monocyte adhesion assay	Reduced monocyte–endothelium adhesion
Clarkson et al (130)	1996	p, r, p-c	Hypercholesterolemic patients ($n = 27$)	21 g L-Arginine or placebo	4 wk	Flow-mediated dilation of brachial artery	Improvement
Theilmeier et al (131)	1997	p, r, p-c	Hypercholesterolemic patients and control subjects ($n = 32$)	8.4 g Arginine HCl	2 wk	Monocyte adhesion assay	Reduced monocyte–endothelium adhesion
Bellamy et al (132)	1998	p, r, p-c	Angina patients and and control subjects ($n = 17$)	14 g L-Arginine or placebo	4 wk	Flow-mediated dilation of brachial artery	Improvement
Lerman et al (133)	1998	p, r, p-c	Recurrent chest pain patients ($n = 26$)	9 g L-Arginine or placebo	6 mo	Agonist (ACH)-mediated coronary blood flow	Improvement
Hambrecht et al (134)	2000	p, r	Chronic heart failure patients ($n = 20$)	8 g L-Arginine	4 wk	Agonist (ACH)-mediated dilation of radial artery	Improvement
Mullen et al (135)	2000	p, r, p-c	Patients with type 1 diabetes ($n = 84$)	14 g L-Arginine	6 wk	Flow-mediated dilation of brachial artery	No improvement
Blum et al (135)	1999	p	Patients with intractable angina pectoris ($n = 10$)	9 g L-Arginine	3 mo	Soluble ICAM-1, VCAM-1, E-, P-, and L-selectin	Reduction in plasma ICAM-1, P-, and E-selectin concentrations
Blum et al (137)	2000	p, r, p-c	CHD patients ($n = 29$)	9 g L-Arginine or or placebo	1 mo	Flow-mediated dilation of brachial artery	No improvement
						Soluble ICAM-1, VCAM-1, E-selectin	No change
Blum et al (138)	2000	p, r, p-c	Postmenopausal women ($n = 10$)	9 g L-Arginine or placebo	1 mo	Flow-mediated dilation of brachial artery	No improvement
						Soluble ICAM-1, VCAM-1, E-selectin	No change

[1] p, prospective; r, randomized; p-c, placebo-controlled; CHD, coronary heart disease; ICAM-1, intercellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1; ACH, acetylcholine.

vasodilation was not affected by the high postprandial triacylglycerol concentrations in the group that received folic acid supplementation. Urinary malondialdehyde, a measure of oxidative stress, was significantly elevated in the placebo group but not in the folic acid–treated group. Recently, Thambyrajah et al (125) evaluated the effects of supplementation with 5 mg folic acid/d or placebo for 12 wk on endothelial dysfunction in 100 patients with predialysis renal failure. Endothelial dysfunction was assessed by measuring *1*) endothelium-dependent dilation of the brachial artery, *2*) combined serum nitrite and nitrate concentrations, and *3*) plasma vWF concentrations. The treatment lowered but did not normalize hyperhomocysteinemia in these patients. There was no significant change in any of the 3 measures of endothelial dysfunction. The authors concluded that treatment with high doses of folic acid might not be able to reverse endothelial dysfunction in patients with renal failure.

The overall results of these studies suggest that folic acid supplementation has a beneficial effect on endothelial function, as measured by flow-mediated vasodilation or hemostatic markers, in healthy subjects or patients with elevated homocysteine. The observed benefit is probably explained largely by the homocysteine-lowering effect of folic acid. However, other mechanisms may contribute to the benefit. A previous study showed that acute administration of folic acid improved endothelial function without any effects on homocysteine concentrations (126). Folic acid was shown to have antioxidant properties and direct scavenging effects in vitro (126) and may directly improve nitric-oxide production by enhancing enzymatic acitivity of nitric-oxide synthase (127).

L-Arginine

L-Arginine is a semiessential amino acid that is important during periods of growth and is required for the urea cycle in protein catabolism (128). L-Arginine is also the substrate for nitric-oxide synthase in the production of nitric oxide and is therefore essential to the maintenance of proper vascular function. In hypercholesterolemic animal models, dietary supplementation with L-arginine improves endothelium-dependent dilation, decreases platelet aggregation and monocyte adhesion, and reduces the development of atherosclerosis (129).

