Assessing Endothelial Function As a Risk Factor for Cardiovascular Disease

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Current Atherosclerosis Reports 2003, **5:**506–513
Current Science Inc. ISSN 1523–3804

The vascular endothelium plays a key role in the prevention of atherosclerosis. Endothelial dysfunction is an antecedent of clinical cardiovascular disease and can be viewed as the final pathway between coronary risk factors and the development of atherosclerosis. The development of a noninvasive method of assessing endothelial health (*ie*, measurement of flow-mediated dilation of the brachial artery) has enabled investigators to evaluate the effects of dietary patterns on vascular function. Emerging evidence indicates that dietary fats may acutely impair endothelial function, but this effect is dependent on the type of fat and on the other nutrients consumed. Although inconclusive, studies in cohorts of modest size suggest that antioxidants, L-arginine, and folic acid may modulate endothelial function. Additional research is needed to define the impact of complex, long-term dietary patterns on the vascular endothelium.

Introduction

The vascular endothelium, the monolayer of cells that lines blood vessels, plays a key role in the prevention of atherosclerosis. Traditional risk factors for coronary disease, including increased lipids, tobacco use, hypertension, diabetes, age, and male gender, contribute to endothelial dysfunction. Dietary factors also modulate endothelial function, and their effect may be the mechanism by which nutrients modify the risk of coronary events. Thus, measures of endothelial function may serve as a useful endpoint in studies evaluating the efficacy of dietary interventions in decreasing cardiovascular risk.

This paper reviews the physiology of the vascular endothelium, describes methods of measuring endothelial function, discusses the prognostic significance of endothelial dysfunction, and briefly summarizes recent literature evaluating the effects of nutrients on endothelial function.

Physiology of the Endothelium

Once considered an inert diffusional barrier between blood and vascular smooth muscle, the endothelium is now recognized as a vital endocrine and paracrine organ that plays a key role in the prevention of atherosclerosis. Among the important functions of the endothelium are the maintenance of vascular tone, regulation of vascular cell growth, regulation of leukocyte and platelet adhesion, regulation of thrombosis and fibrinolysis, and mediation of inflammation [1–4].

The normal endothelium senses changes in hemodynamic forces (*ie*, pressure and shear stress) and the hormonal environment (*ie*, vasoactive substances and mediators released from blood cells and platelets), and then synthesizes and releases biologically active substances that maintain vascular homeostasis. Vascular tone is regulated by the production and release of several relaxing and constricting factors. The most important endogenous vasodilator is nitric oxide (NO). NO is generated by conversion of the amino acid L-arginine to NO by the endothelial isoform of the enzyme NO synthase (eNOS). NO activates guanylyl cyclase in vascular smooth muscle, leading to increased concentrations of cGMP. cGMP, in turn, activates cGMP-dependent protein kinase, which decreases cytosolic calcium concentration, causing smooth muscle relaxation and decreased vascular tone. In addition to its importance as a vasodilator, NO mediates many of the vasoprotective effects of the normal endothelium. Most in vivo indices of endothelial function assess the vasodilating capacity of blood vessels, but reduced vascular reactivity probably also reflects an impaired defense against vascular injury, inflammation, and thrombosis.

Dysfunction of the vascular endothelium results from reduced bioavailability of NO due to decreased formation and enhanced degradation. Many vascular disease states are characterized by excess generation of reactive oxygen species, including superoxide anion and oxidized low-density lipoprotein (LDL) cholesterol, which inactivate NO.

Endothelial injury with resulting dysfunction plays a critical role in all stages of atherosclerosis. Traditional markers of cardiovascular risk, such as age, hyperlipidemia, hypertension, diabetes, and tobacco use, as well as novel risk factors such as inflammation and hyperhomocysteinemia,

are associated with damaged endothelium in asymptomatic subjects. Modification of these risk factors, in turn, induces improvement in endothelial function. Endothelial dysfunction may be viewed as the common pathway between clinical risk factors and the development of atherosclerosis. In addition to contributing to the pathogenesis of atherosclerosis, endothelial dysfunction plays a key role in the clinical manifestations of coronary disease by promoting coronary vasoconstriction and thrombosis.

Measurement of endothelial function

In vivo assessments of endothelial function have relied on measurements of vascular responses to vasodilators, such as acetylcholine, or physical stimuli, such as increased shear stress, which are mediated by the endothelium.

