Tea, flavonoids, and cardiovascular health: endothelial protection[1–4]

Davide Grassi, Giovambattista Desideri, Paolo Di Giosia, Martina De Feo, Emanuela Fellini, Paola Cheli, Livia Ferri, and Claudio Ferri

ABSTRACT

Several studies have suggested that tea consumption might protect against the development and progression of cardiovascular disease, one of the leading causes of morbidity and mortality worldwide. The endothelium plays a pivotal role in arterial homeostasis. Reduced nitric oxide (NO) bioavailability with endothelial dysfunction is considered the earliest step in the pathogenesis of atherosclerosis. Endothelial dysfunction has been considered an important and independent predictor of future development of cardiovascular risk and events. The association between brachial NO-dependent flow-mediated dilation (FMD) and cardiovascular disease risk has been investigated in several prospective studies, suggesting that FMD is inversely associated with future cardiovascular events. Dietary flavonoids and tea consumption have been described to improve endothelial function and FMD. A proposed mechanism by which dietary flavonoids could affect FMD is that they improve the bioactivity of the endothelium-derived vasodilator NO by enhancing NO synthesis or by decreasing superoxide-mediated NO breakdown. This could be of clinical relevance and may suggest a mechanistic explanation for the reduced risk of cardiovascular events and stroke observed among tea drinkers in the different studies. The purpose of this article is to provide an overview of the relation between tea consumption and cardiovascular disease, with a focus on clinical implications resulting from the beneficial effects of tea consumption on endothelial function. *Am J Clin Nutr* 2013;98(suppl):1660S–6S.

INTRODUCTION

Cardiovascular disease is a leading cause of death worldwide (1). The majority of cardiovascular disease results from complications of atherosclerosis (1). The structural integrity and functional activity of the endothelium play a pivotal role in atherogenesis and related adverse outcomes. The endothelium is the common target of all cardiovascular risk factors, and functional impairment of the vascular endothelium in response to injury occurs long before the development of visible atherosclerosis. Cardiovascular risk conditions contribute to oxidative stress, which causes a disruption in the balance between nitric oxide (NO)[5] and reactive oxygen species, with a resulting relative decrease in NO bioavailability (2). Smoking, hypertension, hypercholesterolemia, diabetes, obesity, social deprivation, physical activity, and dietary habits are recognized risk factors for cardiovascular disease and endothelial dysfunction (2, 3). Furthermore, changes in nutritional habits of many populations have been considered, among others, to be responsible for increased cardiovascular disease incidence (3). The Framingham Heart Study first identified the contribution of diet and sedentary lifestyles to the risk of cardiovascular disease (4). According to this evidence, Lim et al (5) recently reported that in 2010 the 3 leading risk factors for global disease burden were high blood pressure, tobacco smoking, and alcohol use, whereas dietary risk factors and physical inactivity collectively accounted for 10% of global deaths and disability-adjusted life-years, with the most prominent dietary risks being diets low in fruit and those high in sodium (5). To date, in addition to maintaining a healthy body weight, numerous guidelines increasingly suggest dietary recommendations including the consumption of a wide variety of plant-based foods. These recommendations are based on the observation that high intakes of fruit and vegetables are associated with a decreased risk of cardiovascular disease, stroke, and hypertension (3–5).

Evidence from epidemiologic studies and randomized controlled trials together with in vitro data on vascular bioactivity support a potential role for some flavonoids in the reduction of cardiovascular risk (6, 7). Flavanols (flavan-3-ols), such as epicatechin and catechin, and their oligomers, the procyanidins, represent a major class of flavonoids widely distributed in plants. They are found in high concentrations in certain foods, such as tea, cocoa, and grapes. The profile of flavonoids in these foods is

[1] From the Department of Life, Health, and Environmental Sciences, University of L'Aquila, L'Aquila, Italy (DG, GD, PDG, MDF, EF, PC, and CF); and University "Sapienza"–II Faculty of Medicine, Rome, Italy (LF).

[2] Presented at the conference "Fifth International Scientific Symposium on Tea and Human Health," held at the US Department of Agriculture, Washington, DC, 19 September 2012. The conference was organized by Jeffrey Blumberg, Tufts University, Boston, MA, and a Steering Committee including representatives from each of the symposium cosponsors: the American Cancer Society, the American College of Nutrition, the American Institute for Cancer Research, the American Medical Women's Association, the American Society for Nutrition, and the Linus Pauling Institute. The symposium was underwritten by the Tea Council of the USA. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the Tea Council of the USA or the cosponsoring organizations.

