INTRODUCTION
This review represents a summary of a presentation I made at the 1st International Conference on Polyphenols and Health, in Vichy, France, on November 20, 2003. The review provides an overview of epidemiologic data supporting a relationship between higher intake of polyphenolic flavonoids and reduced risk of cardiovascular disease. The remainder of the review focuses on possible mechanisms for this beneficial effect, including improved endothelial function and reduced platelet aggregation.

EPIDEMIOLOGIC STUDIES OF FLAVONOIDS CONSUMPTION
Many epidemiologic studies have investigated the relationship between flavonoid intake and cardiovascular disease risk, and they have provided mixed results (1–12). The subject has been reviewed (13–17); overall, the evidence suggests that individuals with the highest flavonoid intake have modestly reduced risks for cardiovascular disease (1, 3, 4, 7–9). In some studies, the apparent benefit of flavonoids was quite large. For example, Sesso et al (8) examined the relationship between tea and coffee consumption and myocardial infarction. A total of 340 subjects with well-documented myocardial infarction and 340 matched control subjects from the Boston Area Health Study provided dietary information. The investigators observed that individuals who drank more than one cup of tea per day had a 44% reduction in cardiovascular risk. In contrast, coffee consumption was not significantly associated with decreased or increased cardiovascular disease.

In addition to apparent benefits of flavonoid intake in the setting of primary prevention, one recent study suggested that flavonoid intake in the form of tea might have benefit among individuals with established cardiovascular disease. Mukamal et al (10) examined tea consumption in the Myocardial Infarction Onset Study, a prospective cohort study that examined 1900 individuals with established cardiovascular disease. Mukamal et al observed that individuals who drank more than one cup of tea per day had a 44% reduction in cardiovascular risk. In contrast, coffee consumption was not significantly associated with decreased or increased cardiovascular disease.

Analysis of why tea drinking might have benefit and the possible mechanisms have been reviewed. This review summarizes data suggesting that flavonoids improve endothelial function and inhibit platelet aggregation in humans. The vascular endothelium is a critical regulator of vascular homeostasis, and endothelial dysfunction contributes to the pathogenesis and clinical expression of coronary artery disease. Platelet aggregation is a central mechanism in the pathogenesis of acute coronary syndrome, including myocardial infarction and unstable angina. For these reasons, the observed effects of flavonoids on endothelial and platelet function might explain, in part, the observed beneficial effects of flavonoids on cardiovascular disease risk.

KEY WORDS Polyphenols, cardiovascular disease, endothelium, platelets, flavonoids, tea

Joseph A Vita

ABSTRACT
Epidemiologic studies suggest that higher polyphenol intake from fruits and vegetables is associated with decreased risk for cardiovascular disease. The mechanisms explaining this observation remain unclear. This review summarizes data suggesting that flavonoids improve endothelial function and inhibit platelet aggregation in humans. The vascular endothelium is a critical regulator of vascular homeostasis, and endothelial dysfunction contributes to the pathogenesis and clinical expression of coronary artery disease. Platelet aggregation is a central mechanism in the pathogenesis of acute coronary syndromes, including myocardial infarction and unstable angina. For these reasons, the observed effects of flavonoids on endothelial and platelet function might explain, in part, the observed beneficial effects of flavonoids on cardiovascular disease risk.


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From Evans Department of Medicine and Whitaker Cardiovascular Institute, Boston University School of Medicine, Boston.


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Address correspondence to JA Vita, Section of Cardiology, Boston Medical Center, 88 East Newton Street, Boston, MA 02118. E-mail: jvita@bu.edu.
well-nourished populations, such as US health professionals or college alumni, also tended to show less benefit of flavonoids (5, 12). In those studies, it is possible that confounding factors masked benefits of flavonoid consumption. For example, tea consumption is more common among individuals of low socioeconomic status in the United Kingdom, and such individuals are known to have increased risk for cardiovascular disease. Furthermore, baseline flavonoid intake may affect the results. If the population has a relatively high level of flavonoid consumption, then even subjects in the lowest quartile of consumption may be receiving the maximal benefits of flavonoid intake. This might provide an explanation for the lack of effect of tea observed in the United Kingdom, where consumption is high.