Measurement of Coronary Endothelial Function

Coronary endothelial function can be studied by cannulating a coronary artery and measuring the effects of an infusion of an endothelium-dependent vasodilator, such as acetylcholine, on either large epicardial vessels or on the arterial microvasculature. The dilation of epicardial coronary arteries is quantified by measuring the change in luminal diameter by quantitative angiography. The response of resistance arterioles, which govern the coronary flow rate, can be measured with a Doppler flow wire inserted into the vessel. Endothelium-independent vasodilation is measured after infusion of nitroglycerin or sodium nitroprusside.

In a landmark study published nearly two decades ago, Ludmer *et al*. [5] demonstrated vasodilation of normal coronary arteries but paradoxical vasoconstriction of diseased vessels during infusion of acetylcholine. Subsequent studies of coronary endothelial function have confirmed the dynamic nature of coronary stenoses, elucidated relationships between coronary atherosclerosis and risk factors, and demonstrated the efficacy of risk reduction through improved endothelial function. However, the research utility of this method is limited by the need for cardiac catheterization. Coronary cannulation is impractical in studies requiring sequential measurements, in studies of subjects without clinical evidence of coronary artery disease, or in observational studies of large cohorts.

Measurement of endothelial function by forearm plethysmography

Although the study of atherogenic risk factors and assessment of therapeutic interventions is often targeted toward understanding coronary artery disease, atherosclerosis is a systemic process and dysfunction of the endothelium in the peripheral vessels reflects poor vascular health in general. The properties of the forearm microvasculature can be assessed in a manner analogous to that described for the coronary circulation. With this technique, endothelium-dependent (*eg*, acetylcholine) or endothelium-independent (*eg*, nitroglycerin) vasodilators are infused by an intra-arterial catheter into the brachial artery. Changes in forearm blood flow are quantified by venous occlusion plethysmography.

The forearm plethysmography technique has provided important insights into the physiology of microvascular reactivity in healthy adults. Advantages of this method include the ability to examine dose-response relationships and study the effects of specific agonists and antagonists. Although less invasive than measurements of coronary endothelial function, this approach is nonetheless time consuming and requires cannulation of a brachial artery. In addition, the mechanisms by which acetylcholine induces dilation of the forearm microvasculature are not well established, and factors other than stimulation of NO release may be important. Moreover, a close relationship between forearm microvasculature reactivity and coronary reactivity has not been demonstrated.

Flow-mediated dilation of the brachial artery

The need for a noninvasive index of endothelial function that would permit repeated measurements in healthy individuals and that would be applicable to large observational studies led to the development of a technique using high-frequency ultrasound to measure flow-mediated dilation (FMD) of the brachial artery [6••]. This method is based on the finding that high shear stress due to increased flow stimulates the release of NO by endothelial cells. When flow through the brachial artery is increased due to reactive hyperemia, the diameter of the brachial artery increases by up to 20%. This flow-mediated dilation is due to NO production [7], and reflects the vasodilator response to acetylcholine in coronary arteries [8]. Changes in vascular diameter in response to sublingual nitroglycerin may also be measured, and they represent an index of endothelium-independent vasodilation.

Ultrasound images of the brachial artery are acquired with a system configured with software for two-dimensional imaging, color and spectral Doppler, electrocardiogram (ECG) monitoring, and a high-frequency (at least 7 MHz) vascular transducer. Reactive hyperemia of the arm is produced by arterial occlusion, utilizing a blood pressure cuff positioned either on the upper arm or the forearm. After a baseline resting image of the brachial artery is acquired, the cuff is inflated to a suprasystolic pressure, typically for 5 minutes. This results in ischemia of the lower arm musculature and dilation of the resistance vessels so that flow through the brachial artery is transiently enhanced following cuff deflation. The increase in arterial diameter caused by the resultant increase in shear stress is determined by a second measurement of arterial diameter, acquired approximately 60 seconds following cuff release. After 10 to 15 minutes of rest, arterial diameter can be measured before and about 4 minutes after the administration of 0.4 mg of sublingual nitroglycerin to determine the response to an endothelium-independent vasodilator.

Flow-mediated dilation is usually expressed as the percent change in diameter from baseline. Small arteries tend to dilate relatively more than large arteries, however, so any responses should be adjusted for baseline diameter in studies comparing individuals or groups with different vessel sizes. In our laboratory, the use of specialized software with edge detection capabilities (Brachial Tools, Medical Imaging Applications, Iowa City, IA) has proven useful in identifying the maximum vascular dilation and improving the reproducibility of measurements.

