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INVITED REVIEW

Nutritional improvement of the endothelial control of vascular tone by polyphenols: role of NO and EDHF

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Abstract Numerous studies indicate that regular intake of polyphenol-rich beverages (red wine and tea) and foods (chocolate, fruit, and vegetables) is associated with a protective effect on the cardiovascular system in humans and animals. Beyond the well-known antioxidant properties of polyphenols, several other mechanisms have been shown to contribute to their beneficial cardiovascular effects. Indeed, both experimental and clinical studies indicate that polyphenols improve the ability of endothelial cells to control vascular tone. Experiments with isolated arteries have shown that polyphenols cause nitric oxide (NO)-mediated endothelium-dependent relaxations and increase the endothelial formation of NO. The polyphenol-induced NO formation is due to the redox-sensitive activation of the phosphatidylinositol3-kinase/Akt pathway leading to endothelial NO synthase (eNOS) activation subsequent to its phosphorylation on Ser 1177. Besides the phosphatidylinositol3-kinase/Akt pathway, polyphenols have also been shown to activate eNOS by increasing the intracellular free calcium concentration and by activating estrogen receptors in endothelial cells. In addition to causing a rapid and sustained activation of eNOS by phosphorylation, polyphenols can increase the expression level of eNOS in endothelial cells leading to an increased formation of NO. Moreover, the polyphenol-induced endothelium-dependent relaxation also involves endothelium-derived hyperpolarizing factor, besides NO, in several types of arteries. Altogether, polyphenols have the capacity to improve the endothelial control of vascular tone not only in several experimental models of cardiovascular diseases such as hypertension but also in healthy and diseased humans. Thus, these experimental and clinical studies highlight the potential of polyphenol-rich sources to provide vascular protection in health and disease.

Keywords Polyphenols · eNOS · NO · EDHF · Hypertension

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Introduction

Diet, one of the most important lifestyle risk factors, can strongly influence the incidence of cardiovascular diseases [10, 96]. Indeed, accumulating data from numerous epidemiological studies indicate that regular intake of polyphenol-rich beverages and foods such as red wine, tea, and chocolate, and fruit and vegetables is associated with an improved cardiovascular prognosis [29, 48, 75, 95].

Studies aiming to characterize the beneficial effects of polyphenols on the cardiovascular systems have started several decades ago [14, 32]. The beneficial effects of polyphenols on the cardiovascular system have been attributed to their ability to reduce vascular oxidative stress, in particular, not only through their direct superoxide anion scavenging properties and interaction with other reactive oxygen species such as hydroxy and peroxy radicals [50, 81, 88, 102] but also through their stimulatory effect on endogenous antioxidant enzymes [81] and their inhibitory effect on xanthine oxidase and NAD(P)H oxidase, two major enzymes generating large amounts of reactive oxygen species [81, 82]. In addition to the antioxidant effects of polyphenols, both experimental and clinical studies indicate that polyphenols might also protect the

cardiovascular system by improving the endothelial function. As it has been first demonstrated by Furchgott and Zawadzki [45], the endothelium plays a key role in the control of vascular tone by releasing several vasorelaxing factors, which have been identified later on as nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF) [19, 38, 51, 83, 101]. Therefore, the aim of this review is to focus on the polyphenol-induced endothelial formation of NO and stimulation of EDHF-mediated responses, two major vasoprotective factors. In addition, this review will summarize both the experimental and the clinical evidences indicating that polyphenols might improve the endothelial function in health and disease.

Polyphenols cause NO-mediated endothelium-dependent relaxations

It was initially shown that various grape products including wines, grape juices, and grape skin extracts are able to induce concentration-dependent relaxations in rat aortic rings with endothelium but only minor relaxations in rings without endothelium [41]. The fact that the polyphenol-induced relaxation is associated with an increase in the cyclic GMP content in intact aortic rings and that both the relaxation and the formation of cyclic GMP are prevented by NO synthase inhibitors indicates that grape-derived products increase the endothelial NO synthase activity leading to the formation of NO, which subsequently relaxes the vascular smooth muscle via the cGMP-mediated pathway. In addition, the endothelium-dependent relaxation appears to be strongly correlated with the concentration of polyphenols in red wines [17].