Overall, the evidence does suggest that higher consumption of flavonoids is associated with modest reduction of cardiovascular risk, despite the negative results of some studies. For tea, this conclusion is supported by a meta-analysis by Peters et al (17), which suggested an overall reduction in cardiovascular disease risk of ~11% with consumption of 3 cups of tea per day. A growing body of work has provided mechanistic information about how polyphenols might reduce cardiovascular disease, which provides additional support for a biological effect of polyphenols, rather than a simple association of polyphenol intake with a healthier lifestyle or other confounding factors.

**PATHOGENESIS OF CARDIOVASCULAR DISEASE EVENTS**

When considering how polyphenols might reduce cardiovascular risk, it is important to consider recent insights into the pathogenesis of atherosclerotic cardiovascular disease. Atherosclerosis is a chronic inflammatory disease that develops in lesion-prone regions of medium-sized arteries (21). Atherosclerotic lesions may be present and clinically silent for decades before becoming active and producing clinical events such as acute myocardial infarction, unstable angina, or sudden cardiac death. Such events are often caused by acute rupture or erosion of a vulnerable plaque, which exposes the highly thrombogenic subendothelium to flowing blood. The result is acute, platelet-rich, mural thrombosis that occludes or partially occludes the arterial lumen to produce infarction or ischemia. The precise mechanisms accounting for plaque vulnerability and rupture remain incompletely understood, but the available data suggest that local inflammation within the plaque, thinning of the fibrous cap, and accumulation of plaque lipid may contribute (22). Once plaque rupture occurs, the extent of thrombosis formation and acute changes in vascular tone may determine the extent of ischemia/infarction.

The development of a vulnerable plaque and the subsequent ischemic events represent a profound loss of vascular homeostasis. Recent studies focusing on vascular biological features provide a fruitful approach for development of novel treatments and prevention strategies for cardiovascular disease. In particular, there is great interest in antithrombotic therapies and therapies that influence the function of endothelial cells, which are key regulatory cells in the vessel wall. Relevant to the current review, there is increasing evidence that flavonoids may have beneficial effects on endothelial control of thrombosis, inflammation, and vascular tone. Flavonoids also have beneficial effects on platelets, which occlude the arterial lumen in the setting of acute coronary syndromes.

**ENDOTHELIAL FUNCTION AND CARDIOVASCULAR RISK**

The vascular endothelium plays a key role in the regulation of vascular homeostasis, and increasing evidence suggests that alterations in endothelial function contribute to the pathogenesis and clinical expression of cardiovascular disease (23). Endothelial cells regulate vascular homeostasis by producing factors that act locally in the vessel wall and lumen, and a key endothelial product is nitric oxide (24). Nitric oxide was first described as an endothelium-derived vasodilator, but it is now clear that nitric oxide regulates other important aspects of vascular homeostasis (25). For example, nitric oxide prevents adherence of leukocytes to the endothelial surface and inhibits expression of leukocyte adhesion molecules at the endothelial surface. Nitric oxide prevents platelet adhesion and platelet aggregation. Nitric oxide also inhibits vascular smooth muscle cell proliferation and alters expression of noncellular components that constitute the matrix of the vascular wall, making nitric oxide relevant to lesion formation, hypertrophy of the vessel wall, and vascular compliance. Therefore, endothelium-derived nitric oxide has important vasodilator, antiinflammatory, antithrombotic, and growth-suppressing properties that are relevant to all stages of atherosclerosis (23).

Other endothelium-derived products regulate vascular homeostasis, including other substances that influence vascular tone (eg, prostacyclin and endothelin), fibrinolytic factors (tissue plasminogen activator and plasminogen activator inhibitor-1), factors that affect coagulation (tissue factor, heparans, and von Willebrand factor), and proinflammatory factors (eg, adhesion molecules and inflammatory cytokines) (26). In general, loss of nitric oxide is paralleled by changes in these other regulatory mechanisms, leading to the development of a pathologic endothelial phenotype. These observations suggest that the state of the endothelium may be an indicator of vascular health and that examining endothelial vasomotor function may have clinical utility (23).