The noninvasive nature of ultrasound measurement of brachial artery FMD makes this technique well suited to the assessment of dietary interventions on endothelial health. Despite its conceptual simplicity, however, assessment of FMD is a technically difficult procedure that requires a considerable investment on the part of the laboratory and sonographer. The key to generating reliable, reproducible data is the acquisition of high-quality images, which depends on both equipment quality and the skill and experience of the operator. Even in experienced laboratories, the biologic and technical variability of the technique is significant, and 20 to 30 patients in a crossover study or 40 to 60 patients in a parallel group design study are required to demonstrate an improvement of 1.5% to 2.0% in FMD [6••]. Many studies evaluating the effects of nutrients on endothelial function have not been adequately powered to detect significant changes in FMD.

Prognostic Significance of Endothelial Dysfunction

The value of indices of endothelial function as surrogate endpoints in interventional studies is based on the hypothesis that endothelial dysfunction plays a causal role in cardiovascular events. As recently reviewed by Kuvin and Karas [9•], observational studies have demonstrated that endothelial dysfunction in either coronary or peripheral vascular beds predicts adverse outcomes, independent of traditional coronary risk factors.

In a study of 308 patients undergoing coronary angiography, Halcox *et al.* [10] measured changes in coronary vascular resistance and epicardial coronary diameter in response to infusion of acetylcholine and nitroglycerine or adenosine. Over a subsequent follow-up period of almost 4 years, acute vascular events (cardiovascular death, myocardial infarction, stroke, and unstable angina) occurred in 35 patients. Both the change in coronary resistance and the increase in conduit vessel diameter were significant predictors of events, even after accounting for traditional coronary risk factors. Responses to nitroglycerin had no significant relationship to outcomes.

Other studies have demonstrated that an impaired forearm blood flow response to acetylcholine predicts cardiovascular events in patients with hypertension and in subjects with coronary artery disease [11,12]. More recently, Gokce *et al.* [13•] prospectively examined the association of brachial artery FMD with cardiac events in patients undergoing peripheral vascular surgery. Of the 187 patients, 54 had a postoperative event. Preoperative FMD was significantly lower in those who subsequently suffered a cardiovascular complication than in those who did not, whereas the response to nitroglycerin was similar in both groups. Lower FMD remained a predictor of adverse events even after adjustment for other clinical characteristics.

Influence of Nutritional Factors on Endothelial Function

Improvement in endothelial function is a plausible mechanism by which dietary patterns influence the risk of cardiovascular events. Investigation of the effects of nutritional factors on the endothelium have focused on dietary fats and on specific nutrients, such as antioxidants, L-arginine, and folic acid [14••,15••].

Effects of dietary fat

The key role of dietary fat consumption in the current coronary heart disease epidemic has stimulated interest in the effect of fats on the vascular endothelium. Vogel *et al.* [16] were the first to demonstrate that endothelial function is acutely impaired following ingestion of a high-fat meal. Ten healthy, normocholesterolemic men and women had measures of FMD before and after a high-fat fast food breakfast (one Egg McMuffin, one Sausage McMuffin [McDonald's, Oak Brook, IL], two hash brown patties, and a noncaffeinated beverage totaling 900 calories and 50 grams of fat). FMD fell by approximately 50% at 4 hours after the meal, and the change in vascular reactivity was correlated with the increase in serum triglyceride levels. This postprandial impairment of endothelium-dependent vasodilation was not observed following a low-fat meal.

Subsequent studies have focused on the capacity of antioxidants to prevent short-term deterioration in endothelial function associated with fatty meals. Plotnick *et al.* [17] demonstrated that consumption of vitamins C and E immediately prior to a high-fat breakfast blunted the adverse impact of fat on FMD. A more recent study from the same group demonstrated a similar effect with daily administration for 4 weeks of a powdered fruit juice concentrate, with or without a supplement providing antioxidants and various herbal extracts [18]. Red wine, which is rich in antioxidant polyphenolic flavonoids, may also inhibit the detrimental effects of a fatty diet [19]. These findings suggest that the adverse effect of fatty meals on the vascular endothelial function may be mediated by enhanced production of free radical superoxide anions, perhaps stimulated by triglyceride-rich lipoproteins. Asymmetric dimethylarginine, an endogenous inhibitor of NO production, may also play a critical role in postprandial endothelial dysfunction [20].

