Review

Dietary polyphenols regulate endothelial function and prevent cardiovascular disease

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\textbf{A B S T R A C T}

Vascular endothelial cell (EC) dysfunction strongly induces development of cardiovascular and cerebrovascular diseases. Epidemiologic studies demonstrated a preventative effect of dietary polyphenols toward cardiovascular disease. In studies using cultured vascular ECs, polyphenols were recognized to regulate nitric oxide and endothelin-1 (ET-1) production. Furthermore, epigallocatechin-3-gallate inhibited the expression of adhesion molecules by a signaling pathway that is similar to that of high-density lipoprotein and involves induction of Ca\textsuperscript{2+}/calmodulin-dependent kinase II, liver kinase B, and phosphatidylinositol 3-kinase expression. The effects of polyphenols on ECs include antioxidant activity and enhancement of the expression of several protective proteins, including endothelial nitric oxide synthase and paraoxonase 1. However, the observed effects of dietary polyphenols in vitro do not always translate to an in vivo setting. As such, there are many questions concerning their physiological mode of action. In this review, we discuss research on the effect of dietary polyphenols on cardiovascular disease and their protective effect on EC dysfunction.

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\textbf{Introduction}

Polyphenols are found in a variety of foods, including fruits, vegetables, soybeans, tea, wine, and cocoa. These foods are thought to have a preventative effect on the development of cardiovascular disease (CVD)\textsuperscript{[1]}, which is supported by the so-called “French paradox,” wherein, despite their consumption of large amounts of milk fat that is associated with higher rates of CVD-related death, the French CVD mortality rate is lower, presumably due to their higher rates of wine intake compared with other European countries. Taking the French paradox into consideration, polyphenol and epidemiologic studies exploring disease development carried out in several countries found that CVD mortality rates were inversely related to dietary polyphenol intake\textsuperscript{[1–4]}. Although the French paradox generally is considered related to the consumption of red wine, a recent study indicated that the French paradox also applies to Mediterranean regions\textsuperscript{[5]}. Furthermore, red wine consumption alone cannot explain the French paradox, so other components of the typical French diet could be responsible for the decreased CVD mortality seen in these regions\textsuperscript{[6,7]}. In epidemiologic studies, polyphenols inhibited vascular system cell injury, including defects in vascular endothelial cell (EC) functions, indicating that polyphenols may have a preventative effect on CVD\textsuperscript{[8]}. Polyphenol molecules and components typically carry several hydroxyl groups and more than 4000 to 7000 varieties are present in plants. Polyphenols can be categorized into flavonoids and non-flavonoids (Fig. 1)\textsuperscript{[9]}. The flavonoid group has a phenyl chroman frame (C 6-C 3-C6), and based on differences in side-chain structures can be classified into flavones (e.g., apigenin, luteolin), isoflavones (e.g., genistein, daidzein), flavanones (e.g., hesperidin, naringenin), flavonols (e.g., quercetin, myricetin, catechin, and epigallocatechin), and anthocyanins (e.g., delphinidin, malvidin) (Fig. 2)\textsuperscript{[9]}. Typical non-flavonoids include tannins, chlorogenic acid, gallic acid, caffeic acid, curcumin, and resveratrol.

In vitro studies, protective actions of polyphenols toward ECs and vascular smooth muscle cells (SMCs) have been demonstrated. These reports indicated that polyphenols help maintain normal EC functions, and contribute to a critical inhibition of CVD. Polyphenols have an antioxidant effect in vascular
ECs, and induce nitric oxide (NO) production by promoting EC nitric oxide synthase (eNOS) expression, generating vascular relaxing factors such as prostacyclin (PGI₂) and inhibiting synthesis of the vasoconstrictor endothelin-1 (ET-1) [10,11]. Red wine polyphenols increase NO production by enhancing calcium release [12], which is important for NO-producing signaling pathways. Furthermore, the flavonol quercetin attenuated ET-1 release while simultaneously enhancing tissue plasminogen activator (tPA) and PGI₂ levels in vascular ECs [13]. However, the physiological action of dietary polyphenols is quite complex and many questions concerning their precise mechanism of action remain.

There is a wide variety of polyphenols present in foods, and their absorption and metabolism vary according to the polyphenolic type. Furthermore, the pharmacokinetics of different polyphenols differ and provide additional complicating factors to understanding polyphenol activity. This review considers the results of studies that explored the effects of polyphenols present in fruits and vegetables as well as their effects on vascular EC function.

**Epidemiologic studies**

In light of the French paradox, epidemiologic studies to determine the relationship between myocardial or cerebral infarction and polyphenol uptake from fruits, vegetables, and wine were performed. These studies found that consumption of larger amounts of fruits and vegetables was inversely related to development of ischemic heart disease [14–16].