[3] This symposium was supported by the American Cancer Society, the American College of Nutrition, the American Institute for Cancer Research, the American Medical Women's Association, the American Society for Nutrition, the Linus Pauling Institute, the University of Western Australia, and the Unilever Food and Health Research Institute.

[4] Address correspondence to D Grassi, University of L'Aquila, Department of Life, Health, and Environmental Sciences, Viale S Salvatore, Delta, 6 Medicina, 67100 Coppito, L'Aquila, Italy. E-mail: davide.grassi @cc.univaq.it.

[5] Abbreviations used: cGMP, cyclic guanosine-5'-monophosphate; EGCG, epigallocatechin-3-gallate; eNOS, endothelial nitric oxide synthase; FMD, flow-mediated dilation; NO, nitric oxide.

First published online October 30, 2013; doi: 10.3945/ajcn.113.058313.

variable, and it can be modified as a consequence of food processing (8). In this context, green and black teas have received much attention, because tea leaves contain a large amount of polyphenols (~30% in the dried tea leaves), mainly flavonoids. The major class of flavonoids in tea are flavanols, which include catechin, epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin-3-gallate (EGCG) (8–10). Total polyphenol content is similar in different types of tea, but the individual components vary, based in part on the degree of polyphenol oxidation during the manufacturing process. Catechins represent ~80–90% and flavanols ~10% of the total flavonoids in green tea, whereas theaflavins account for 50–60% and catechins only 20–30% of total flavonoids in black tea (8, 9, 11). Moreover, in addition to flavonoids, tea contains other potentially relevant active substances that positively affect vascular function. In this regard, Siamwala et al (12) showed that L-theanine administration in vitro enhanced NO production and thereby vasodilation in the arteries, suggesting that L-theanine might mediate the vascular health benefits of tea. There has been considerable interest in the possibility that the consumption of tea reduces the risk of cardiovascular disease (13–15). A wide range of biological actions of flavanol-rich foods supports the potential cardiovascular protection (8, 9). A suggested mechanism that may partly explain these effects on cardiovascular protection is the direct effect of tea on the vasculature, particularly on the endothelium (8, 9, 15). The purpose of this article is to provide an overview of the relation between tea consumption and cardiovascular disease, particularly focusing on clinical implications that result from the beneficial effects of tea consumption on endothelial function.

