Published in final edited form as:
J Am Coll Nutr. 2007 August ; 26(4): 366S–372S.

Effects of Flavonoid-Containing Beverages and EGCG on Endothelial Function

Sherene M. Shenouda, PhD and **Joseph A. Vita, MD, FACN**
Evans Department of Medicine and Whitaker Cardiovascular Institute, Boston University School of Medicine, Boston, MA

Abstract

Abnormalities of the vascular endothelium contribute to all stages of atherosclerosis from lesion development to clinical cardiovascular disease events. Recognized risk factors, including diabetes mellitus, hypertension, dyslipidemia, cigarette smoking, and sedentary lifestyle are associated with endothelial dysfunction. A variety of pharmacological and behavioral interventions have been shown to reverse endothelial dysfunction in patients with cardiovascular disease. A large number of epidemiological studies suggest that dietary factors, including increased intake of flavonoid-containing foods and beverages, reduce cardiovascular risk, and recent studies have shown that such beverages have favorable effects on endothelial function. These studies have engendered interest in the development of dietary supplements or drugs that would allow for more convenient and higher dose administration of flavonoids and might prove useful for prevention or treatment of cardiovascular disease. In this paper, we will review the contribution of endothelial dysfunction to the pathogenesis and clinical expression of atherosclerosis and recent data linking flavonoid and EGCG consumption to improved endothelial function and reduced cardiovascular risk.

Keywords

EGCG; epigallocatechin gallate; flavonoids; endothelial function

INTRODUCTION

Endothelial Regulation of Vascular Homeostasis

The endothelium regulates vascular tone, thrombosis, local inflammation, composition of the vascular wall, and angiogenesis by producing a variety of paracrine factors [1-4]. For example, the endothelium controls vascular tone by producing vasoconstrictors such as endothelin-1 and

Address for correspondence and reprints: Joseph A. Vita, MD Boston University School of Medicine 88 East Newton Street, C-818 Boston, MA 02118 Phone: 617-638-8742 Fax: 617-638-8756 E-mail: jvita@bu.edu.

Disclosures: Joseph Vita has received research support from DSM Nutritional Products, Welch's, Inc. and Ocean Spray, Inc.

TEACHING POINTS

- Endothelial dysfunction contributes to the pathogenesis of cardiovascular disease
- Epidemiological studies suggest that flavonoids may reduce cardiovascular risk
- Recent clinical studies have shown that consumption of flavonoid-containing foods leads to improved endothelial function
- Experimental studies have provided insights into the specific mechanisms that account for improved endothelial function following flavonoid treatment, and a simple antioxidant effect does not provide an adequate explanation
- These data suggest that flavonoid supplements might have promise for prevention and management of cardiovascular disease, but further studies are needed

angiotensin 2 and vasodilators such as prostacyclin, endothelium-derived hyperpolarizing factor, and nitric oxide. Endothelial cells influence the local inflammatory response via expression of adhesion molecules and chemotactic factors. A variety of endothelial products affect thrombosis and fibrinolysis, including von Willebrand Factor, thrombomodulin, heparans, nitric oxide, tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1). Under normal conditions, the endothelium maintains blood fluidity and opposes vasoconstriction and leukocyte adhesion. In the setting of cardiovascular disease risk factors, the endothelium adopts a pathological phenotype characterized by expression of an array of factors that promote vasospasm, thrombosis, and local inflammation that is relevant to the development of atherosclerosis.

The ability of the endothelium to produce bioactive nitric oxide in particular has emerged as an important indicator of endothelial phenotype and overall vascular health [5,6]. Nitric oxide is synthesized from L-arginine by the endothelial isoform of nitric oxide synthase (eNOS). This enzyme is constitutively expressed in endothelial cells and is normally in a relatively quiescent state with a low level of basal nitric oxide production. Nitric oxide production rapidly increases over a period of seconds following stimulation with a variety of clinically relevant receptor-dependent agonists such as acetylcholine, serotonin, thrombin, and angiotensin 2 and following changes in local shear stress, which is the frictional force produced by flowing blood. Nitric oxide plays a homeostatic role and generally opposes the vasoconstrictor, prothrombotic, proinflammatory, and growth-promoting effects of such receptor-dependent agonists. The importance of shear stress-dependent nitric oxide production is emphasized by the observation that atherosclerosis tends to develops at branch points and other locations in the arterial tree with a loss of normal laminar flow and non-physiological levels of shear stress [7].