In a cohort study conducted in Japan, soy isoflavones were reported to reduce the critical risk for myocardial infarction (MI) [17]. In this study, the consumption of dietary polyphenols by ~40,500 Japanese postmenopausal women (ages 40–59 y) from 1990 to 1992 (with a follow-up survey in 2002) was monitored and the incidence of cerebral infarctions and MIs relative to polyphenol intake was examined. This study showed that an increase in soybean isoflavone intake reduced the critical risk of cerebral infarctions and MIs. An Ohsaki National Health Insurance cohort study examining the relationship between green tea consumption and CVD mortality for ~40,530 Japanese individuals, ages 40 to 79 y (without anamnesis, e.g., stroke, ischemic heart disease, and cancer) during 1995 to 2005 found an inverse relationship between the amount of green tea consumed and the incidence of stroke [18]. Furthermore, another study [19] examined the beneficial effects of green tea in ~1400 Japanese men living in the same area and found that serum triacylglycerol and total cholesterol were reduced and high-density lipoprotein (HDL) levels increased with increasing amounts of green tea intake. One study evaluated 340 individuals to determine whether risk for MI could be reduced by tea consumption compared with caffeinated and decaffeinated coffee. Individuals who drank more than one cup of tea per day had a 44% reduction in CVD compared with those who drank nothing, although no significant relationship between CVD and coffee intake was observed [20].

**Fig. 1.** Chemical structures of the polyphenols.
Another cohort study found that, compared with healthy individuals, CVD patients with irregular alcohol consumption patterns had significantly increased rates of vascular disorders as well as an increased mortality risk. On the other hand, appropriate consumption of alcoholic drinks having rich polyphenol content such as wine and beer reduced mortality risk, suggesting that the polyphenols in these beverages may exert a protective effect on vascular tissues [21]. Similarly, a cohort study of ~129,000 adults living in California between 1978 and 1985 found that wine drinkers had a reduced mortality risk compared with those who drank beer and spirits. Red wine has about 10-fold more polyphenols than white wine and this increased level is suggested to decrease the risk for death from CVD by preventing vascular dysfunction [22,23].

The effect of tea, apple, and onion consumption on the development of CVD was followed in a study involving 805 older men [24]. The contents of quercetin, kaempferol, myricetin, apigenin, and luteolin in the consumed foods were measured and the study participants provided a diet history from which an average daily flavonoid intake was estimated to be ~26 mg. The major dietary flavonoid sources were tea (61%), onion (13%), and apple (10%). This study showed that among the 693 men in this study who had no history of previous MI, during the 1985 to 1990 period, 43 died of ischemic heart disease while 38 developed MI. Based on these results, an increased intake of flavonoids appears to be inversely related to mortality from ischemic heart disease. Development of MI in particular was inhibited by flavonoid uptake. However, the inhibition of mortality from ischemic heart disease induced by tea, onion, and apple uptake was weak, which highlights the importance of consistent uptake of these foods. Together, these studies show that uptake of flavonoids from foods such as fruits and vegetables reduces the risk for death by ischemic heart disease, particularly in older men.

As shown in Table 1, the quantity of polyphenols in food differs [25–34]. For example, one study [28] investigated the effects of soy protein isolate beverage (60 g/d for 28 d) versus casein in 20 men who were randomly allocated into two groups. Gas chromatography-mass spectroscopy measurements of plasma after soy protein supplementation showed that the soy protein-intake group had increased levels of plasma isoflavone concentrations compared with the casein control group, with lower amounts of daidzein (498 ± 102 nM, n = 10) than genistein (907 ± 245 nM, n = 10). Although these results indicate that soy protein supplementation can increase isoflavone levels, this may not necessarily be sufficient to counter the negative effects of high plasma cholesterol and platelet aggregation. Furthermore, it has been demonstrated that after ingestion of 1.2 g green tea, polyphenol levels in urine and plasma samples from four human volunteers as measured by high-performance liquid chromatography (HPLC) ranged from 46 to 268 ng/mL epigallocatechin-3-gallate (EGCG), 82 to 206 ng/mL epigallocatechin, and 48 to

Fig. 2. Chemical structures of the flavonoids.
factors can contribute to many vascular diseases, including hypertension, atherosclerosis, ischemic heart disease, and diabetes \[40,42,43\]. Furthermore, angiography in advance of atherosclerosis development supports that these diseases likely arise from EC dysfunction \[44\]. As such, EC function appears to be a signature initial feature of CVD and raises the possibility that ECs could be a therapeutic target for preventing CVD and cerebrovascular disorders \[45\]. Another study that determined the amount of circulating EC progenitor cells found that reduced levels of these cells might be associated with increased risk for CVD \[46\]. Taken together, these findings suggest that the maintenance of systemic vascular health and levels of ECs are strongly related to the risk for developing CVD.