A common feature of otherwise diverse cardiovascular disease risk factors is their adverse effects on the endothelium. In this regard, dyslipidemia, hypertension, diabetes mellitus, smoking, aging, physical inactivity, systemic inflammation and infectious processes, hyperhomocysteinemia, and the postmenopausal state are all associated with endothelial dysfunction. Genetic and environmental factors might influence the effects of risk factors on endothelial function. Genetic variation in the activity of antioxidant enzymes or nitric oxide synthase might influence the effects of risk factors on endothelial function (27). Diet might also influence the effects of risk factors on endothelial function; therefore, polyphenol intake could be important in determining the risk for cardiovascular disease events.

Prospective studies have shown that endothelial dysfunction is associated with an increased risk of cardiovascular events (28–38). One study suggested that improved endothelial function after treatment of hypertension was associated with improved outcomes (35). Many interventions known to reduce cardiovascular disease risk have the ability to reverse endothelial dysfunction. For example, lipid-lowering therapy, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, smoking cessation, and exercise have all been shown to reverse endothelial dysfunction among patients with atherosclerosis or...
cardiovascular risk factors (26). These findings suggest that endothelial function may have utility as a surrogate marker of cardiovascular risk. Furthermore, endothelial function has evolved into a clinically useful endpoint for studies of potential interventions for the prevention or treatment of cardiovascular disease (23).

**STUDIES OF ANTIOXIDANTS AND ENDOTHELIAL DYSFUNCTION**

With respect to the mechanism of endothelial dysfunction, there has recently been great interest in the importance of oxidative stress. These studies fit well with the recognition that oxidative stress contributes to atherogenesis (39). The importance of oxidative stress as a cause of endothelial dysfunction has prompted many investigations into the effects of antioxidants on endothelial function. For ascorbic acid in particular, there is extensive evidence that acute treatment reverses endothelial dysfunction in many disease states, including atherosclerosis, diabetes mellitus, hypertension, congestive heart failure, renal failure, hyperhomocysteinemia, hypercholesterolemia, and others (40).

Gokce et al (41) examined the effect of ascorbic acid on brachial artery flow-mediated dilation in a randomized, placebo-controlled, double-blind study of patients with coronary artery disease. Flow-mediated dilation reflects shear stress-mediated nitric oxide production by the endothelium (42). This physiologically relevant response is impaired in the setting of coronary risk factors and is correlated with abnormal responses in the coronary circulation (43); abnormal responses in the brachial artery predict future cardiovascular disease events among high- and low-risk patients (34–36, 38). In the study by Gokce et al (41), brachial artery flow-mediated dilation was impaired at baseline. Flow-mediated dilation improved 2 h after an acute 2-g dose of ascorbic acid, and the effect was sustained after 30 d of treatment with 500 mg/d. The responses were unchanged after acute and chronic treatment with placebo. Mechanistic in vitro studies suggested that the benefit of ascorbic acid may be attributable, in part, to increased activity of nitric oxide synthase resulting from stabilization of tetrahydrobiopterin, an essential cofactor for enzyme activity (44).

Other water-soluble antioxidant compounds have beneficial effects on endothelial function among patients with cardiovascular disease, including N-acetylcysteine (45, 46), glutathione (47), and the cysteine donor L-2-oxothiazolidine-4-carboxylic acid (48). In contrast, the effects of lipid-soluble antioxidants on endothelium-dependent dilation among human subjects with atherosclerosis and cardiovascular risk factors have been relatively disappointing, although benefits have been reported for select, high-risk groups, including patients with type 1 diabetes mellitus (49) or multiple risk factors (50).