Extensive studies in experimental models and in human subjects have shown that cardiovascular disease risk factors reduce the bioavailability of endothelium-derived nitric oxide and impair endothelium-dependent vasodilation [1]. As has been recently reviewed, there is considerable information about the mechanisms that account for impaired nitric oxide bioavailability in atherosclerosis [6]. In general, risk factors are associated with a state of increased oxidative stress in endothelial cells and other cells in the vascular wall. Reactive oxygen species may react directly with nitric oxide and eliminate its biological activity. In addition, alterations in cellular redox state have the potential to reduce eNOS expression, decrease the availability of essential co-factors, and uncouple eNOS from its normal dimeric configuration into a monomeric form that produces superoxide anion, rather than nitric oxide [6]. Furthermore, increased oxidative stress in the setting of risk factors may increase levels of endogenous inhibitors of eNOS and produce critical modifications in guanylyl cyclase and reduce tissue responses to nitric oxide. Although acute administration of high doses of radical scavengers has been shown to restore endothelial vasodilator function in experimental models and human subjects [8,9], interventions that inhibit the enzymatic sources of reactive oxygen species appear to be a more effective strategy to maintain nitric oxide bioavailability [6,10].

Clinical Studies of Endothelial Function and Their Clinical Relevance

Given the importance of endothelial function for regulation of vascular homeostasis and its contribution to atherosclerosis, there has been considerable interest in studying endothelial function in human subjects. A variety of invasive and non-invasive methods have been developed that examine endothelium-dependent vasodilation in the coronary and peripheral circulations [11,12]. The most specific methods involve intra-arterial infusion of endothelium-dependent vasodilators and inhibitors of nitric oxide production, and in these studies, vasodilation is assessed by angiography, intra-arterial Doppler, or venous occlusion plethysmography. A variety of non-invasive methods have also developed, and the most frequently-used approach uses cuff occlusion to increase local arterial flow and shear stress in

the brachial artery and high-resolution ultrasound to measure flow-mediated dilation [13]. Recently, there has been considerable interest in the observation that endothelial progenitor cells circulate in blood and have a role in arterial repair following injury and in angiogenesis. The number of circulating endothelial cells correlates with endothelial vasodilator function and endothelial progenitor cell count is reduced in patients with risk factors, and thus appears to be another important measure of vascular health [14].

These invasive and non-invasive approaches have been used to confirm in human subjects that cardiovascular disease risk factors and established coronary artery disease are associated with impaired endothelium-dependent vasodilation [15-17]. Importantly, endothelial dysfunction precedes the development of atherosclerosis assessed by angiography or intravascular ultrasound [16,18]. In addition to the classical risk factors, endothelial dysfunction is present in individuals with more recently recognized risk factors such as obesity, the metabolic syndrome, sedentary lifestyle, and elevated markers of inflammation. As has been reviewed, a variety of pharmacological interventions have been shown to reverse endothelial dysfunction in coronary and peripheral arteries including lipid lowering drugs, angiotensin converting enzyme inhibitors, angiotensin receptor blockers, and insulin sensitizing drugs [1]. Lifestyle interventions such as weight loss, increased physical activity, and smoking cessation also reverse endothelial dysfunction. It is interesting that these diverse interventions have the ability to restore endothelial function and reduce cardiovascular disease risk, providing evidence that endothelial dysfunction plays a critical role in the pathogenesis of cardiovascular disease events [19].

Additional evidence that endothelial dysfunction plays a pathophysiological role in the cardiovascular events is provided by prospective outcome studies. Several groups of investigators have reported increased cardiovascular risk in patients with an abnormal vasomotor response to acetylcholine, an agonist for endothelial production of nitric oxide, in the coronary [20-22] or forearm circulations [23,24]. Impaired brachial artery flow-mediated dilation, as measured by ultrasound, is also associated with increased risk for cardiovascular events [25-27]. A recent study demonstrated increased cardiovascular risk in patients with lower numbers of circulating endothelial progenitor cells [28]. Thus, systemic vascular health and regenerative capacity appears to be highly relevant to cardiovascular disease.

Overall, the available evidence suggests that measuring endothelial function in human subjects provides clinically useful information. Although no method is sufficiently standardized to provide prognostic information or track the response to therapy in an individual patient, studies of endothelial function provide important data about mechanisms of cardiovascular disease and interventions that have the potential to reduce cardiovascular risk. In the next sections, we will review the data supporting a link between increased flavonoid intake and decreased cardiovascular risk and studies providing evidence that improved endothelial function is a possible mechanistic explanation for those observations.