Dietary polyphenols can enhance vascular function and contribute to vascular health, which is largely regulated by NO and PGI2 produced by ECs. NO is formed from L-arginine by the constitutive EC-dependent eNOS in response to several physiologic and pathologic stimuli of ECs. Polyphenols have been shown to have antioxidant activity and increase the formation of NO that is catalyzed by eNOS \[47\]. Several reports demonstrated that dietary polyphenols induced endothelium-dependent vasorelaxation that was associated with increased cyclic guanosine monophosphate (cGMP) formation. The effect of different polyphenols on NO production from ECs and relaxation of isolated vessels from porcine coronary tissues has been examined \[48\]; enhanced NO activity was observed that was produced by flavan-3-ols with free hydroxyl-residues at C3, C3′, C4′, C5, and C7 \[48\]. Furthermore, an effect on ECs by the green tea polyphenol EGCG was indicated in a study using bovine aortic ECs that found that EC PGI2 was increased in the presence of EGCG with the monophosphates with free hydroxyl-residues at C3, C3′, C4′, C5, and C7 \[48\]. EGCG also enhanced generation of NO via the Fyn/PI3K/Akt signaling pathways in rat ECs \[49\]. EGCG also enhanced generation of NO via the Fyn/PI3K/Akt signaling pathways in rat ECs \[50\]. Similarly, EGCG could attenuate ET-1 expression and secretion by the PI3K-dependent transcription factor FOXO1 in human aortic ECs \[51\] and this EGCG-dependent reduction in ET-1 synthesis may increase NO bioavailability. Thus, dietary polyphenols may regulate the release of factors from ECs, and support EC health, which subsequently reduces the risk for CVD.

### Effect of polyphenol-induced NO production on endothelial cell dysfunction and maintenance of vascular health

ECs are present between blood and smooth muscle vasculature and release many vasoactive factors to maintain and regulate vascular structure and function \[38\]. The activity of these EC factors is diverse and they participate in vascular relaxation, anticoagulation, and regulation of blood pressure and blood flow to tissues \[39\]. In particular, EC cell-derived NO, PGI2, and endothelium-derived relaxing factor regulate vascular tone. Under normal conditions, vascular health is maintained by NO and PGI2 produced by EC cells, with EC NO providing a protective effect for vasculature. Deficiencies in NO production can lead to the development of atherosclerosis and subsequently increased risk for CVD \[40\]. On the other hand, ET-1 is a vasoconstrictive factor that can induce vasospasm, which in turn can promote fibrosis, arterial remodeling, and vascular injury. Indeed, increased ET-1 plasma levels are associated with hypertension or heart failure \[41\]. Abnormalities in the release of EC-derived factors can contribute to many vascular diseases, including hypertension, atherosclerosis, ischemic heart disease, and diabetes \[40,42,43\]. Furthermore, angiography in advance of atherosclerosis development supports that these diseases likely arise from EC dysfunction \[44\]. As such, EC function appears to be a signature initial feature of CVD and raises the possibility that ECs could be a therapeutic target for preventing CVD and cerebrovascular disorders \[45\]. Another study that determined the amount of circulating EC progenitor cells found that reduced levels of these cells might be associated with increased risk for CVD \[46\]. Taken together, these findings suggest that the maintenance of systemic vascular health and levels of ECs are strongly related to the risk for developing CVD.

### Preventive effects of dietary polyphenols on early EC dysfunction

Although many studies evaluating the effect of polyphenols on vascular ECs have been performed, several in vitro results did not translate to effects in humans. Quercetin and iso-flavones are absorbed by the small intestine relatively quickly \[52\]. Quercetin in vegetables is present as a glycoside and in the intestine the binding saccharide is vulnerable to enterobacterial activity. After processing, quercetin is excreted into the blood,