EVIDENCE FROM EPIDEMIOLOGIC STUDIES

A number of studies have examined the relation between tea and flavonoid consumption and cardiovascular risk. In this regard, the prospective Zutphen Elderly Study (5-y follow-up of a cohort of 805 men aged 65–84 y) reported that flavonoid intake was inversely associated with mortality from coronary heart disease and also reported a trend toward an inverse relation with the incidence of myocardial infarction (16). This prospective cohort study showed a marked reduction in cardiovascular risk in the tertile of subjects with the highest flavonoid consumption compared with individuals in the lowest tertile. Specifically, authors showed that individuals consuming >29 mg flavonoids/d had a 68% reduction in cardiovascular risk, after adjustment for other known risk factors (age, BMI, smoking, total and HDL cholesterol, blood pressure, physical activity, coffee consumption, and intakes of energy, vitamin C, vitamin E, β-carotene, and dietary fiber) (16). In keeping with this, Hertog et al (17) also showed an inverse relation between flavonoid intake and coronary heart disease mortality after the 25-y follow-up of 12.763 men from the 16 cohorts of the Seven Countries Study. Of note, flavonoid intake explained 25% of the variance in coronary heart disease among cohorts (17). Of particular interest, the major source (61%) of flavonoid intake in the Zutphen Elderly Study was represented by black tea (16). Thus, it is conceivable that tea ingestion significantly contributed to the observed cardiovascular protection exerted by a high flavonoid intake. In the Rotterdam Study (18), an increased intake of flavonoids (mainly derived from black tea) in 4807 subjects with no history of myocardial infarction contributed in reducing the relative risk of a first myocardial infarction. Flavonoid intake was negatively associated with the occurrence of fatal rather than nonfatal myocardial infarction (18). Similarly, a prospective cohort study in 1900 patients [1019 consuming no tea (nondrinkers), 615 consuming <14 cups/wk (moderate tea drinkers), and 266 consuming ≥14 cups/wk (heavy tea drinkers)] hospitalized with a confirmed acute myocardial infarction reported a 31% and 39% reduction in age- and sex-adjusted cardiovascular mortality in moderate and heavy tea drinkers, respectively. Additional adjustment for clinical and sociodemographic characteristics did not change this association. Furthermore, the association of tea and mortality was similar for total and cardiovascular mortality (19). This finding suggested that tea can also reduce vascular events in secondary prevention. Indeed, in the same Determinants of Myocardial Infarction Onset Study, Mukamal et al (20) reported a lower prevalence of ventricular arrhythmia during hospitalization for myocardial infarction among tea drinkers, possibly indicating less severe infarction and providing a potential explanation for the reduction in cardiovascular mortality observed during subsequent follow-up. Moreover, Sesso et al (21) in the case-control Boston Area Health Study examined the relation between tea and coffee consumption and myocardial infarction. They evaluated tea and coffee intake by questionnaire in 340 subjects and 340 matched controls and observed a 44% reduction in risk of myocardial infarction in individuals who drank >1 cup of tea/d compared with those who drank no tea. Of note, the effects of tea were independent of other established coronary risk factors, and there was no significant relation between coffee consumption and cardiovascular disease. A study from a Norwegian cohort, conducted in 9856 men and 10,233 women without a history of cardiovascular disease or diabetes from the county of Oppland, showed that deaths from coronary heart disease and all-cause mortality rates were both slightly lower among persons drinking ≥1 cup of tea/d compared with persons drinking no tea or <1 cup of tea/d (22). In this study, Stensvold et al (22) also observed significant inverse correlations between tea consumption and blood pressure and cholesterol concentrations (which were lower among individuals consuming ≥1 cup/d). Consistent with this finding, de Koning Gans et al (23) evaluated the associations of coffee and tea consumption with risk of morbidity and mortality of stroke and coronary heart disease and with all-cause mortality in a large cohort of 37,514 participants in The Netherlands. After a 13-y follow-up, they observed that tea consumption (mainly black tea; 3–6 cups daily) was inversely associated with coronary heart disease, with the lowest HR for >6 cups/d. However, no association between tea consumption and all-cause mortality was reported. In that study, tea drinking was also found to be associated with a higher educational level, higher physical activity level, a healthier diet, and a lower prevalence of smoking, hypercholesterolemia, and diabetes (23). With regard to green tea, a Japanese study in 8552 men and women showed a 28% reduction in deaths from cardiovascular disease in participants consuming >10 cups/d compared with those consuming <3 cups/d (24). The Ohsaki National Health Insurance Cohort Study (25), which followed 40,530 Japanese adults for 11 y, showed that green tea consumption was inversely associated with mortality due to all causes and due to cardiovascular disease. Furthermore, the authors reported a dose

relation between increasing green tea consumption and reduced total and cardiovascular mortality. The inverse association with all-cause mortality was stronger in women. The inverse association with cardiovascular disease mortality was stronger than that with all-cause mortality. Stroke mortality was reported to be particularly reduced in tea drinkers (with the strongest inverse association) (25). In agreement with the above findings, Liang et al (26) in a case-control study conducted in southern China in 374 patients with incident ischemic stroke observed a significant decrease in ischemic stroke risk for drinking at least 1 cup of tea weekly when compared with infrequent or nondrinkers; the largest risk reduction was found in those who drank 1 to 2 cups of green or oolong tea daily. Significant inverse dose-response relations were also reported for years of drinking and the amount of dried tea leaves brewed (26). Furthermore, a recent study in 76,979 Japanese adults examined the relation between cardiovascular mortality and the consumption of green, black, and oolong teas (27). The authors observed that compared with nondrinkers of green tea, the risk of coronary heart disease among women drinking 1–6 cups/wk, 1–2 cups/d, 3–5 cups/d, and ≥6 cups/d decreased by 66%, 72%, 61%, and 58%, respectively. With regard to oolong tea, they also reported that consumption of >1 cup of oolong tea/d was associated with reduced risk of total cardiovascular disease among men (27).