Flavonoids and Cardiovascular Disease

Flavonoids are naturally occurring polyphenolic compounds found in numerous fruits, vegetables, and specific beverages (e.g. tea, grape juice, wine, cocoa, and cranberry juice). Flavonoids have been reported to have anti-platelet, anti-inflammatory, and antioxidant activities. Numerous epidemiological studies have explored the relationship between flavonoid consumption and cardiovascular disease risk [29-38]. In regard to tea consumption, studies have reported up to a 58% reduction in cardiovascular risk for individuals consuming the highest compared to the lowest amount of tea [30]. On the other hand, several studies have reported no relationship between tea or flavonoid consumption and cardiovascular risk [33, 39-41], although those studies may have been confounded by socioeconomic factors and cohort nutritional status [42]. In a recent meta-analysis that included both positive and negative

studies, Peters and colleagues concluded that higher tea consumption is associated with a significant, but modest 11% reduction in risk [43]. Similar epidemiological data suggest a beneficial effect on cardiovascular disease risk from other sources of flavonoids, including red wine [44,45]. Based in part on these findings, the American Heart Association has recommended diets rich in fruits and vegetables as a strategy to prevent cardiovascular disease [46], and the Beverage Guidance Panel recommends tea consumption as the preferred beverage following water [47].

Despite the recognized benefits associated with consumption of flavonoid-containing beverages, the responsible mechanisms remain uncertain. Flavonoids have strong antioxidant properties *in vitro*, and investigators hypothesized that reduced cardiovascular risk might reflect an antioxidant effect, including an ability to scavenge reactive oxygen species and prevent oxidative modification of low density lipoprotein, a critical step in atherogenesis [48]. For example, it has been reported that low density lipoprotein isolated from patients consuming grape juice is less susceptible to oxidation [49]. However, the relevance of these findings may be questioned since alpha tocopherol also has a similar protective effect, but has consistently failed to reduce the risk of cardiovascular disease in randomized trials [50,51]. In contrast to studies with grape juice, studies with tea have largely failed to demonstrate an effect on systemic markers of lipid peroxidation [52]. Investigators have also examined changes in the antioxidant capacity of plasma following beverage consumption as an indicator of an *in vivo* antioxidant effect. As was recently reviewed, a variety of flavonoid-containing foods have been shown to increase total antioxidant capacity, however, it remains unclear whether component flavonoids are actually responsible for this effect [53].

Flavonoids and Endothelial Function

One possible explanation for reduced cardiovascular risk in individuals consuming higher amounts of flavonoids is a favorable effect of these compounds on endothelial function. There is now strong evidence from multiple studies that flavonoid-containing beverages and foods have such an effect (Table 1). For example, Duffy and colleagues observed that endothelium-dependent flow-mediated dilation of the brachial artery improved two hours after consumption of black tea and that this effect was sustained following daily consumption for four weeks in patients with proven coronary artery disease [54]. Interestingly, this favorable effect could not be related to a systemic reduction in oxidative stress as reflected by plasma antioxidant capacity or other systemic markers of oxidative stress [55]. However, it remains possible that flavonoids could have an antioxidant effect at the tissue level in the vasculature. The effect of tea consumption also was not attributable to caffeine or non-specific effects on the function of vascular smooth muscle [54].

Hodgson and colleagues showed a favorable effect of tea consumption on endothelial function in otherwise healthy subjects with cardiovascular risk factors [56]. Those investigators also found no evidence that an antioxidant effect accounted for these observations [57]. Other investigators have observed favorable vascular effects of black and green tea [58,59]. As outlined in the Table 1, improved endothelial function has also been demonstrated following consumption of other flavonoid-containing beverages and foods, including grape juice [49, 60] wine,[61], dealcoholized red wine [62], cocoa [63-65], and chocolate [66].

Several studies sought to determine the specific components of flavonoid-containing beverages that are responsible for improved endothelial function. The flavonoids found in tea can be divided into two subclasses, the flavanols and flavonols [67]. Flavanols are found in monomer as well as polymer form. Monomer flavanols found in tea include catechin, epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG) whereas polymer forms found in tea include theaflavins and thearubigins. The most abundant flavonols found in tea include quercetin and kaempferol.

Several recent studies have examined the effects of specific catechins on endothelial function. Schroeter and colleagues demonstrated improved flow-mediated dilation of the brachial artery and fingertip microvessels up to four hours after administration of pure EC [65]. Since the effects of pure EC mimicked the effects of flavanol-rich cocoa and because circulating EC and EC metabolite levels correlated with the degree of improvement in endothelial function, they concluded that EC accounts, in part, for the benefits of cocoa. Widlansky and colleagues observed an improvement in brachial artery flow-mediated dilation following acute administration of EGCG [68]. Consistent with measured plasma levels and the known pharmacokinetics of EGCG, this effect was not sustained following two-week administration of EGCG twice daily, if measurements were made twelve hours after the last dose (trough time point). These findings support the concept that pure catechins can improve endothelial function. Since tea has a sustained benefit with chronic treatment, however, the lack of a sustained benefit of EGCG suggests that some other tea component accounts for the chronic benefit. Further investigations will be needed to determine whether single flavonoids, food flavonoid extracts, or whole foods/beverages have better clinical utility.