Thereafter, NO-mediated endothelium-dependent relaxations induced by polyphenols from grape-derived products, including wines and grape seeds, have been observed in various types of blood vessels including the rat and rabbit aorta, the perfused rat mesenteric artery, and in the porcine and human coronary arteries [4, 24, 40, 43, 78, 94]. Electron paramagnetic resonance spectroscopy and NO-selective microsensor provided direct evidence that grape-derived polyphenols cause an increase in the endothelial formation of NO in intact segments of rat aorta and porcine coronary artery and in cultured endothelial cells [77, 98, 99]. In addition, polyphenols from various sources such as cocoa, tea, hawthorn, maritime pine bark, honey, and propolis have also been shown to induce endothelium-dependent NO-mediated relaxations in arteries [6, 21, 33, 39, 42, 57, 60, 67, 99].

Berries and red fruits are another interesting rich source of polyphenols, mainly anthocyanins and ellagitannins. Indeed, blackcurrant juice induced pronounced endothelium-dependent relaxations of isolated porcine coronary arteries (Fig. 1). Similarly, red raspberry caused endothelium-dependent relaxations of rabbit aortic rings, and the biological activity was predominantly associated with the fraction enriched in ellagitannins [76]. Endothelium-dependent relaxations have also been observed in response to anthocyanin-enriched extracts of chokeberry and of bilberry whereas that of elderberry had only minor effects [15] suggesting that the phenolic composition of berries is determinant for their vasorelaxant activity. In addition, a procyanidin-rich extract from apples also induced endothelium-dependent NO-mediated relaxations in rat aortic rings [71].

Endothelium-dependent relaxations have also been observed in response to several authentic polyphenolic compounds including curcumin [111], apigenin [54], resveratrol [65, 87], and soy isoflavones [103].

Polyphenols increase endothelial NO synthase activity

Role of intracellular calcium

Activation of endothelial NO synthase in response to circulating hormones, local autocoids, substances released by platelets, by the coagulation cascade, and by the autonomic nervous system is mostly dependent upon an increase in the free cytosolic calcium concentration ($[Ca^{2+}]_i$) in endothelial cells [74]. A polyphenol extract from red wine and delphinidin, a major anthocyanin present in red wine, at a concentration of 10 mg/l induced a significant increase in $[Ca^{2+}]_i$ leading to the endothelial formation of NO [69, 98] (Fig. 2). Nevertheless, the amplitude of the calcium signal, less than 100 and 200 nM for the polyphenol extract and delphinidin, respectively, is relatively

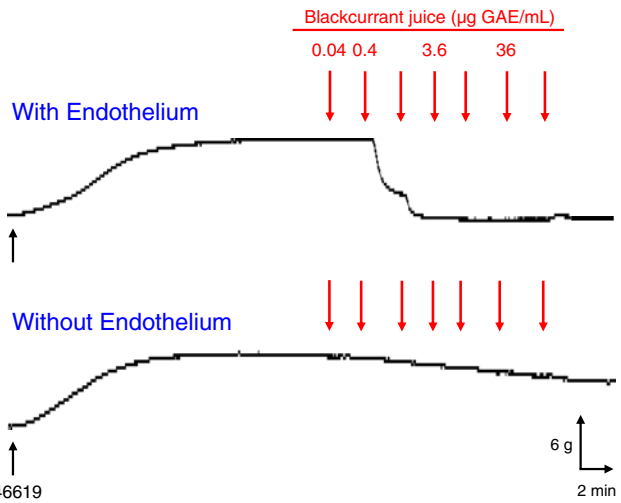

Fig. 1 Original tracings indicating that blackcurrant juice causes concentration-dependent relaxations of porcine coronary artery rings with endothelium but only minor effects in rings without endothelium