Not all fats impair endothelial function. Both observational studies and controlled trials indicate that eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA), which

are long-chain omega-3 fatty acids found in coldwater fish, reduce the risk of cardiovascular events. Emerging evidence also suggests that these compounds exert a beneficial effect on endothelial function. In a recent observational study of 326 men and women without overt cardiovascular disease, Leesson *et al.* [21] examined the relationships of plasma levels of DHA and EPA to brachial artery reactivity. No correlation between omega-3 fatty acids and FMD was observed for the study cohort as a whole. However, among the smokers and those with higher fasting insulin, glucose, or triglyceride levels (a subgroup at high risk of cardiovascular events), increased DHA levels were associated with greater FMD. A beneficial effect of fish oil supplements was demonstrated by Goodfellow *et al.* [22], who found improved FMD in hypercholesterolemic patients who were treated with 4 g/d of omega-3 fatty acids for 4 months.

In contrast to omega-3 fatty acids, trans fatty acids, which are isomers of fatty acids found in solid margarines and shortening, increase the risk of coronary heart disease. De Roos *et al.* [23] examined the effect of trans fatty acids on endothelial function in healthy volunteers who participated in a crossover controlled feeding study. Replacement of dietary saturated fatty acids with trans fatty acids significantly lowered FMD, an effect that may have been mediated by a decrease in serum high-density lipoprotein (HDL) cholesterol.

These studies in aggregate suggest that the impact of a meal on endothelial function depends on the amount and type of fat consumed, and the fact that other ingredients in the meal may ameliorate the adverse effects of fats. This concept is supported by data from Vogel *et al.* [24], who measured postprandial FMD in healthy subjects after five different meals. Each meal contained 50 g of fat. FMD was reduced by 31% when the fat source was olive oil, but was not impaired by meals containing omega-3 fatty acid-enriched canola oil or salmon. Moreover, no significant change in FMD was observed when the olive oil meal contained either vitamins C and E or salad and balsamic vinegar.

Few studies have examined the impact of complex dietary patterns on endothelial function. Recently, Fuentes *et al.* [25••] studied the effects of a diet rich in monounsaturated fats, as commonly consumed in Mediterranean countries. FMD was measured in 22 hypercholesterolemic men fed a baseline diet high in saturated fat, then assigned in a crossover design to two diets for 28 days: 1) a low-fat, low-saturated fat National Cholesterol Education Program stage 1 (NCEP-1) diet; and 2) a Mediterranean diet. FMD was higher after the Mediterranean diet than after the saturated fat diet; a nonsignificant trend toward improved FMD was observed following the NCEP-1 diet.

Effects of specific nutrients

Several excellent previous overviews have reviewed studies evaluating the impact of antioxidant vitamins and flavonoids, L-arginine, and folic acid on the vascular endothelium [14••,15••,26]. The results of trials published since 2000 that measured the chronic effects of these nutrients on coronary or peripheral arterial endothelial function are summarized in Table 1.

Antioxidants

Antioxidant vitamins and flavonoids with antioxidant properties have the potential to modulate endothelial function, and thus reduce the risk of cardiovascular disease by scavenging superoxide free radicals and inhibiting the oxidation of LDL cholesterol.

Vitamin C is a water-soluble antioxidant that is plentiful in citrus fruits, melons, berries, and green vegetables. Vitamin E is a lipid-soluble vitamin with antioxidant properties found in vegetable oils, nuts, seeds, whole grains, and avocados. Observational studies have found that consumption of antioxidant vitamins, especially vitamin E, is associated with a reduced risk of cardiovascular disease. Clinical trials, however, have not consistently demonstrated benefit. A number of investigations have suggested a role for vitamins C and E in preserving endothelial function in patients with vascular disease or cardiovascular risk factors. Gokce *et al.* [27], for example, demonstrated a significant improvement in FMD in patients with established coronary artery disease treated with 500 mg/d of vitamin C for 4 weeks. Similarly, patients with type 1 diabetes mellitus exhibited increased FMD after 3 months of 1000 IU/d of vitamin E in a study reported by Skyrme-Jones *et al.* [28]. Others, however, have failed to demonstrate a benefit of chronic vitamin supplementation on vascular reactivity in the fasting state. The results of recent studies are outlined in Table 1. Most have examined small numbers of subjects, and the findings have been conflicting.