### Table 1

<table>
<thead>
<tr>
<th>Polyphenol Source</th>
<th>Polyphenol intake (mg)</th>
<th>Plasma maximum concentration (μM)</th>
<th>Excretion to urine (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soy proteins 60 g/d for 28 d</td>
<td>80</td>
<td>0.5</td>
<td></td>
<td>[26]</td>
</tr>
<tr>
<td>Soy milk</td>
<td>19</td>
<td>0.74</td>
<td>19.8</td>
<td>[27]</td>
</tr>
<tr>
<td>Grapefruit juice, 120 L</td>
<td>43</td>
<td>&lt;4</td>
<td>8.8</td>
<td>[28]</td>
</tr>
<tr>
<td>Grapefruit and orange juice, 1250 mL each</td>
<td>689</td>
<td>6.8</td>
<td></td>
<td>[29]</td>
</tr>
<tr>
<td>Green tea infusion, 1.2 g</td>
<td>88</td>
<td>0.33</td>
<td>ND</td>
<td>[30]</td>
</tr>
<tr>
<td>Green tea infusion, 5 g</td>
<td>105</td>
<td>0.13–0.31</td>
<td></td>
<td>[31]</td>
</tr>
<tr>
<td>Onion</td>
<td>68</td>
<td>0.74</td>
<td>1.34</td>
<td>[32]</td>
</tr>
<tr>
<td>Onion</td>
<td>139</td>
<td>1.34</td>
<td>0.8</td>
<td>[33]</td>
</tr>
<tr>
<td>Apple</td>
<td>98</td>
<td>0.30</td>
<td>0.44</td>
<td>[32]</td>
</tr>
<tr>
<td>Red wine, 300 mL</td>
<td>218</td>
<td>1.0–6.7</td>
<td></td>
<td>[34]</td>
</tr>
</tbody>
</table>

EGCG, epigallocatechin-3-gallate; ND, not detected

Plasma and urine were hydrolyzed by acid or enzymes before high-performance liquid chromatography analysis.
whereupon glucuronic acid or sulfate conjugation occurs in the liver [9]. In the blood, quercetin is more likely to be present in the form of aglucon. Indeed, most polyphenols in vivo are present as metabolites, although the exact effects of polyphenol metabolites remain unclear. In this section, we describe the preventive effect of polyphenols on initial disorders in cultured ECs.

Adhesion of leukocytes to ECs occurs during the early stages of arteriosclerosis, and dietary polyphenols may have effects during this initial adhesion. In human umbilical vein ECs (HUVECs), a 24-h treatment with oxidized low-density lipoprotein (LDL; 100 mg/mL) induced ICAM-1 gene expression and reactive oxygen species (ROS) production. Pretreatment of the cells for 2 h with anthocyanin (delphinidin and cyanidin), flavonol (myricetin and quercetin), flavone (luteolin and apigenin), or isoflavones (genistein and daidzein) inhibited the increases in ICAM-1 expression and ROS production [53]. This study found that the total number of hydroxyl groups in the structure, the B ring 3',4'-ortho-dihydroxy group and the hydroxyl group at the three position of the C ring were involved in inhibition of these effects. In particular, delphinidin and myricetin had strong ROS scavenging effects and also inhibited ICAM-1 gene expression. Together, these results again support a role for dietary polyphenols in maintaining vascular EC function. Additionally, protective effects on expression levels of ICAM-1 and VCAM-1 were observed for cyanidin, quercetin, luteolin, and apigenin. In another study, high glucose (30 mM) plus tumor necrosis factor (TNF-α) also promoted VCAM-1 expression and adhesion of U937 cells to human ECs and these effects could be strongly inhibited by the parsley flavonoid apigenin, which inhibited the activity of the transcription factor nuclear factor kappa B (NF-B) [54]. Similarly, apigenin as well as related flavonoid structures could inhibit high glucose plus TNF-α induction of lectin oxidation receptor-1 expression [55]. At a molecular level, these effects required a double bond in the flavonoid frame C ring and A ring hydroxyl group [56]. These results show that polyphenols can attenuate the expression of adhesion molecules such as VCAM-1, and these changes in expression may be relevant during the early stages of arteriosclerosis.

Green tea flavonoids (catechin) represent ~30% of the dry weight of new tea leaves [57]. The major components of green tea catechin are EGCG, epigallocatechin, epicatechin gallate, and epicatechin [58]. Many reports have studied the effect of green tea in cancer prevention and these compounds are thought to have anti-inflammatory, antibacterial, antioxidant, and antiangiogenic activities [59–63]. Many of these effects were attributable to EGCG [64]. In vascular EC cells derived from human aortas, addition of EGCG at low concentrations (2.5 μM) inhibited expression of VCAM-1 induced by TNF-α, while heme oxygenase-1 expression was significantly increased [65]. Additionally, by examining the effects of several inhibitors, the protective action of EGCG against TNF-α-induced VCAM-1 expression was shown to be associated with Nrf-2 and p38 MAPK-dependent pathways as well as with increased expression of heme oxygenase-1.