Several clinical experimental studies examined the effect of L-arginine supplementation on endothelial function in humans (129–138) (**Table 6**). Clarkson et al (130) showed that supplementation with 21g L-arginine/d for 4 wk resulted in a 3.9% improvement in flow-mediated dilation in hypercholesterolemic patients. In CAD patients supplemented with 21 g L-arginine/d, Adams et al (129) found a 4.7% improvement in flow-mediated endothelium-dependent dilation and a significant reduction in monocyte adherence to the endothelium. Theilmeier et al (131) supplemented hypercholesterolemic patients and normocholesterolemic subjects with 8.4 g L-arginine/d and assessed ex vivo monocyte adhesion to endothelial cells. At baseline, monocytes from the hypercholesterolemic patients adhered by 50% more

than did those from the normocholesterolemic subjects. After L-arginine supplementation, the monocytes from the hypercholesterolemic patients had reduced adhesion comparable with control levels, suggesting that the increased adhesiveness of mononuclear cells induced by hypercholesterolemia can be reversed in vivo by L-arginine. In another study (132), patients with angina, normal results of a coronary arteriogram, and a positive exercise test were supplemented with 14 g L-arginine/d for 4 wk. These patients' flow-mediated dilation was improved by 3.4%, to physiologically normal levels. Lerman et al (133) assessed coronary blood flow in patients with recurrent chest pain after supplementation with L-arginine or placebo (9 g/d) for 6 mo. The treatment group had a 149% greater acetylcholine-mediated coronary blood flow compared with the placebo group. Hambrecht et al (134) supplemented patients with chronic heart failure with 8 g L-arginine/d for 4 wk and found an 8.8% improvement in acetylcholine-mediated vasodilation of the radial artery. However, in a study by Mullen et al (135) in 84 normocholesterolemic patients with type 1 diabetes, 7 g L-arginine twice daily had no significant effect on endothelial function as measured by flow-mediated dilation of the brachial artery. In contrast, treatment with atorvastatin significantly lowered LDL cholesterol and improved endothelial dysfunction.

Recently, Blum et al (136–138) conducted 3 studies in different patient populations to examine the effect of L-arginine supplementation on soluble adhesion molecule concentrations and flow-mediated dilation. In an earlier study of 10 patients with intractable angina pectoris (136), supplementation with 9 g L-arginine/d for 3 mo resulted in a significant reduction in plasma concentrations of ICAM-1 and P- and E-selectin. However, Blum et al's 2 most recent studies showed no effect of L-arginine on either flow-mediated vasodilation or soluble adhesion molecules (137, 138). In one study (137), 30 patients with coronary artery disease were randomly assigned to receive 9 g L-arginine or placebo daily for 1 mo. Although the plasma arginine concentration increased significantly in the treatment group, serum nitrogen oxides, flow-mediated brachial artery dilation, and concentrations of cell adhesion molecules did not change significantly. In the other study (138), 10 healthy postmenopausal women received 9 g L-arginine or placebo daily for 1 mo. The treatment had no effects on serum nitrogen oxide concentrations. The flow-mediated vasodilation and serum concentrations of soluble cell adhesion molecules were also similar between the treatment and placebo groups. The authors speculated that the relatively low dosage (9 g/d) used in their studies might have accounted for the lack of effects of L-arginine on endothelial function.

Taken together, the results of these studies suggest a potential beneficial effect of L-arginine supplementation on endothelial function in patients with hypercholesterolemia or existing coronary artery disease. However, the benefit has not been shown in subjects with diabetes or healthy subjects. In a recent epidemiologic study, dietary arginine intake (which is far below the supplement dosages) did not appear to predict CHD mortality (139). However, in the Nurses' Health Study (140), a moderately high consumption of dietary protein (median: 24% of energy), compared with a low consumption (median: 15% of energy), was associated with a modest but significantly lower risk of CHD during 14 y of follow-up. In several large epidemiologic studies, a high consumption of nuts was associated with a significantly lower risk of CHD (141). The relatively high arginine content of nuts has been suggested as one of the potential bio-

logical mechanisms for their cardioprotective effect. So far, no clinical trials have assessed the effect of supplementary arginine on cardiovascular events. Clearly, this would be an interesting and promising line of investigation.

SUMMARY

Endothelial dysfunction, clinically assessed by measuring plasma concentrations of soluble endothelial adhesion molecules or by endothelium-dependent vasodilation, is present in patients with all types of CVD, including CHD, peripheral arterial disease, chronic heart failure, and stroke. Furthermore, endothelial dysfunction is present in patients who do not have clinically manifested CVD but who have coronary risk factors such as smoking, hypertension, hypercholesterolemia, hyperhomocysteinemia, and diabetes mellitus. Because abnormal endothelial function is an early marker of CVD, the endothelium appears to be an ideal target for preventive therapy. Several pharmacologic and nonpharmacologic strategies were effective in improving vascular function. The literature summarized here suggests that dietary intervention is a promising strategy for improving endothelial function in patients at risk of CVD and those with existing CVD. Note that most of the studies we reviewed in this article were published in the past 2–3 y, reflecting emerging and growing interest in the area of diet and endothelial function.