**EFFECTS OF POLYPHENOLS ON ENDOTHELIAL FUNCTION**

The extensive prior work demonstrating a beneficial effect of ascorbic acid on endothelial function prompted us to consider that other water-soluble antioxidants, such as flavonoids, might have beneficial effects on endothelial function. To explore this possibility, we investigated the effects of tea consumption on endothelial function. Tea contains an assortment of water-soluble antioxidant flavonoids, including catechins, quercetin, kaempferol, and other polyphenols, particularly thearubigins. We examined the short-term and long-term effects of tea consumption on flow-mediated dilation in the brachial artery among 50 subjects with angiographically proven coronary artery disease, in a placebo-controlled, crossover study (51). Subjects taking antioxidant supplements were excluded, and subjects were asked to refrain from drinking tea and red wine during the study. All subjects were receiving prescribed medications for coronary artery disease, and 77% were undergoing lipid-lowering therapy. Short-term effects of tea were examined by measuring flow-mediated dilation before and 2 h after the subjects consumed 450 mL of freshly brewed black tea. Long-term effects were examined by measuring flow-mediated dilation again after the subjects had consumed 900 mL of black tea per day for 30 d. The study used water as a control beverage, and the beverage order was randomized.

Both short-term and long-term tea consumption improved endothelial function, whereas water consumption had no effect. There also were no effects of tea consumption on nitroglycerin-mediated dilation, baseline arterial diameter, or the extent of reactive hyperemia, which confirmed that tea consumption affected endothelial function, rather than the function of vascular smooth muscle or the stimulus for dilation. Flow-mediated dilation was not affected by an acute dose of caffeine, which suggested that the caffeine content of tea did not account for the results. Blood pressure, serum glucose concentrations, and serum lipid concentrations remained stable during tea consumption. Although catechins represent a relatively minor component of black tea, they are measurable in plasma. Our study demonstrated that total catechin amounts were increased ~20% after tea consumption; however, there was no relationship between changes in total catechin amounts and improvements in endothelial function.

Although tea contains flavonoid antioxidants, it remains unclear whether an antioxidant effect explains the observed benefits of tea. Our study demonstrated no effect of tea consumption on plasma antioxidant capacity (51). There also was no effect on plasma concentrations of F2-isoprostanes, markers of systemic lipid peroxidation, or 8-hydroxydeoxyguanosine, a marker of DNA oxidation. These findings are consistent with several other well-conducted studies that failed to demonstrate a reduction in markers of oxidative stress after tea consumption (52).

Several other studies demonstrated that flavonoid-containing beverages have beneficial effects on endothelial function. For example, Hodgson et al (53) examined the effect of tea consumption on brachial artery flow-mediated dilation among a group of otherwise healthy subjects with modest hypercholesterolemia. Those investigators observed that consumption of 5 cups of black tea per day for 5 wk led to improved flow-mediated dilation. Interestingly, tea consumption was also associated with an improvement in nitroglycerin-mediated dilation, which suggested that tea improved the bioactivity of endothelium-derived nitric oxide and/or had a beneficial effect on the function of vascular smooth muscle.

Other flavonoid-containing beverages, particularly grape products, have been shown to improve endothelial function. Stein et al (54) observed that consumption of grape juice for 14 d was associated with improved brachial artery flow-mediated dilation among 15 adults with angiographically proven coronary
artery disease. In that study, the susceptibility of LDL to ex vivo oxidation was reduced, which suggested an antioxidant effect. A second study from the same group also indicated beneficial effects of purple grape juice on endothelial function (55).

Agewall et al (56) observed an improvement in brachial artery flow-mediated dilation < 1 h after consumption of dealkohol- ized red wine among healthy volunteers. In that study, consumption of wine (containing alcohol) was associated with vasodilatation and increased blood flow but no observable increase in flow-mediated dilation. However, the effects of wine on baseline diameter and flow might have obscured an effect on endothelial function.

In a recent study, Heiss et al (57) examined the effects of cocoa on flow-mediated dilation. Among patients with at least one cardiovascular disease risk factor, impaired endothelial function was observed. Two hours after the patients consumed cocoa containing 176 mg/dL flavan-3-ols, the investigators observed a significant increase in flow-mediated dilation. They also observed increases in nitrosylated and nitrosated species in plasma, which suggested an increase in nitric oxide production.