Furthermore, EGCG promoted NO-dependent vasodilation in ECs through a mechanism involving src-family kinase Fyn (but not Src) that led to activation of PI3K, Akt, and eNOS with ROS production [51]. These findings may explain the beneficial effects of green tea consumption on vascular EC function. 7-ketocholesterol (7 KC) induces monocyte adhesion to ECs, which in turn induces arteriosclerosis. The 7KC-induced monocyte cell adhesion was accompanied by increased expression of ICAM-1 and MCP-1 and decreased eNOS and Ca2+ /calmodulin-dependent kinase (CaMKII) expression. Meanwhile, EGCG has a protective effect against arteriosclerosis, which may be due to its capacity to block 7KC-dependent monocyte cell adhesion to ECs, and to induce expression of eNOS as well as several genes involved in the CaMKII pathway [66]. As shown in Figure 3, the EGCG action in this setting was associated with changes in the expression of genes such as CaMKII, LKD1, and PI3K, which could in turn affect ROS production [66]. CaMKII, LKD1, and PI3K participate in signaling pathways that are important for the effects of HDL in ECs [67]. Additionally, ROS production is a significant feature of the action of EGCG, suggesting that it may induce expression of proteins such as CaMKII, which is associated with HDL signaling. Namely, EGCG appears to inhibit expression of adhesion molecules and enhance eNOS expression via a signaling pathway similar to that of HDL [67]. Polyphenol-induced ROS production is involved in intracellular signaling. In the case of EGCG, ROS signaling did appear to affect EC functionality by altering NO production [50]. Beneficial actions of EGCG on the endothelium were induced through ROS production. For instance, EGCG induced endothelium-dependent NO-mediated relaxation of coronary artery rings through Akt-dependent activation of eNOS in ECs. This response to EGCG was initiated by the intracellular formation of superoxide anions and hydrogen peroxide [68]. Furthermore, Concord grape juice (CGJ), which contains abundant polyphenols, induced eNOS expression and this effect was correlated with elevated rates of NO formation in ECs. Simultaneously, CGJ induces ROS formation in ECs. Together, these results indicate that the stimulatory effect of CGJ is redox sensitive, and thereby prevents repression of eNOS gene expression [69]. EGCG and CGJ induce eNOS/NO expression in response to ROS production. However, damage caused by oxidative stress was not observed in these studies. Although the details of these effects remain unclear, the ROS production induced by EGCG and polyphenols may not induce cell injury.

eNOS activation is increased by increasing [Ca2+]i concentrations and eNOS phosphorylation mediated by the PI3-kinase/Akt pathway [7]. Polyphenols may regulate NO production by promoting NOS activity via these two mechanisms. Figure 3 shows results indicating that EGCG contributed to ROS production and changes in expression of genes such as CaMKII, LKD1,
and PI3K in the presence of the antioxidant N-acetyl-cysteine. Therefore, polyphenols likely also have effects on ROS production.

Similarly, a report indicated that the addition of interleukin (IL)-1β, carotenoid β-carotene, or both to cultured human ECs affected HDL signal transduction pathways and EC-U937 adhesion [70], β-carotene also induces expression of CaMKII, PI3K, PFK1, LKB1, eNOS, and PON1, while reducing expression levels of ICAM-1 and MCP-1. Furthermore, β-carotene induced phosphorylation of 5′ adenosine monophosphate-activated (AMP)-activated protein kinase (p-AMPK), eNOS (p-eNOS), and PON1 proteins. PON1 is an HDL-associated enzyme that has antioxidant and antiatherogenic properties [71]. These findings suggest that EGCG and β-carotene can regulate the expression of PON1, eNOS, and adhesion molecules by activating the CaMKK pathway, β-carotene may contribute to the functional maintenance of vascular ECs in a signaling pathway similar to HDL to protect them from negative stimuli such as those produced by IL-1β.

In epidemiologic studies, polyphenols found in black tea and cocoa improved EC function in acute and short-term intervention trials in humans [72–74]. Although polyphenols are direct free radical scavengers [75], a recent study indicated that polyphenols can modulate pathways that promote increased eNOS levels in ECs [76]. These acute effects of polyphenols are important to inhibit aggravated EC disorders.

**Preventive effects against endothelial dysfunction by the polyphenol family: flavonoids and non-flavonoids**

Polyphenols can be classified into flavonoids and non-flavonoids (Fig. 1) [9]. The flavonoid group has a phenyl chroman frame, and based on differences in side-chain structures can be classified into flavones, isoflavones, flavanones, flavonols, and anthocyanins [9]. Typical non-flavonoids include tannins, chlorogenic acid, gallic acid, caffeic acid, curcumin, and resveratrol. In this section, we explore the effects of many flavonoids and non-flavonoids in fruits and vegetables on vascular EC function (Table 2).