Supporting this, Kokubo et al (28) investigated the association between green tea intake and stroke incidence in a general population (82,369 Japanese aged 45–74 y) and showed that compared with seldomly drinking green tea, the multivariable-adjusted HRs (95% CIs) of all strokes were 0.86 (0.78, 0.95) and 0.80 (0.73, 0.89) for consumption of 2–3 and ≥4 cups green tea/d, respectively. Moreover, higher green tea consumption was associated with inverse risks of cardiovascular disease and stroke subtypes (28). Consistent with this finding, a recent study performed in Sweden (29), after a mean follow-up of 10.2 y, reported that high tea consumption was associated with a significantly lower risk of total stroke, without observing a dose-response relation. Compared with no tea consumption, the multivariable RR for ≥4 cups/d was 0.79 (95% CI: 0.62, 0.998). The corresponding RRs were 0.80 (95% CI: 0.61, 1.04) for cerebral infarction and 0.68 (95% CI: 0.35, 1.30) for hemorrhagic stroke.

To explain the observed discrepancy and the possible potential confounding factors, including lifestyle pattern as well as baseline tea intake in the different national cohorts, the wide dose range of flavonoids, and the different kinds of tea flavonoid ingested, Peters et al (13) performed a meta-analysis investigating the relation between tea consumption with coronary heart disease, myocardial infarction, and stroke on the basis of 10 cohort and 7 case-control studies (even including negative studies). The authors estimated that the incidence rate of myocardial infarction decreased by 11% with consumption of 3 cups of tea/d. However, some caution was suggested by the authors in the interpretation of the results, particularly for a specific geographic heterogeneity. Studies on coronary heart disease and myocardial infarction conducted in continental Europe reported much stronger inverse associations than did studies conducted elsewhere (13). Of interest, these findings were not specific to green or black tea, but most of the tea consumed in the populations included in this analysis was black tea (13). Again, a recent meta-analysis that considered data from 9 studies involving 4378 strokes among 194,965 individuals showed that, regardless of their country of origin, individuals consuming ≥3 cups of tea/d had a 21% lower risk of stroke than did those consuming <1 cup/d (14).

On the other hand, a more recent meta-analysis did not suggest a substantial association between black tea and coronary artery disease risk and showed only limited evidence for an association of green tea and coronary artery disease (an increase in green tea consumption of 1 cup/d was associated with a 10% decrease in risk) (30). Similarly, the most recent meta-analysis aiming to define the association between tea consumption and the risk of stroke reported a modest but significant inverse association between tea consumption and risk of stroke. In particular, an increase of 3 cups/d in tea consumption was associated with a 13% decreased risk of stroke. Nevertheless, there was substantial heterogeneity in the results, with a substantial proportion of studies that did not suggest an association (31).

ENDOTHELIAL DYSFUNCTION AND CARDIOVASCULAR DISEASE

The vascular endothelium is probably the most extensive tissue in the body, and its continuous smooth and nonthrombogenic surface forms a highly selective impermeable barrier. A single layer of endothelial cells lines the entire vascular system, and normality of both endothelial cell structure and functions is of great importance in the maintenance of vessel wall integrity (2).

In this regard, endothelial cells actively regulate vascular reactivity by responding to mechanical forces and neurohormonal mediators with the release of a variety of relaxing and contracting factors (2, 32–34). Endothelial synthesis and the release of vasoactive mediators have been widely recognized as one of the main mechanisms involved in the regulation of vascular tone (2, 32–34). The most important endothelium-derived product is represented by NO, an endogenous gas that is synthesized by endothelial NO synthase (eNOS) starting from L-arginine. Although NO was first described with regard to its vasodilator effects, it is now clear that it has other very important and relevant effects on vascular homeostasis. NO is an antiinflammatory compound that prevents adherence of leukocytes to the endothelial surface. It is an antithrombotic compound that prevents platelet adhesion and platelet aggregation. NO inhibits the proliferation of vascular smooth muscle cells and the formation of other noncellular components that comprise the matrix of the vascular wall and thus is relevant to lesion formation and vascular compliance (2, 32–35). NO not only affects conduit arteries where lesions develop but also affects microvessels regulating blood flow to tissues (2, 32–34). Therefore, an impairment of NO formation would have adverse effects on the cardiovascular system. When produced in appropriate amounts, NO is an antiatherosclerotic, antithrombotic, and vasodilator molecule. NO is constitutively generated in endothelial cells from its precursor L-arginine by the action of eNOS (converting L-arginine to citrulline) in the presence of cofactors such as tetrahydrobiopterin (2, 32–34). NO diffuses to the vascular smooth muscle cells and activates the soluble guanylate cyclase, which leads to cyclic guanosine-5′-monophosphate (cGMP)–mediated vasodilatation. cGMP acts as a second messenger, leading to many of the biological effects of NO such as relaxation of smooth muscle and inhibition of platelet aggregation