Mechanisms of Benefit

Recent *in vitro* studies provide insights into how flavonoids improve endothelial function at the molecular level. Lorenz et al. examined the effect of EGCG on endothelial function on isolated rat aorta segments and cultured endothelial cells [69]. They reported that EGCG activates eNOS and produces endothelium-dependent relaxation of arterial segments. Using pharmacological inhibitors, they gained evidence that EGCG activates eNOS in a manner that depends on phosphatidylinositol 3-kinase (PI3-K), cyclic adenosine monophosphate-dependent protein kinase (PKA), and Akt. Using a more molecular approach, Anter and colleagues further investigated the cellular signaling mechanisms that account for eNOS activation by catechins and a concentrated preparation of black tea polyphenols [70,71]. They determined that activation of eNOS depends on p38 mitogen-activated protein kinase (p38 MAPK) and ligand-independent activation of estrogen receptor-α, which leads to activation of PI 3-K/Akt pathway and a specific pattern of eNOS phosphorylation that is associated with increased nitric oxide synthesis. Thus, in addition to putative benefits resulting from the ability of flavonoids to scavenge radicals, it appears that flavonoids activate specific signaling pathways in endothelial cells that improve multiple aspects of endothelial function.

CONCLUSIONS

Thus, the available data strongly supports the importance of endothelial dysfunction in the pathogenesis of cardiovascular disease and the potential benefits of interventions that reverse endothelial dysfunction. Epidemiological studies have shown that increased consumption of flavonoid-containing beverages and foods reduce cardiovascular risk. There is now strong evidence from multiple studies that such beverages and foods have favorable effects on the endothelium. Although not discussed in the present review, anti-thrombotic and anti-inflammatory effects of flavonoids may also help reduce cardiovascular risk [67]. The current dietary guidelines support increased consumption of whole fruits and vegetables, including beverages such as tea and grape juice, and there is considerable interest about the possibility that consumption of chocolate may also have health benefits.

Based on the current state of knowledge, it would be logical to consider the use of flavonoid supplements as an approach to reduce cardiovascular risk. Randomized trials would be needed, however, before such a recommendation could be made. Furthermore, the best form and type of flavonoid supplement to be used in such trials remain unclear. Recent studies using surrogate markers, such as endothelial function, have raised the possibility that whole food extracts or flavonoid-enriched foods might have more promise that purified single flavonoids.

Nevertheless, it is now clear from experimental studies that flavonoids have powerful effects at the cellular level and offer a great deal of promise as a novel approach for the prevention and management of cardiovascular disease.

ACKNOWLEDGEMENTS

Dr. Shenouda is supported by the Boston University School of Medicine Basic Science Cardiovascular Training Program (T32 HL 07224). Dr. Vita is supported by NIH grants HL75795, HL081587, HL083801, HL083269, and HL083781 and research grants from DSM Nutritional Products, Welch's, Inc. and Ocean Spray, Inc.

REFERENCES

1. Widlansky ME, Gokce N, Keaney JF Jr. Vita JA. The clinical implications of endothelial dysfunction. J Am Coll Cardiol 2003;42:1149–1160. [PubMed: 14522472]
2. Huang AL, Vita JA. Effects of systemic inflammation on endothelium-dependent vasodilation. Trends Cardiovasc Med 2006;16:15–20. [PubMed: 16387625]
3. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. Circulation 2007;115:1285–1295. [PubMed: 17353456]
4. Lerman A, Zeiher AM. Endothelial function: cardiac events. Circulation 2005;111:363–368. [PubMed: 15668353]
5. Vita JA, Keaney JF Jr. Endothelial function: A barometer for cardiovascular risk? Circulation 2002;106:640–642. [PubMed: 12163419]
6. Hamburg, NM.; Vita, JA. Endothelial dysfunction in atherosclerosis: Mechanisms of impaired nitric oxide bioactivity. In: Loscalzo, J., editor. Molecular mechanisms of atherosclerosis. Taylor & Francis; London: 2006.
7. Malek AM, Alper SL, Izumo S. Hemodynamic shear stress and its role in atherosclerosis. JAMA 1999;282:2035–2042. [PubMed: 10591386]
8. Sherman DL, Keaney JF Jr. Biegelsen ES, Duffy SJ, Coffman JD, Vita JA. Pharmacological concentrations of ascorbic acid are required for the beneficial effects on endothelial vasomotor function in hypertension. Hypertension 2000;35:936–941. [PubMed: 10775565]
9. Jackson TS, Xu A, Vita JA, Keaney JF Jr. Ascorbate prevents the interaction of superoxide and nitric oxide only at very high physiological concentrations. Circ Res 1998;83:916–922. [PubMed: 9797340]
10. Münzel T, Keaney JF Jr. Are ACE-inhibitors a "magic bullet" against oxidative stress? Circulation 2001;104:1571–1574. [PubMed: 11571254]
11. Vita JA. Nitric oxide-dependent vasodilation in human subjects. Methods Enzymol 2002;359:186–200. [PubMed: 12481571]
12. McMackin CJ, Vita JA. Update on nitric oxide-dependent vasodilation in human subjects. Methods Enzymol 2005;396:541–553. [PubMed: 16291261]
13. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery. A report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol 2002;39:257–265. [PubMed: 11788217]
14. Hill JM, Zalos G, Halcox JP, Schenke WH, Waclawiw MA, Quyyumi AA, Finkel T. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. N Engl J Med 2003;348:593–600. [PubMed: 12584367]
15. Ludmer PL, Selwyn AP, Shook TL, Wayne RR, Mudge GH, Alexander RW, Ganz P. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. N Engl J Med 1986;315:1046–1051. [PubMed: 3093861]
16. Vita JA, Treasure CB, Nabel EG, McLenachan JM, Fish RD, Yeung AC, Vekshtein VI, Selwyn AP, Ganz P. Coronary vasomotor response to acetylcholine relates to risk factors for coronary artery disease. Circulation 1990;81:491–497. [PubMed: 2105174]