**Table 2**

<table>
<thead>
<tr>
<th>Category</th>
<th>Typical components</th>
<th>Authors &amp; References</th>
<th>Preventive effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>Apigenin</td>
<td>Yamagata et al. [79]</td>
<td>Increases vascular relaxation</td>
</tr>
<tr>
<td></td>
<td>Luteolin</td>
<td>Park et al. [81]</td>
<td>Inhibits ROS level</td>
</tr>
<tr>
<td></td>
<td>Genistein</td>
<td>Xiang et al. [83]</td>
<td>Induces relaxation via PPAR-γ and ER-β</td>
</tr>
<tr>
<td>Isoflavones</td>
<td>Daidzein</td>
<td>Cho et al. [86]</td>
<td>Increase NO levels, vasodilation, and reduce endothelin-1 levels</td>
</tr>
<tr>
<td></td>
<td>Kaempferol</td>
<td>Crespo et al. [98]</td>
<td>Reduces production of MCP-1, VCAM-1, and eNOS</td>
</tr>
<tr>
<td></td>
<td>Quercetin Q3GA</td>
<td>Li et al. [94]</td>
<td>Inhibits ICAM-1, VCAM-1, and E-selectin expression</td>
</tr>
<tr>
<td></td>
<td>ECGG</td>
<td>Mizugaki et al. [49]</td>
<td>Inhibits ROS production, activates IRS-1 and NO production</td>
</tr>
<tr>
<td></td>
<td>ClG</td>
<td>Wang et al. [104]</td>
<td>Induces vascular relaxation through PGI2 production</td>
</tr>
<tr>
<td>Non-flavonoids</td>
<td>Resveratrol</td>
<td>Wallether et al. [108]</td>
<td>Inhibits cholesterol, 7-oxysterol accumulation and block superoxide production</td>
</tr>
<tr>
<td></td>
<td>Curcumin</td>
<td>Xia et al. [109]</td>
<td>Reduces NO production through the Sirt1/Foxo transcription factor</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alhosin et al. [113]</td>
<td>Reduces oxidative stress and vasodilation, inhibits apoptosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pu et al. [116]</td>
<td>induces eNOS and AMPK phosphorylation, up-regulates UCP2, and reduces ROS production</td>
</tr>
</tbody>
</table>

C3G, cyanidin-3-glucoside; ECGG, epigallocatechin-3-gallate; eNOS, endothelial cell nitric oxide synthase; ER, estrogen receptor; NO, nitric oxide; PPAR, peroxisome proliferator-activated receptor; ROS, reactive oxygen species

**Flavone**

The flavone family member apigenin markedly inhibits attenuated endothelium-dependent relaxation produced by superoxide anions in rat aorta cells [77]. Apigenin can increase vascular relaxation to promote neovascularization that increases blood flow to tissues. In HUVECs, apigenin inhibits hypoxia-induced expression of vascular endothelial growth factor (VEGF) that is associated with stabilization of hypoxia–induced factor-1α [78]. Apigenin reduces hypoxic reoxygenation-induced EC dysfunction via apelin and fatty acid uptake in human ECs [79]. Thus, these actions of apigenin suggest that it inhibits oxidative stress produced by ischemic conditions and relieves EC dysfunction. Similarly, the flavonoid luteolin attenuates relaxation inhibition induced by high glucose in isolated rat aortic rings [80]. Luteolin significantly inhibited increases in ROS levels and NO decreases that occur with high glucose. Additionally, luteolin inhibits neovascularization in the retina that is induced by ROS production [81]. Thus, luteolin may induce endothelium-dependent relaxation that enhances NO activity and inhibits oxidative stress in ECs.

**Isoflavone**

Genistein and daidzein are isoflavone compounds. Genistein can bind to the estrogen receptor (ER) to mimic various biological effects of estradiol, although with weaker transcriptional potency [82]. Genistein binding to ERs can affect their interaction with estrogen response elements to enhance eNOS expression, and subsequently produce protective cardiovascular effects. Indeed, genistein induces relaxation of phenylephrine-enhanced vascular contraction in female rat aorta tissue by stimulating peroxisome proliferator-activated receptor-γ and ER-β [83]. Clinical studies have demonstrated that genistein induces increased NO levels and endothelium-dependent vasodilation to reduce endothelin-1 levels [84]. As with other dietary polyphenols, these isoflavones influence vascular reactivity by affecting eNOS and redox-sensitive gene expression [85]. Similarly, the effects of genistein and daidzein were compared by examining MCP-1, VCAM-1, and eNOS production in HUVECs stimulated with TNF-α [86]. Genistein reduced MCP-1 and...
VCAM-1 production in a dose-dependent manner, whereas daidzein slightly decreased MCP-1 production. Daidzein and genistein inhibited reduction of eNOS levels induced by TNF-α, and conversely NO expression was increased. However, the effect on NO production by daidzein was low compared with genistein. A previous study demonstrated that a daidzein metabolite can stimulate NO production via phosphorylation of eNOS at cytosolic Ca²⁺ levels to activate the ERK1/2, PI3-kinase/Akt, and PKA signaling pathways [87]. Another study demonstrated the effect of genistein on hyperglycemia-induced vascular inflammation and found that genistein inhibits hyperglycemia-enhanced monocyte adhesion to human aortic ECs through the cAMP signaling pathway and ameliorates vascular inflammation in obese diabetic mice [88]. As such, genistein may play a beneficial role in vascular function in human ECs via the PKA/CREB/eNOS/NO signaling pathway [89].