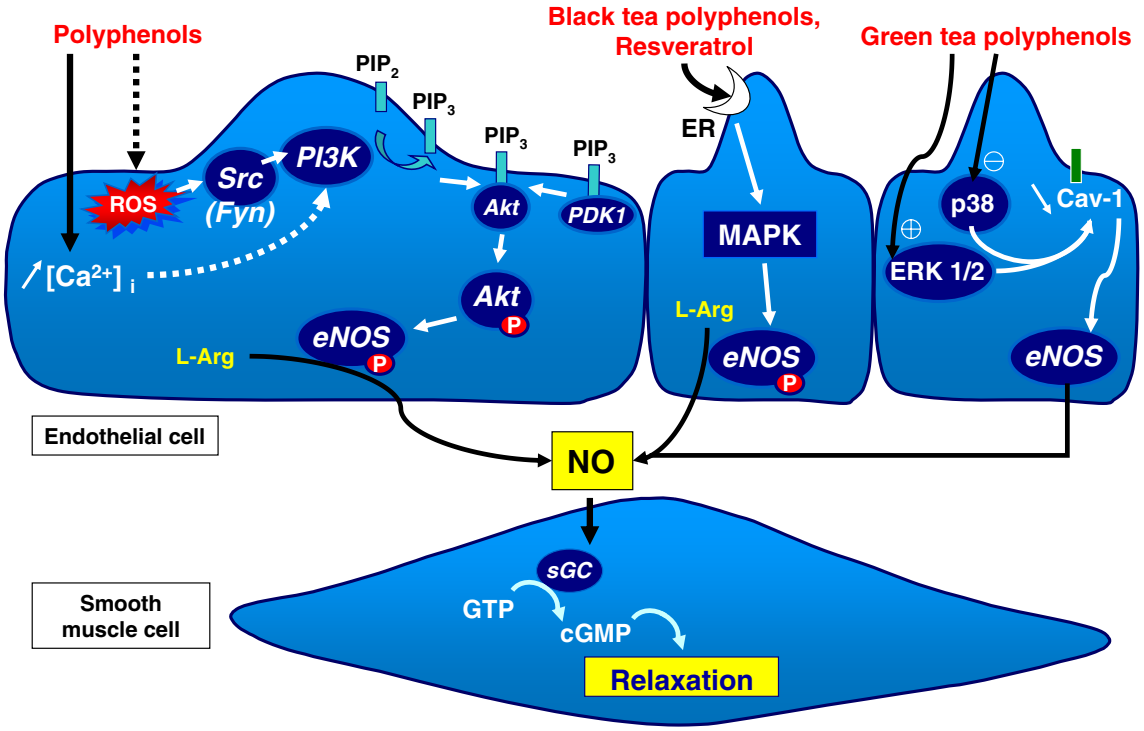

Fig. 2 Schematic indicating that polyphenols are potent inducers of the endothelial formation of NO involving different intracellular signaling pathways. *ROS* reactive oxygen species; *PI3K* phosphatidylinositol 3-kinase; *PDK1* phosphoinositide-dependent kinase 1; *eNOS* endothelial NO synthase; *NO* nitric oxide; *ER* estrogen receptor; *Cav-1* caveolin-1; *sGC* soluble guanylyl cyclase

low in comparison to that induced by physiological agonists such as bradykinin. Therefore, although an increase in $[Ca^{2+}]_i$ in endothelial cells is an important pathway leading to eNOS activation, it is likely that additional mechanisms contribute to mediate the stimulatory effect of polyphenols on endothelial NO synthase.

Role of the PI3-kinase/Akt pathway

Besides the calcium signal, the PI3-kinase/Akt pathway is another important signal pathway leading to the activation of endothelial NO synthase such as in response to the shear stress induced by blood flow, estrogens, and vascular endothelial growth factor [18, 30, 72]. The PI3-kinase/Akt causes a rapid activation of endothelial NO synthase through its phosphorylation at Ser1177 [30].

It has been shown that red wine polyphenols (RWPs) induce the activation of the PI3-kinase/Akt pathway in endothelial cells, which, in turn, causes phosphorylation of eNOS at Ser1177 (an activator site) and dephosphorylation of eNOS at Thr495 (an inhibitor site), leading to an increased formation of NO [77] (Fig. 2). The PI3-kinase/Akt signaling pathway is triggered by the polyphenol-induced intracellular formation of reactive oxygen species predominantly superoxide anions in endothelial cells [5, 68, 77]. A similar mechanism of eNOS activation has been observed in response to a grape seed extract [35], grape skin extract [68], purple grape juice [5], strawberry powder rich in polyphenols [36], and epigallocatechin-3-gallate [67], a major flavonoid from the flavan-3-ol subclass found in green tea. In addition, polyphenols cause within minutes the phosphorylation of eNOS, and this effect persists for several hours indicating a sustained activation of eNOS. In contrast, activation of G protein-coupled receptors by bradykinin or thrombin caused only a transient formation of NO, which reached a peak value within 1 min and thereafter vanished rapidly [27, 91].

Additional investigations identified Src kinase as a redox-sensitive mediator, which acts upstream of the PI3-kinase/Akt pathway leading to eNOS activation in response to grape-derived polyphenols [5, 6, 68] (Fig. 2). Moreover, Fyn, a member of the Src family, mediated the epigallocatechin gallate-induced PI3-kinase/Akt-mediated activation of eNOS [59].