17. Benjamin EJ, Larson MG, Kupka MJ, Mitchell GF, Keaney JF Jr. Vasan RS, Lehman B, Fananapazir L, Osypiuk E, Vita JA. Cross-sectional correlates of brachial artery endothelial function in the community: The NHLBI's Framingham Heart Study. Circulation 2002;104:II–152.
18. Nishimura RA, Lerman A, Chesebro JH, Ilstrup DM, Hodge DO, Higano ST, Holmes DR, Tajik J. Epicardial vasomotor responses to acetylcholine are not predicted by coronary atherosclerosis as assessed by intracoronary ultrasound. J Am Coll Cardiol 1995;26:41–49. [PubMed: 7797774]
19. Levine GN, Keaney JF Jr. Vita JA. Cholesterol reduction in cardiovascular disease: Clinical benefits and possible mechanisms. N Engl J Med 1995;332:512–521. [PubMed: 7830734]
20. Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR, Lerman A. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. Circulation 2000;101:948–954. [PubMed: 10704159]
21. Schachinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long- term outcome of coronary heart disease. Circulation 2000;101:1899–1906. [PubMed: 10779454]
22. Halcox JPJ, Schenke WH, Zalos G, Mincemoyer R, Prasad A, Waclawiw MA, Nour KRA, Quyyumi AA. Prognostic value of coronary vascular endothelial function. Circulation 2002;106:653–658. [PubMed: 12163423]
23. Heitzer T, Schlinzig T, Krohn K, Meinertz T, Munzel T. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. Circulation 2001;104:2673–2678. [PubMed: 11723017]
24. Perticone F, Maio R, Ceravolo R, Cosco C, Cloro C, Mattioli PL. Relationship between left ventricular mass and endothelium-dependent vasodilation in never-treated hypertensive patients. Circulation 1999;99:1991–1996. [PubMed: 10209003]
25. Gokce N, Keaney JF Jr. Menzoian JO, Watkins M, Hunter L, Duffy SJ, Vita JA. Risk stratification for postoperative cardiovascular events via noninvasive assessment of endothelial function. Circulation 2002;105:1567–1572. [PubMed: 11927524]
26. Yeboah J, Crouse JR, Hsu FC, Burke GL, Herrington DM. Brachial flow-mediated dilation predicts incident cardiovascular events in older adults: the Cardiovascular Health Study. Circulation 2007;115:2390–2397. [PubMed: 17452608]
27. Anderson TJ. Prognostic significance of brachial flow-mediated vasodilation. Circulation 2007;115:2373–2375. [PubMed: 17485590]
28. Werner N, Kosiol S, Schiegl T, Ahlers P, Walenta K, Link A, Bohm M, Nickenig G. Circulating endothelial progenitor cells and cardiovascular outcomes. N Engl J Med 2005;353:999–1007. [PubMed: 16148285]
29. Stensvold I, Tverdal A, Solvoll K, Foss OP. Tea consumption. relationship to cholesterol, blood pressure, and coronary and total mortality. Prev Med 1992;21:546–553. [PubMed: 1409496]
30. Hertog MGL, Feskens EJM, Hollman PCH, Martijn B, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen Elderly Study. Lancet 1993;342:1007–1011. [PubMed: 8105262]
31. Keli SO, Hertog MGL, Feskens EJM, Kromhout D. Dietary flavanoids, antioxidant vitamins, and incidence of stroke: the Zutphen study. Arch Intern Med 1996;156:637–642. [PubMed: 8629875]
32. Knekt P, Jarvinen R, Reunanen A, Maatela J. Flavonoid intake and coronary mortality in Finland: a cohort study. Br Med J 1996;312:478–481. [PubMed: 8597679]
33. Hertog MG, Sweetnam PM, Fehily AM, Elwood PC, Kromhout D. Antioxidant flavonols and ischemic heart disease in a Welsh population of men: The Caerphilly Study. Am J Clin Nutr 1997;65:1489–1494. [PubMed: 9129481]
34. Sesso HD, Gaziano JM, Buring JE, Hennekens CH. Coffee and tea intake and the risk of myocardial infarction. Am J Epidemiol 1999;149:162–167. [PubMed: 9921961]
35. Yochum L, Kushi LH, Meyer K, Folsom AR. Dietary flavonoid intake and risk of cardiovascular disease in postmenopausal women. Am J Epidemiol 1999;149:943–949. [PubMed: 10342803]
36. Geleijnse JM, Launer LJ, Van der Kuip DA, Hofman A, Witteman JC. Inverse association of tea and flavonoid intakes with incident myocardial infarction: the Rotterdam Study. Am J Clin Nutr 2002;75:880–886. [PubMed: 11976162]