The chronic effects of flavonoids on the endothelium have been less extensively studied. Flavonoids are water-soluble phenolic compounds, many of which have antioxidant properties, which are derived from plants. Dietary sources include tea, soybeans, grape skins and seeds, cranberries, and citrus fruits. A recent report by Duffy *et al.* [29] described the effects of black tea on endothelial function in patients with coronary artery disease. Compared with a placebo and an equivalent dose of caffeine, consumption of 450 mL of black tea acutely or 900 mL of black tea daily for 4 weeks led to improved FMD. In contrast, daily supplementation with the soy flavonoid genestein was of no benefit on endothelial function in a cohort of healthy postmenopausal women [40].

L-arginine

L-arginine is a biochemical precursor of NO that also has antioxidant properties. Dietary sources of this amino acid include nuts, rice, cocoa, soy, grapes, whole wheat, corn, and seeds. The typical American diet contains about 5 g of L-arginine; supplementation with an additional 6 to 21 g/d has led to improved endothelial function in some studies of patients with hypercholesterolemia or established coronary disease. Mullen *et al.* [31], however, in a study of 84 normocholesterolemic patients with type 1 diabetes mellitus, found no

Table 1. Recent clinical studies of chronic nutrient consumption and endothelial function

Study / year	Design	Subjects	Dosage	Duration	Method	Result
Vitamin C						
Singh *et al.* [33] / 2002	R, C	Elderly (*n*=54)	1 g/d or placebo	6 wk	VOP	VOP improved with Mediterranean diet but not with vitamin C
Pullin *et al.* [34] / 2002		Homocystinurea (*n*=5); age/sex-matched control subjects (*n*=5)	1 g/d	6 mo	FMD	FMD improved in subjects with homocystinurea
Darko *et al.* [35] / 2002	R, C	Type 2 diabetes (*n*=35)	1.5 g/d	3 wk	VOP	No effect
Fennessy *et al.* [36] / 2003	X	Young smokers (*n*=15)	2 g/d of vitamin C or taurine	5 d	FMD	FMD improved
Fang *et al.* [37] / 2002	R, C	Heart transplant (*n*=40)	1 g/d of vitamin C + 800 IU/d of vitamin E	1 y	Coronary reactivity	No effect
Duffy *et al.* [38] / 2001	R, C	Hypertensives (*n*=39)	500 mg/d	1 mo	FMD	No effect
Raitakari *et al.* [39] / 2000	R, X	Young smokers (*n*=20)	1 g/d	8 wk	FMD	No effect
Vitamin E						
McKechnie *et al.* [40] / 2002	R, C	Nonsmoking, nondiabetic patients with CAD (*n*=18)	500 mg/d of vitamin C + 400 IU/d of vitamin E + 12 mg/d of beta-carotene; or 1 g/d of vitamin C + 800 IU/d of vitamin E + 24 mg/d of beta-carotene	12 wk	FMD	No effect
Stein *et al.* [41] / 2001	R	Elderly, high LDL (*n*=37)	400 IU/d ± statin	6 mo	FMD	No improvement with vitamin E, C, or statin
	R	Elderly, high LDL (*n*=17)	400 IU/d ± statin + vitamin C + statin	6 mo	FMD	No improvement with vitamin E, C, or statin
Borovnicar *et al.* [42] / 2000	C	Hypercholesterol-emic men	400 mg/d	6 mo	FMD	Improved FMD
Skyrme-Jones *et al.* [28] / 2000	R, C	Type 1 diabetes mellitus (*n*=41)	1000 IU/d	3 mo	FMD (*n*=41); VOP (*n*=21)	Improved FMD and VOP
Flavonoids						
Hale *et al.* [30] / 2002	R, C	Healthy menopausal women (*n*=29)	80 mg/d of soy isoflavone	2 wk	FMD	No effect
Duffy *et al.* [29] / 2001	R, X	CAD patients (*n*=66)	900 mL/d of black tea	4 wk	FMD	Improved FMD
L-arginine						
Maxwell *et al.* [43] / 2002	R, C, X	CAD patients (*n*=36)	2 nutrition bars (6.6 g/d of L-arginine)	2 wk	FMD	Improved FMD
Bennett-Richards *et al.* [44] / 2002	R, C, X	Children with CRF (*n*=21)	2.5 g/m^2/d	4 wk	FMD	No effect
Walker *et al.* [45] / 2001	C	CAD patients (*n*=40)	15 g/d	2 wk	VOP	No effect
Maxwell *et al.* [46] / 2000	C	Hypercholesterol-emic patients (*n*=43)	2 nutrition bars (6.6 g/d of L-arginine)	1 wk	FMD	Improved FMD
Mullen *et al.* [31] / 2000	2x2 factorial	Type 1 diabetes mellitus (*n*=84)	14 g/d	6 wk	FMD	FMD improved with atorvastatin; no change with L-arginine