(32–34). Shear stress is a key factor for eNOS activation in normal physiology, and this adapts organ perfusion to changes in cardiac output. Under homeostatic conditions, the endothelium maintains normal vascular tone and blood flow, and there is little or no expression of proinflammatory factors. In normal vascular physiology, NO favors the maintenance of the vascular wall in a quiescent state by inhibiting inflammation, cellular proliferation, and thrombosis (2, 32–34). The endothelium maintains vascular homeostasis through multiple complex interactions with cells in the vessel wall and lumen. Furthermore, a "healthy" endothelium maintains vascular tone and structure by regulating the balance between vasodilation and vasoconstriction (2, 32–34). On the contrary, in pathologic conditions, particularly in the presence of cardiovascular risk factors, the endothelium undergoes functional and structural alterations, thus losing its protective role and becoming a proatherosclerotic structure (2, 32–34). In the earliest stages, the principal endothelial alteration is merely functional and is defined as "endothelial dysfunction." The fundamental feature of this condition is impaired NO bioavailability leading to the loss of normal endothelium-dependent and NO-mediated vasodilation in the arteries (32–34). This can be the consequence of either a lowered production by eNOS or, more frequently, of an increased breakdown by reactive oxygen species (2, 32–34). Furthermore, it is interesting to note that eNOS, which normally helps maintain the quiescent state of the endothelium, can switch to generate reactive oxygen species in certain circumstances as part of endothelial dysfunction (2, 31–33). In this case, eNOS itself can paradoxically produce superoxide, a process referred to as "eNOS uncoupling." Reduced concentrations of tetrahydrobiopterin or L-arginine lead to uncoupling of reduced NADPH oxidation and NO synthesis, with oxygen as the terminal electron acceptor instead of L-arginine, resulting in the generation of superoxide by eNOS (2, 32–34). Starting from this evidence, we could affirm that intact function and integrity of the endothelium might play a pivotal role in cardiovascular health. In patients at high cardiovascular risk, the decline in endothelial NO bioavailability is attributed to the following factors: *1*) the reduced expression of eNOS, *2*) the deficiency of substrate or cofactors for eNOS and a deficient activation of eNOS caused by impaired cellular signaling, *3*) the decreased capacity of endothelial cells to synthesize and/or release NO, or *4*) the inactivation of synthesized NO by reactive oxygen species (2, 32–34). All of these abnormalities might promote endothelial dysfunction, which is considered the earliest step in the pathogenesis of atherosclerosis. Endothelial dysfunction seems to fuel a ''vicious circle'' in which inflammatory factors promote monocyte and T cell adhesion, foam cell formation, extracellular matrix digestion, as well as vascular smooth muscle migration and proliferation that lead to atherosclerotic plaque formation (2, 32–34). Many cardiovascular risk factors, such as hypercholesterolemia, diabetes, hypertension, aging, smoking, and hyperglycemia, have been shown to be accompanied by endothelial dysfunction (2, 32–35). It has been suggested that this endothelium-dependent vascular imbalance is critical, not only in the initiation and progression of atherosclerosis but also in the transition from a stable to an unstable disease state with the precipitation of acute vascular events (2, 32–35). Furthermore, a dysfunctional endothelium may favor plaque activation, promoting greater plaque vulnerability, thus successively inducing plaque destabilization and rupture (2). Endothelial dysfunction is also relevant to the later stages of the disease and seems to play a role in acute coronary syndromes (35–37). As a consequence, reversal of endothelial dysfunction has been suggested to slow down atherogenesis and improve individual cardiovascular prognosis (2, 32–35).