37. Mukamal KJ, Maclure M, Muller JE, Sherwood JB, Mittleman MA. Tea consumption and mortality after acute myocardial infarction. Circulation 2002;105:2476–2481. [PubMed: 12034652]
38. Mink PJ, Scrafford CG, Barraj LM, Harnack L, Hong CP, Nettleton JA, Jacobs DR Jr. Flavonoid intake and cardiovascular disease mortality: a prospective study in postmenopausal women. Am J Clin Nutr 2007;85:895–909. [PubMed: 17344514]
39. Brown CA, Bolton-Smith C, Woodward M, Tunstall-Pedoe H. Coffee and tea consumption and the prevalence of coronary heart disease in men and women: Results from the Scottish Health Study. J Epidemiol Community Health 1993;47:171–175. [PubMed: 8350026]
40. Rimm EB, Katan MB, Ascherio A, Stampfer MJ, Willett WC. Relation between intake of flavonoids and risk for coronary heart disease in male health professionals. Ann Intern Med 1996;125:384–389. [PubMed: 8702089]
41. Sesso HD, Paffenbarger RS Jr. Oguma Y, Lee IM. Lack of association between tea and cardiovascular disease in college alumni. Int J Epidemiol 2003;32:527–533. [PubMed: 12913023]
42. Vita JA. Polyphenols and cardiovascular disease: effects on endothelial and platelet function. Am J Clin Nutr 2005;81:292S–297S. [PubMed: 15640493]
43. Peters U, Poole C, Arab L. Does tea affect cardiovascular disease? a meta-analysis. Am J Epidemiol 2001;154:495–503. [PubMed: 11549554]
44. Klatsky AL, Friedman GD, Armstrong MA, Kipp H. Wine, liquor, beer, and mortality. Am J Epidemiol 2003;158:585–595. [PubMed: 12965884]
45. Di Castelnuovo A, Rotondo S, Iacoviello L, Donati MB, de Gaetano G. Meta-analysis of wine and beer consumption in relation to vascular risk. Circulation 2002;105:2836–2844. [PubMed: 12070110]
46. Lichtenstein AH, Appel LJ, Brands M, Carnethon M, Daniels S, Franch HA, Franklin B, Kris-Etherton P, Harris WS, Howard B, Karanja N, Lefevre M, Rudel L, Sacks F, Van Horn L, Winston M, Wylie-Rosett J. Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee. Circulation 2006;114:82–96. [PubMed: 16785338]
47. Popkin BM, Armstrong LE, Bray GM, Caballero B, Frei B, Willett WC. A new proposed guidance system for beverage consumption in the United States. Am J Clin Nutr 2006;83:529–542. [PubMed: 16522898]
48. Diaz MN, Frei B, Vita JA, Keaney JF Jr. Antioxidants and atherosclerotic heart disease. N Engl J Med 1997;337:408–417. [PubMed: 9241131]
49. Stein JH, Keevil JG, Wiebe DA, Aeschlimann S, Folts JD. Purple grape juice improves endothelial function and reduces the susceptibility of LDL cholesterol to oxidation in patients with coronary artery disease. Circulation 1999;100:1050–1055. [PubMed: 10477529]
50. MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 20,536 high-risk individuals: a randomised placebo-controlled trial. Lancet 2002;360:23–33. [PubMed: 12114037]
51. Yusuf S, Dagenais G, Pogue J, Bosch J, Sleight P. Vitamin E supplementation and cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. N Engl J Med 2000;342:154–160. [PubMed: 10639540]
52. O'Reilly JD, Mallet AI, McAnlis GT, Young IS, Halliwell B, Sanders TA, Wiseman H. Consumption of flavonoids in onions and black tea: lack of effect on F(2)-isoprostanes and autoantibodies to oxidized LDL in healthy humans. Am J Clin Nutr 2001;73:1040–1044. [PubMed: 11382657]
53. Lotito SB, Frei B. Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: Cause, consequence, or epiphenomenon? Free Rad Biol Med 2006;41:1727–1746. [PubMed: 17157175]
54. Duffy SJ, Keaney JF Jr. Holbrook M, Gokce N, Swerdloff PL, Frei B, Vita JA. Short- and long-term black tea consumption reverses endothelial dysfunction in patients with coronary artery disease. Circulation 2001;104:151–156. [PubMed: 11447078]
55. Widlansky ME, Duffy SJ, Hamburg NM, Gokce N, Warden BA, Wiseman S, Keaney JF Jr. Frei B, Vita JA. Effects of black tea consumption on plasma catechins, markers of oxidative stress and inflammation in patients with coronary artery disease. Free Radic Biol Med 2004;38:499–506. [PubMed: 15649652]
56. Hodgson JM, Puddey IB, Burke V, Watts GF, Beilin LJ. Regular ingestion of black tea improves brachial artery vasodilator function. Clin Sci (Lond) 2002;102:195–201. [PubMed: 11834139]