5-MTHF—5-methyl tetrahydrofolic acid; C—controlled; CAD—coronary artery disease; CRF—chronic renal failure; FMD—flow-mediated dilation; IV—intravenous; LDL—low-density lipoprotein; R—randomized; VOP—venous occlusion plethysmography; X—crossover.

Table 1. Recent clinical studies of chronic nutrient consumption and endothelial function *(Continued)*

Study / year	Design	Subjects	Dosage	Duration	Method	Result
Folate						
Mangoni *et al.* [47] / 2002	R, C	Smokers (*n*=24)	5 mg/d	4 wk	VOP	VOP improved
Hirsch *et al.* [48] / 2002	C	Hyperhomocysteinemic males (*n*=20); age-matched males (*n*=20)	0.6 mg/d	8 wk	FMD	No effect in either group
Buccianti *et al.* [49] / 2002		CRF, hemodialysis (*n*=15)	45 mg/wk IV of 5-MTHF	10 wk	FMD	FMD improved
Willems *et al.* [50] / 2002	R, C	Hyperhomocysteinemia and CAD	5 mg/d + cobalamin	6 mo	Coronary reactivity	Coronary blood flow improved
Doshi *et al.* [51] / 2001	R, C, X	CAD patients (*n*=52)	5 mg/d	6 wk	FMD	FMD improved
Chambers *et al.* [52] / 2000	R, C	Men with CAD (*n*=89)	5 mg/d + vitamin B_{12}	8 wk	FMD	FMD improved
Title *et al.* [53] / 2000	R, C	CAD patients (*n*=75)	5 mg/d ± vitamin C + vitamin E	4 mo	FMD	FMD improved
Thambyrajah *et al.* [32] / 2000	R, C	Predialysis renal failure patients (*n*=100)	5 mg/d	12 wk	FMD	No effect

5-MTHF—5-methyl tetrahydrofolic acid; C—controlled; CAD—coronary artery disease; CRF—chronic renal failure; FMD—flow-mediated dilation; IV—intravenous; LDL—low-density lipoprotein; R—randomized; VOP—venous occlusion plethysmography; X—crossover.

improvement in FMD after supplementation with 7 g of L-arginine twice daily, although treatment with atorvastatin significantly improved endothelial function. Other studies in patients with chronic kidney disease or diabetes mellitus have failed to demonstrate an improvement with L-arginine.

Folic acid

Folic acid is a nutrient found abundantly in green leafy vegetables; smaller quantities are also found in fruits, beans, and eggs. Folic acid decreases the plasma levels of homocysteine, an independent risk factor for coronary disease that reduces NO bioavailability by remethylation of homocysteine back to methionine. Although several observational studies have suggested an inverse relationship between folic acid intake and cardiovascular risk, there have not been clinical trials demonstrating benefit in reducing cardiac events. Recent studies have confirmed earlier reports suggesting improved endothelial function with 5 to 10 g/d of folic acid in patients with hyperhomocysteinemia, hypercholesterolemia, or established coronary disease (Table 1). Thambyrajah *et al.* [32], however, found no improvement in FMD in a large cohort of patients with predialysis renal disease who were randomized to 5 mg/d of folate or placebo.

Conclusions

As a key organ in the prevention of atherosclerosis and clinical cardiovascular disease, the vascular endothelium is an attractive therapeutic target for dietary interventions. Emerging evidence suggests that dietary fats acutely impair endothelial function, but the magnitude of this effect depends on the type of fat and on the other nutrients consumed. Studies of antioxidants, L-arginine, and folic acid have not consistently demonstrated beneficial effects of these nutrients on endothelial function, perhaps in part due to modest sample sizes. There are little data on the influence of complex dietary patterns on endothelial function. Large-scale, carefully controlled studies of specific nutrients, and investigation of the effect of complex diets, including low-fat diets, the Dietary Approaches to Stop Hypertension (DASH) diet, the Mediterranean diet, and low-carbohydrate diets, are needed to better understand the relationships between diet and cardiovascular disease.

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