n−3 Fatty acids have been studied extensively in epidemiologic and experimental studies. Overall, the prospective cohort studies showed that n−3 fatty acids are more protective in fatal CHD than in nonfatal MI. The DART and the GISSI-Prevenzione trial showed that supplementation with n−3 fatty acids or increased fish consumption can reduce mortality in MI patients. The protective effect of fish oil is probably due in part to improved endothelial function. The results of in vitro studies, very consistently, support the idea that n−3 fatty acids decrease expression of adhesion molecules on the endothelium and also decrease leukocyte-endothelium interactions. In vitro studies also showed that n−3 fatty acids, particularly DHA, increase nitric-oxide production and improve endothelium-dependent relaxation. The clinical experimental studies were less consistent with regard to the effects of n−3 fatty acids on soluble cell adhesion molecules, but they consistently showed that consumption of fish oil improves hemostatic factors associated with endothelial function (65, 66). The favorable effects of n−3 fatty acids on markers of endothelial activation observed in subjects with diabetes by Abe et al (64) are promising and need to be confirmed by future studies.

Antioxidant vitamins, both water and lipid soluble, have the potential to reduce oxidative stress within the body and have therefore become the subject of intensive study with respect to CVD and endothelial function. Several prospective cohort studies showed a consistent inverse association between vitamin E intake and the risk of CHD. However, randomized clinical trials on vitamin E supplementation and CVD are less consistent. Nonetheless, it is postulated that the action of antioxidants on the endothelium has an important role in mediating their potential cardioprotective effects. In vitro studies showed clearly that antioxidant vitamins have beneficial effects on endothelial function by reducing cell adhesion molecule expression and monocyte adhesion and improving endothelium-dependent vasodilation. In addition, clinical experimental studies consistently showed that oral supplementation with vitamins C or E (lasting from 1 wk to 3 mo) improves endothelial function in high-risk

patients. Although the experimental studies and epidemiologic evidence are generally positive, application to the public should await the results of the ongoing clinical trials, and the proper dosages and possible interactions between antioxidants should be investigated further in clinical studies.

There is growing evidence supporting potential benefits of folic acid in reducing the risk of CVD. The reduction in cardiovascular risk is attributable primarily to the ability of folate to reduce concentrations of plasma homocysteine, which adversely affect the endothelium by increasing adhesion molecule expression and platelet aggregation and decreasing nitric-oxide production. Clinical studies consistently showed improved flow-mediated dilation and a reduction in inflammatory markers after folic acid supplementation in patients with elevated homocysteine.

L-Arginine is the substrate for nitric-oxide synthase in the production of nitric oxide and is essential to normal endothelium-dependent vasomotion. Several clinical experimental studies showed that L-arginine supplementation improves endothelial function in patients with CVD or hypercholesterolemia. To date, however, no clinical trials have examined the effects of L-arginine on cardiovascular outcomes.

In conclusion, substantial evidence suggests that n−3 fatty acids, antioxidant vitamins, folic acid, and L-arginine have beneficial effects on endothelial function. The mechanisms by which these nutrients influence endothelial function are likely to be multiple and complex, including inhibition of monocyte adhesion and platelet activation, increased nitric-oxide production and improvement of vasodilation, and blockage of lipid oxidation. These mechanisms may contribute to the role of these nutrients in reducing the incidence of CHD that has been observed in epidemiologic studies and some controlled clinical trials. The overall evidence to support the benefit of n−3 fatty acids in CVD appears more convincing than that for other nutrients, owing largely to the positive results from the DART and the GISSI-Prevenzione trial. At this point, it is reasonable to conclude that fish oil can have a therapeutic role among patients with MI and can be recommended to these patients. Although definitive data on the role of antioxidants and folic acid in reducing CVD are not yet available, a balanced diet that provides a sufficient supply of these nutrients is likely to have substantial benefits for CVD and other chronic illnesses.

Clearly, more clinical experimental studies are needed to elucidate the role of diet in endothelial functions, especially studies with a longer duration of treatment. Meanwhile, future epidemiologic studies and clinical trials should incorporate measures of endothelial dysfunction into the study design so that one can evaluate both the ability of such measures to predict cardiovascular outcomes and the relation between long-term dietary intake and measures of endothelial function. Moreover, these studies can shed light on whether the effects of dietary components on CVD endpoints are mediated through improvement in endothelial function.

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