57. Hodgson JM, Croft KD, Mori TA, Burke V, Beilin LJ, Puddey IB. Regular ingestion of tea does not inhibit in vivo lipid peroxidation in humans. J Nutr 2002;132:55–58. [PubMed: 11773508]
58. Hirata K, Shimada K, Watanabe H, Otsuka R, Tokai K, Yoshiyama M, Homma S, Yoshikawa J. Black tea increases coronary flow velocity reserve in healthy male subjects. Am J Cardiol 2004;93:1384–8. A6. [PubMed: 15165919]
59. Nagaya N, Yamamoto H, Uematsu M, Itoh T, Nakagawa K, Miyazawa T, Kangawa K, Miyatake K. Green tea reverses endothelial dysfunction in healthy smokers. Heart 2004;90:1485–1486. [PubMed: 15547040]
60. Chou EJ, Keevil JG, Aeschlimann S, Wiebe DA, Folts JD, Stein JH. Effect of ingestion of purple grape juice on endothelial function in patients with coronary heart disease. Am J Cardiol 2001;88:553–555. [PubMed: 11524068]
61. Hashimoto M, Kim S, Eto M, Iijima K, Ako J, Yoshizumi M, Akishita M, Kondo K, Itakura H, Hosoda K, Toba K, Ouchi Y. Effect of acute intake of red wine on flow-mediated vasodilatation of the brachial artery. Am J Cardiol 2001;88:1457–60. A9. [PubMed: 11741577]
62. Agewall S, Wright S, Doughty RN, Whalley GA, Duxbury M, Sharpe N. Does a glass of red wine improve endothelial function? Eur Heart J 2000;21:74–78. [PubMed: 10610747]
63. Heiss C, Dejam A, Kleinbongard P, Schewe T, Sies H, Kelm M. Vascular effects of cocoa rich in flavan-3-ols. JAMA 2003;290:1030–1031. [PubMed: 12941674]
64. Heiss C, Kleinbongard P, Dejam A, Perre S, Schroeter H, Sies H, Kelm M. Acute consumption of flavanol-rich cocoa and the reversal of endothelial dysfunction in smokers. J Am Coll Cardiol 2005;46:1276–1283. [PubMed: 16198843]
65. Schroeter H, Heiss C, Balzer J, Kleinbongard P, Keen CL, Hollenberg NK, Sies H, Kwik-Uribe C, Schmitz HH, Kelm M. (-)-Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans. Proc Natl Acad Sci U S A 2006;103:1024–1029. [PubMed: 16418281]
66. Engler MB, Engler MM, Chen CY, Malloy MJ, Browne A, Chiu EY, Kwak HK, Milbury P, Paul SM, Blumberg J, Mietus-Snyder ML. Flavonoid-rich dark chocolate improves endothelial function and increases plasma epicatechin concentrations in healthy adults. J Am Coll Nutr 2004;23:197–204. [PubMed: 15190043]
67. Erdman JW Jr. Balentine D, Arab L, Beecher G, Dwyer JT, Folts J, Harnly J, Hollman P, Keen CL, Mazza G, Messina M, Scalbert A, Vita J, Williamson G, Burrowes J. Flavonoids and Heart Health: Proceedings of the ILSI North America Flavonoids Workshop, May 31-June 1, 2005, Washington, DC. J Nutr 2007;137:718S–737S. [PubMed: 17311968]
68. Widlansky ME, Hamburg NM, Anter E, Holbrook M, Kahn DF, Elliott JG, Keaney JF Jr. Vita JA. Acute EGCG supplementation reverses endothelial dysfunction in patients with coronary artery disease. J Am Coll Nutr 2007;26:95–101. [PubMed: 17536120]
69. Lorenz M, Wessler S, Follmann E, Michaelis W, Dusterhoft T, Baumann G, Stangl K, Stangl V. A constituent of green tea, epigallocatechin-3-gallate, activates endothelial nitric oxide synthase by a phosphatidylinositol-3-OH-kinase-, cAMP-dependent protein kinase-, and Akt-dependent pathway and leads to endothelial-dependent vasorelaxation. J Biol Chem 2004;279:6190–6195. [PubMed: 14645258]
70. Anter E, Thomas SR, Schulz E, Shapira OM, Vita JA, Keaney JF Jr. Activation of eNOS by the p38 MAP kinase in response to black tea polyphenols. J Biol Chem 2004;45:46637–46643. [PubMed: 15333638]
71. Anter E, Chen K, Shapira OM, Karas RH, Keaney JF Jr. p38 mitogen-activated protein kinase activates eNOS in endothelial cells by an estrogen receptor alpha-dependent pathway in response to black tea polyphenols. Circ Res 2005;96:1072–1078. [PubMed: 15879307]
72. Matsuo S, Nakamura Y, Takahashi M, Ouchi Y, Hosoda K, Nozawa M, Kinoshita M. Effect of red wine and ethanol on production of nitric oxide in healthy subjects. Am J Cardiol 2001;87:1029–1031. [PubMed: 11306004]
73. Lekakis J, Rallidis LS, Andreadou I, Vamvakou G, Kazantzoglou G, Magiatis P, Skaltsounis AL, Kremastinos DT. Polyphenolic compounds from red grapes acutely improve endothelial function in patients with coronary heart disease. Eur J Cardiovasc Prev Rehabil 2005;12:596–600. [PubMed: 16319551]