POLYPHENOLS AND PLATELET FUNCTION
Platelet aggregation plays a critical role in the pathogenesis of acute coronary syndromes, and there is extensive evidence that antiplatelet therapy reduces cardiovascular disease risk (58). An effect of polyphenols to reduce platelet activity could have a large impact on cardiovascular disease and might provide an important mechanistic explanation for the available epidemiologic data regarding polyphenols and cardiovascular disease.

Several basic studies demonstrated that flavonoids inhibit platelet aggregation (59, 60). Purple grape juice inhibited ex vivo platelet aggregation in whole blood (60). The direct clinical relevance of ex vivo platelet studies is unclear, and an important study by Demrow et al (59) examined the effects of grape juice on platelet function in vivo. Those investigators used the Folts model of unstable coronary stenosis, which involves the creation of endothelial injury and subocclusive stenosis in a dog coronary artery. In this model, transient platelet aggregation and release are reflected in cyclic variations in coronary blood flow; therefore, the model closely mimics a ruptured atherosclerotic plaque causing unstable angina. In this model, acute intraocular administration of red wine or grape juice was associated with marked reductions in cyclic flow variations, which indicates an important antiplatelet effect that is relevant to cardiovascular disease events (59).

Regarding the potential mechanisms of flavonoids on platelet function, Freedman et al (60) examined the effects of grape juice on platelet function. They observed that addition of grape juice to platelets ex vivo was associated with a reduction in platelet aggregation, a decrease in platelet production of superoxide anion, and an increase in platelet nitric oxide production. The beneficial effects appeared to be related to reduced activation of protein kinase C. Importantly, these findings were reproduced when the studies were repeated with platelets isolated from healthy volunteers who had consumed grape juice for 2 wk.

There are mixed data regarding the effects of tea consumption on platelet function. Animal studies of the effects of tea consumption on platelet aggregation in the Folts model suggested that tea may have benefits comparable to those of grape juice, although rather high doses of tea were required (JD Folts, personal communication, 2004). We examined the effects of short-term and long-term tea consumption on ex vivo platelet aggregation in response to ADP or thrombin-related activated peptide among patients with coronary artery disease (61). That study demonstrated no effect of tea consumption on platelet function, although the concurrent aspirin treatment could have influenced the results. Hodgson et al (62) observed that tea consumption reduced plasma concentrations of P-selectin, a marker of in vivo platelet aggregation. It is clear that additional studies will be required to define the effects of tea on platelet function.

OTHER POLYPHENOLIC COMPOUNDS AND OTHER POTENTIAL MECHANISMS
Many other polyphenolic compounds have been reported to reduce cardiovascular disease risk and to have beneficial effects on endothelial and platelet function; a detailed discussion of these compounds is beyond the scope of the present review. For example, soy products, which are rich sources of isoflavones such as genistein and daidzein, have been reported to improve endothelial function, possibly through an effect on the aromatase receptor (63).

There is great interest currently in the importance of systemic inflammation as a pathogenic mechanism of cardiovascular risk (64). Importantly, there is growing evidence that polyphenolic compounds may have antiinflammatory effects. For example, the grape and wine component resveratrol inhibits adhesion molecule expression and monocyte adhesion in vitro (65). Additional study of the antiinflammatory effects of polyphenols is clearly warranted.

CONCLUSIONS
This article has reviewed epidemiologic and mechanistic studies that suggest that polyphenols have beneficial effects on the cardiovascular system and reduce the risk of cardiovascular disease. It should be emphasized that some of the epidemiologic data are conflicting, and this review considered some possible explanations for the reported negative findings for certain well-nourished populations and populations with high flavonoid intake overall. Improved endothelial function, antiplatelet effects, and antiinflammatory effects are among the important mechanisms to be considered for the observed benefits. Overall, the findings of available studies fit well with the recommendations of the American Heart Association that Americans should increase their consumption of fruits and vegetables and other foods with high polyphenol contents (66).

REFERENCES