**Flavanone**

In human ECs, the citrus flavanone hesperetin enhances NO release by eNOS in a concentration-dependent manner [90]. The estrogenic activities of naringenin and hesperetin were recently evaluated for whether they effect EC NO production via ER activation. Whereas naringenin activated both ER-α and ER-β, hesperetin activated only ER-α. These results suggest that hesperetin exerts antiatherogenic activity by affecting ER-mediated eNOS expression and NO generation. Naringenin induces BK (Ca²⁺) channel activation, suggesting that this flavanone has a vasorelaxant effect [91]. Furthermore, a recent study found an inhibitory effect of naringenin and hesperetin metabolites (hesperetin-3′-sulphate, hesperetin-3′-glucuronide, and naringenin-4′-glucuronide) on monocyte adhesion to TNF-α-activated human ECs and on gene expression [92]. Additionally, naringenin inhibited hyperplasia following arterial reconstruction with interpositional vein grafts by decreasing platelet-derived growth factor (PDGF)-BB levels in vascular SMCs [93]. Taken together, these results suggest that naringenin and hesperetin may have beneficial effects on ECs.

**Flavonol**

The flavonol quercetin and its metabolite quercetin-3-O-glucuronide (Q3GA) inhibited oxidative stress caused by ROS production and efficiently restored mitochondrial membrane potential. Simultaneously, quercetin and Q3GA activated the insulin receptor substrate-1, and caused upregulation of insulin-mediated NO production. Furthermore, quercetin and Q3GA inhibited deterioration of insulin stimulation and the attenuation of NO production induced by inflammation [94]. These data indicate that quercetin and Q3GA inhibit ROS-related inflammation, and diminish insulin-resistant EC dysfunction [95]. In addition to Q3GA, other quercetin metabolites, including quercetin-3′-O-glucuronide [96] and quercetin-3′-O-sulfate [97], were reported to have beneficial effects. Similarly, kaempferol inhibited ICAM-1, VCAM-1 and E-selectin expression [98]. Kaempferol activates the opening of Ca²⁺-activated K⁺ channels in HUVECs via a cAMP/PKA-mediated pathway, which subsequently enhances membrane hyperpolarization. This mechanism may to some extent be responsible for the vasodilator effects of kaempferol.

A recent report indicated that quercetin, cyanidin, or kaempferol inhibit cyclic nucleotide phosphodiesterase (PDE) [99], which leads to increased cAMP or cGMP levels and subsequent prostacyclin production. Inhibition of PDE and PG1₂ production inhibits ET-1 in ECs and has a relaxant effect on SMCs [100]. EGCG induces vascular relaxation through PGI₂ production in ECs [49,101]. However, the inhibition of ET-1 secretion from ECs by EGCG is weak. Therefore, the vasodilator action of PGI₂ by EGCG appears to have a relaxant effect on SMCs via adenylate cyclase.

**Anthocyanins**

Anthocyanins are abundant flavonoid constituents of fruits and vegetables. Several epidemiologic studies suggest that consumption of anthocyanins helps prevent several diseases, including vascular disorders. Recent studies have examined the implication of these mechanisms in the preventive effects induced by anthocyanin on vascular disorders. Delphinidin suppresses monocyte-EC cell adhesion induced by oxidized LDLs in ECs via the ROS/p38MAPK/NF-κB pathway [102]. Both delphinidin and cyanidin inhibit PDGF(AB)-enhanced VEGF production in vascular SMCs by inhibiting the activation of p38 MAPK and JNK [103]. Additionally, the anthocyanin cyanidin-3-O-β-glucoside (C3G) prevents hypercholesterolemia-mediated EC dysfunction in apolipoprotein E-deficient mice by inhibiting cholesterol and 7-oxysterol accumulation in the aorta and reducing superoxide production [104]. Furthermore, C3G protected against TNF-α-induced EC dysfunction by inhibiting ROS production and NF-κB activation [105]. In vascular SMCs, C3G inhibited TNF-α-enhanced cell proliferation by inhibiting NOS activator 1 activity [106]. In ECs, malvidin-3-β-glucoside prevented upregulation of EC NOS, inhibition of iNOS and cyclooxygenase-2 expression and inhibited peroxynitrite-enhanced NF-κB activation [107].