74. Wang-Polagruto JF, Villablanca AC, Polagruto JA, Lee L, Holt RR, Schrader HR, Ensunsa JL, Steinberg FM, Schmitz HH, Keen CL. Chronic consumption of flavanol-rich cocoa improves endothelial function and decreases vascular cell adhesion molecule in hypercholesterolemic postmenopausal women. J Cardiovasc Pharmacol 2006;47(Suppl 2):S177–86. [PubMed: 16794456] discussion S206-9.:S177-S186

75. Vlachopoulos C, Aznaouridis K, Alexopoulos N, Economou E, Andreadou I, Stefanadis C. Effect of dark chocolate on arterial function in healthy individuals. Am J Hypertens 2005;18:785–791. [PubMed: 15925737]

Table 1

Studies of Flavonoid-Containing Foods and Endothelial Function

First Author	Food/Flavonoid	Cohort	Sample size	Method	Result
Duffy [54]	Black tea	CAD	50	Brachial FMD	Improved
Hodgson [56]	Black tea	Hypercholesterolemia	21	Brachial FMD	Improved
Hirata [58]	Black tea	Healthy volunteers	10	Coronary flow reserve	Improved
Nagaya [59]	Green tea	Smokers	20	Reactive hyperemia	Improved
Agewall [62]	Dealcoholized red wine	Healthy volunteers	12	Brachial FMD	Improved
Matsuo [72]	Red wine and polyphenol extract	Healthy volunteers	6	Plasma nitric oxide	Improved
Stein [49]	Purple grape juice	CAD	15	Brachial FMD	Improved
Chou [60]	Purple grape juice	CAD	22	Brachial FMD	Improved
Lekakis [73]	Red grape extract	CAD	30	Brachial FMD	Improved
Heiss [63]	Flavanol-rich cocoa	At least one risk factor	26	Brachial FMD	Improved
Heiss [64]	Flavanol-rich cocoa	Smokers	11	Brachial FMD	Improved
Schroeter [65]	Flavanol-rich cocoa Pure EC	Health volunteers Healthy volunteers	16 6	Brachial FMD & Fingertip tonometry	Improved Improved
Wang-Polagruto [74]	Flavanol-rich cocoa	Post-menopausal hypercholesterolemic women	32	Reactive hyperemia	Improved
Engler [66]	Flavonoid-rich dark chocolate	Healthy volunteers	21	Brachial FMD	Improved
Vlachopoulos [75]	Dark chocolate	Healthy volunteers	17	Brachial FMD Augmentation index	Improved
Widlansky [68]	Acute EGCG	CAD	42	Brachial FMD	Improved

FMD = flow-mediated dilation, CAD = coronary artery disease