Endothelial dysfunction can be determined by assessing the degree of flow-mediated dilation (FMD) of the brachial artery (35–38). FMD represents the endothelium-dependent relaxation of the artery, mediated via NO release, in response to a hyperaemic stimulus (increased flow established by the release of a suprasystolic inflated cuff around the arm) and is seen as a direct and reliable measure of vascular reactivity of the macrocirculatory system (35–38). Although it is clear that study of FMD in the arm is less relevant than studies in the coronary circulation, a number of studies have shown that responses in the arm are clinically relevant (36, 37). In particular, Anderson et al (39) reported that endothelial dysfunction in the brachial artery presented a high predictive value for abnormal endothelial function in the coronary circulation, as assessed by intracoronary acetylcholine infusion. Several recent studies have shown that impaired brachial artery FMD identifies patients at increased risk of cardiovascular disease events (35–37). According to this evidence, the association between brachial FMD and cardiovascular disease risk has been investigated in several prospective studies. The majority of these suggest that FMD is inversely associated with future cardiovascular events (36, 37). In this regard, Inaba et al (36), summarizing the evidence of 14 prospective studies, observed that with a 1% higher FMD, the risk of experiencing a cardiovascular event is 13% lower. A very recent meta-analysis, evaluating a total of 23 prospective studies (including 14,753 subjects) that investigated the association between brachial FMD and future cardiovascular events, showed that brachial FMD is inversely associated with future cardiovascular events (37). In particular, for studies reporting continuous risk estimates, the pooled overall cardiovascular disease risk was 8% lower with a 1% higher FMD. The observed association was stronger in diseased populations than in asymptomatic populations (lower by 13% and 4%, respectively, with a 1% higher FMD) (37). Ras et al (37) also concluded that endothelial dysfunction may be considered relevant for classifying subjects in terms of cardiovascular disease risk.

Consistent with this and of particular interest, only 2 studies (40, 41) evaluated the prognostic role of reversible endothelial dysfunction. In particular, Modena et al (40) in a group of postmenopausal hypertensive women with impaired endothelial function, evaluated by brachial artery FMD, reported that, after a 5-y follow-up of antihypertensive therapy, the subgroup who experienced amelioration of endothelial function within 6 mo from the onset of treatment presented with a significantly better cardiovascular prognosis compared with the subgroup without improvement in FMD (40). Patients had increased risk over the next 5 y when endothelial dysfunction was not reversed by 6 mo of antihypertensive therapy. Although treatment was not standardized, the type of antihypertensive therapy or the degree of blood pressure lowering did not explain the difference in prognosis (40). Concordantly, Kitta et al (41) showed that persistent impairment of FMD (after 6 mo of individualized and optimized therapy to reduce risk factors) was an independent predictor of events in patients with newly diagnosed coronary artery disease.

These results indicated that the amelioration of endothelial dysfunction might be considered as a potential target to reduce cardiovascular risk and as a screening test for the primary prevention of cardiovascular disease as well as as a guide to therapy.

TEA, FLAVONOIDS, AND ENDOTHELIAL FUNCTION

Recently, there have been several experimental and clinical studies showing beneficial effects of tea on endothelial function, particularly on endothelium-dependent vasodilation (8, 9).