**Non-flavonoids**

The non-flavonoid group includes tannins, chlorogenic acid, gallic acid, caffeic acid, curcumin, and resveratrol. Notably, resveratrol has shown beneficial effects against most CVDs and cerebrovascular diseases. Resveratrol induces eNOS expression through the Sirt1/Foxo transcription factor axis that in turn increases NO production in cultured human ECs [108,109]. Sirt is an essential mediator of longevity in normal cells that acts when calories are restricted and provides several biological functions, including transcription and cell cycle regulation as well as protection from apoptosis. The Sirt1/Foxo axis is also associated with resveratrol-induced hepatic glucokinase gene expression [110], which prevents pancreatic β-cell dysfunction [111]. Sirt1 expression is reduced with senescence and in ECs during arteriosclerosis, and these levels were dose dependently increased in resveratrol-treated ECs [112]. Furthermore, resveratrol inhibited alterations associated with senescence induced by H₂O₂. This resveratrol-dependent increase in Sirt1 expression, which together with Foxo drives eNOS expression, may reduce the effects of ROS on endothelium injury [113]. Another report noted that resveratrol reduces oxidative stress, improves acetylcholine-induced vasodilation, and inhibits apoptosis in femoral artery cells. These effects of resveratrol indicate that it provides protective effects toward ECs that in this case are mediated by Nrf2 activation in vitro and in vivo [114]. Furthermore, resveratrol competitively blocks cAMP-degrading PDE [115] and induces elevated cAMP levels. Activation of Epac1 by resveratrol induces activity of phospholipase C and the ryanodine receptor Ca²⁺-release channel. As such, resveratrol increases intracellular Ca²⁺ levels through activation of Epac1 and the CamKKβ-AMPK pathway. The resulting effects of resveratrol
are increases in NAD(+) levels and Sirt1 activity [82]. Additionally, curcumin prevents aging-related cerebrovascular dysfunction via the AMPK/UCP2 pathway [116].

Intake of different polyphenols in foods and beverages improved antiatherogenic, antithrombotic, and antihypertensive endothelial functions, although the mechanisms by which these effects are achieved differ according to active polyphenols in each food or beverage. However, in vivo studies indeed demonstrated beneficial effects toward cardiovascular risk factors.

Human and animal studies to examine residual amounts of orally administered apigenin

Apigenin is a substance present in parsley that has a protective effect toward human vascular EC function [71]. Here, we discuss several reports describing the analysis of apigenin pharmacokinetics. In one study, the blood and urine apigenin levels in individuals (five women, six men, average body mass index 23.9 kg/m², with ages ranging from 23 to 41 y) who ingested 2 g/kg parsley after fasting were assessed by HPLC [117]. Plasma apigenin levels were found to be at their highest (127 nM) 7 h after ingestion and for all participants’ apigenin levels were below 2.3 nM 28 h after consumption. The average apigenin level in urine after 24 h was 0.22% (144 nM). The authors of this report suggested that the small amount of apigenin circulating in the body following parsley consumption might be sufficient for biological effectiveness. A Chinese study examined absorption and excretion of luteolin and apigenin following oral administration of Chrysanthemum morifolium extracts (CME) and found that apigenin was absorbed more effectively than luteolin, although both have similarly slow elimination rates [70]. In this report, CME (200 mg/kg) was administered orally to rats, and in the plasma, urine, feces, and biliary levels of luteolin and apigenin were measured by HPLC following treatment with β-glucuronidase/sulfatase. Luteolin and apigenin levels were highest at 1.1 and 3.9 hours, respectively, after dosing while ~38% and ~45% of the original dose of luteolin and apigenin could be recovered, with the majority being present in the feces. Results from this study indicate that for both flavonoids the excretion was slow compared to their absorption and may therefore be retained for longer periods.

Conclusion

In this review, the relationship between the preventive effects of polyphenols and CVD are discussed as well as the results from many studies on dietary polyphenols found in foods. One the one hand, a variety of studies indicated that polyphenols have a beneficial effect on vascular EC function. On the other hand, treatment with the synthetic flavonoid derivative S17834 prevented left-ventricular hypertrophy and diastolic dysfunction similarly to resveratrol by inhibiting NADPH oxidase activity in ECs [118,119]. Future studies will likely uncover the mechanisms by which dietary polyphenols act to provide their protective effects.

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