With regard to experimental evidence, a number of studies reported that plant polyphenols significantly increased endothelium-dependent vasodilation (8, 9). Furthermore, we recently observed that EGCG and epicatechin induced a concentration-dependent vasorelaxation in phenylephrine, precontracted, endothelium-intact preparations of rat isolated aortic rings. Supporting the important role played by flavanols on endothelium/NO mechanisms involved in the regulation of arterial basal tone and in both mediating vasorelaxation and counteracting vasoconstriction, we also observed that EGCG and epicatechin did not significantly affect vasorelaxation in precontracted endothelium-denuded preparations (42). Consistent with this, in endothelium-intact precontracted preparations, Nω nitro-L-arginine, an inhibitor of eNOS activity, abolished the vasorelaxant effect of EGCG and epicatechin. At high concentrations, EGCG and epicatechin elicited a marked relaxation. This was significantly larger in the presence than in the absence of endothelium or in the presence of Nω nitro-L-arginine (42). With respect to tea, Anter et al (43) showed that black tea and black tea polyphenols promoted both eNOS catalytic activity and increased NO bioavailability in cultured vascular endothelial cells. Of particular interest, the authors reported dose- and time-dependent effects of black tea on endothelial NO production by the increase in cellular cGMP content (43). Black tea–induced cGMP accumulation appeared to result from eNOS activation, because it was also characterized by the enhanced conversion of L-[^{3}H]arginine to L-[^{3}H]citrulline and was sensitive to the NOS inhibitor L-nitroarginine methyl ester (L-NAME) (43). On the contrary, treatment with the inactive enantiomer, D-NAME, had no effect on black tea–induced eNOS activation. The effect of black tea was entirely reproduced by the polyphenolic fraction over a physiologic concentration range. Black tea enhanced eNOS activity by 4- to 5-fold (43). Jochmann and Lorenz (44) also reported that both green and black teas significantly increased eNOS activity in bovine aortic endothelial cells. Similar findings were described in rat aortic rings (44). Confirming this, an interesting study by Lorenz et al (45) showed that both teas stimulated eNOS activity and phosphorylation in bovine aortic endothelial cells as well as vasorelaxation in rat aortic rings to a similar extent. In green tea, only EGCG resulted in pronounced NO production and NO-dependent vasorelaxation in aortic rings. During tea processing to produce black tea, the catechins are converted to theaflavins and thearubigins. Individual black tea theaflavins showed a higher potency than EGCG in NO production and vasorelaxation (45). The thearubigins in black tea were highly efficient stimulators of vasodilation and NO production. Green and black tea compounds induced comparable phosphorylation of eNOS and upstream signaling kinases. These results suggested that highly fermented black tea is equally potent as green tea in promoting beneficial endothelial effects. Theaflavins and thearubigins predominantly counterbalanced the lack of catechins in black tea in favoring endothelial beneficial effects.

Many mechanisms of action have been proposed on the basis of in vitro models. However, the importance of most of these mechanisms remains to be determined in vivo. In particular, the bioavailability and biotransformation of tea catechins play a key role in determining the importance of various mechanisms in vivo. Likewise, the biological activity and bioavailability of tea catechin metabolites are important in understanding the potential beneficial effects of tea. Several processes, including intestinal metabolism, microbial metabolism, hepatic metabolism, and chemical degradation, have been found to be involved in the fate of green tea and to be responsible for the low availability of green tea metabolites in animals and most likely also in humans (46). The fact that catechins are rapidly and extensively metabolized emphasizes the importance of showing their specific activity in vivo. In humans, modest transient increases in plasma antioxidant capacity have been observed after the consumption of tea and green tea catechins (46). After multiple doses of black tea, Warden et al (47) showed a significant increase in plasma, urine, and fecal concentrations over baseline concentrations. Moreover, the authors found only 1.68% of the total catechins consumed (400 mg) in the plasma, urine, and feces, providing further evidence to support the hypothesis that catechins undergo considerable metabolism and/or degradation either in the gastrointentinal tract or in the body after absorption. The apparent bioavailability of the gallated catechins was lower than the nongallated forms. Thus, catechins were bioavailable. However, unless they are rapidly metabolized or sequestered, the catechins appeared to be absorbed in amounts that were small relative to intake.

In in vivo studies, a large body of evidence suggests that ingestion of flavonoids and flavonoid-rich products can improve endothelial function in humans as reported by a significant improvement in NO-dependent FMD (8). Specifically, with regard to tea consumption, a study in healthy women (44) showed that ingestion of green and black teas led to significant increments in brachial artery FMD (from a baseline of 5.4 $\pm$ 2.3% to 10.2 $\pm$ 3% 2 h after green tea consumption and from a baseline of 5 $\pm$ 2.6% to 9.1 $\pm$ 3.6% 2 h after black tea consumption; $P <$ 0.001). The observed increments in FMD were not significantly different between the black and green tea preparations (44). Accordingly, our research group (48) reported that black tea (containing increasing doses of flavonoids but very similar amounts of caffeine) consumption dose-dependently improved endothelial function in healthy men. Black tea increased FMD from 7.8% (control) to 9.0%, 9.1%, 9.6%, and 10.3% after the different flavonoid doses (100, 200, 400, and 800 mg tea flavonoids/d), respectively ($P = 0.0001$). Of interest, even 100 mg/d ($<$1 cup of tea) increased FMD compared with control ($P = 0.0113$). Furthermore, FMD after 800 mg/d was significantly higher than after the control ($P < 0.0001$) but was also higher than after administration of 100 mg/d ($P = 0.0121$) and 200 mg/d ($P = 0.0275$) (48). In addition, Duffy et al (49) showed that both acute and prolonged black tea ingestion (either 450 mL of black tea given as a single dose or 900 mL of black tea/d given over a period of 4 wk) significantly improved FMD in patients with coronary artery disease. Of particular relevance, the positive effects of tea on FMD were associated with increased plasma flavonoid concentrations, thereby strongly suggesting

that black tea consumption markedly improved NO bioavailability in vivo because of its high flavonoid content. In a randomized controlled parallel study, Hodgson et al (50) showed a significant and consistent increase in endothelium-dependent vasodilation after regular consumption of 5 cups (250 mL each) of black tea/d, given over a period of 4 wk in mildly dyslipidemic subjects. In agreement, Ardalan et al (51) observed that short-term consumption of black tea significantly improved endothelial function in renal transplant recipients. Thus, these findings suggest that tea consumption could improve endothelial function in healthy subjects as well as restore endothelial dysfunction in patients at high cardiovascular risk. Finally, after a review of all the reported evidence, a recent meta-analysis by Ras et al (15) (considering 9 studies from different research groups with 15 relevant study arms; 7 studies investigated the effect of black tea; only 3 studies investigated green tea) showed that the overall absolute increase in FMD after tea compared with placebo was 2.6% of the arterial diameter ($P < 0.001$) for a median daily dose of 500 mL of tea (2–3 cups). This is a relative increase of ~40% compared with the average FMD of 6.3% under placebo (for crossover trials) or baseline (for parallel trials) conditions. The authors also reported a significant heterogeneity between studies partially explained by the cuff position being either distal or proximal to the area of FMD measurement (15). However, some key limitations should be considered with regard to the experimental design generally proposed in the studies performed until now. The lack of a proper control—ie, the blinding and the absence of a standardized control product with regard to the flavonoid content—and an inconsistent range of designs in terms of the duration of study periods (with a limited duration in most of the trials) as well as the dose of polyphenols investigated. Another barrier is the question of the specific population studied [in 5 of 9 studies considered by Ras et al (15), subjects were healthy or mildly hypercholesterolemic; the other studies included renal transplant recipients, chronic kidney disease patients, or patients with coronary artery disease].

CONCLUSIONS AND FUTURE DIRECTIONS

The endothelium plays a pivotal role in arterial homeostasis. Endothelial dysfunction is considered the earliest step in the pathogenesis of atherosclerosis (2). Endothelial dysfunction has been considered an important and independent predictor of future development of cardiovascular risk and events (35–37). The association between brachial FMD and cardiovascular disease risk has been investigated in several prospective studies. The majority of these suggest that FMD is inversely associated with future cardiovascular events (37–41).

Dietary flavonoids and tea consumption have been described to improve endothelial function and FMD. A proposed mechanism by which dietary flavonoids could affect FMD is that they improve the bioactivity of the endothelium-derived vasodilator NO by enhancing NO synthesis or by decreasing superoxide-mediated NO breakdown (8, 9). Nevertheless, different additional mechanisms could be involved, and the specific mechanism has not yet been fully clarified. Furthermore, different potentially active flavonoids and their metabolites may have different effects (8, 9).

The clinical relevance of the endothelium-dependent effects of tea consumption is yet to be completely clarified. Moreover, to establish the effects of tea and tea flavonoids, large-scale, long-term, well-controlled trials, preferably with clinical endpoints including cardiovascular morbidity/mortality, seem warranted. Nevertheless, all of the available evidence suggests that moderate consumption of tea substantially enhances endothelium-dependent vasodilation. This might indicate a mechanistic explanation for the modified risk of cardiovascular events and stroke observed among tea drinkers in the different studies. In further support of the clinical relevance of the reported findings, the Italian guidelines for the prevention and treatment of stroke [SPREAD (Stroke Prevention and Educational Awareness Diffusion)] (52) as well as the American College of Cardiology Foundation Task Force (53) suggest that moderate amounts (1–2 cups/d) of tea in addition to general nutritional advice could be useful for cardiovascular risk reduction. Consistent with this, a proposed guidance system for beverage consumption in the United States (54) suggests unsweetened tea (with caffeine not to exceed 400 mg/d) as one among various healthful beverage choices.

The authors' responsibilities were as follows—DG, GD, and CF: data acquisition and writing of the draft of the manuscript; and all authors: conception of the review and critical revision of the manuscript. None of the authors had a personal or financial conflict of interest.

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