The polyphenolic-rich fraction of black tea acutely enhanced NO formation subsequently to the phosphorylation eNOS at Ser1177 and the dephosphorylation of eNOS at Thr495 in porcine aortic endothelial cells [8]. This stimulatory effect is calcium-dependent involving both intracellular and extracellular calcium and involves the p38 MAPK upstream of the PI3-kinase/Akt pathway [8]. A calcium-dependent activation of eNOS has been shown in response to the tannin 1-α-*O*-galloylpunicalagin which is associated with the PI3-kinase/Akt pathway [20]. There-

fore, changes in $[Ca^{2+}]_i$ in endothelial cells are likely to contribute to the redox-sensitive activation of eNOS in response to polyphenols via the PI3-kinase/Akt-dependent pathway.

Role of estrogen receptors

It has been shown that low concentrations (in the nanomolar range) of resveratrol, a polyphenolic phytoestrogen found in grapes and wine, as well as black tea polyphenols are able to activate estrogen receptors resulting in activation of p38 MAPK and eNOS in endothelial cells [7, 61] (Fig. 2). In contrast, the estrogen receptor antagonist ICI 182,780 did not alter relaxations to RWPs in intact aortic rings from both male and female rats [55]. In addition, the RWP-induced phosphorylation of Akt and eNOS in porcine coronary artery endothelial cells was not affected by ICI 182,780 [55]. Similar results were observed in the isolated porcine coronary arteries with the Crataegus (Hawthorn species) special extract WS 1442 [6]. Therefore, these data indicate that an estrogen receptor-dependent pathway mediates activation of eNOS in response to some polyphenols including resveratrol and black tea polyphenols, but not to red wine polyphenols and Crataegus polyphenols.

Role of caveolin-1

A recent study has indicated that green tea polyphenols downregulate caveolin-1 protein expression and mRNA levels in both time- and concentration-dependent manners via activation of ERK1/2 and inhibition of p38 MAPK signaling pathways in bovine aortic endothelial cells [66] (Fig. 2). As caveolin-1 is a major negative regulator of eNOS activity, such an effect might contribute to increase eNOS activation by polyphenols.

Polyphenols increase endothelial NO synthase expression

In addition to causing a rapid and sustained activation of eNOS, polyphenols have been shown to increase the expression level of eNOS in cultured endothelial. Indeed, exposure of endothelial cells to red wine increased eNOS expression at both the mRNA and protein levels [105, 107]. The upregulation of eNOS is attributed to polyphenols contained in red wine because ethanol alone has no such effect [107]. Moreover, eNOS upregulation is also observed in response to a red wine extract without alcohol [63]. However, it must be underlined that large concentrations of red wine are required to increase eNOS expression (1% *v/v* in culture medium for 10 days, 3% *v/v* for 24 h, and 10% *v/v* for 12 h) [107]. The analysis of the active polyphenols in red wine indicated the involvement of several polyphenolic compounds, in particular, trans-resveratrol and also to a lesser extent cinnamic and hydroxycinnamic acids, cyanidin, and several phenolic acids [106].

Resveratrol upregulated eNOS expression at concentrations ranging from 10 to 100 μM [49, 105]. The stimulatory effect is mainly mediated by an increase in the activity of the eNOS promoter (transcriptional effect) and a stabilization of eNOS mRNA (post-transcriptional effect) [105]. In addition, lower concentrations of resveratrol such as 0.1 μM have also been shown to increase the expression of eNOS mRNA in cultured human umbilical vein endothelial cells [80]. Moreover, it has been reported that in cultured human coronary arterial endothelial cells, resveratrol upregulates the expression of SIRT1, a NAD^+-dependent protein deacetylase, and induces its enzymatic activation leading to an upregulation of eNOS mRNA expression and mitochondrial biogenesis; all these events might contribute to improve the endothelial function [25, 71].

The evaluation of the effectiveness of 33 different polyphenols (procyanidins, monomeric flavan-3-ols, flavonols, a flavone, a flavanone, a chalcone, a stilbene, and phenolic acids) to enhance eNOS mRNA expression at 100 μM in a hybrid human endothelial cell line EA.hy926 revealed a significant stimulatory effect only in response to four polyphenols including resveratrol, quercetin, epicatechin-gallate, and epigallocatechin-gallate. No clear relationship between the structure of the active polyphenols and the stimulatory activity was observed [9].

Polyphenols induce EDHF-mediated responses in arteries

The component of arterial endothelium-dependent relaxations, which is resistant to inhibitors of NO synthase and cyclooxygenases, has been attributed to EDHF. It is now commonly admitted that the importance of the EDHF phenomenon increases as the vessel size decreases [93]. Several mechanisms have been proposed to explain the nature of the EDHF phenomenon (see related chapters in the present issue), but one hallmark of EDHF-mediated responses is that these relaxations are associated to an endothelium-dependent hyperpolarization of the vascular smooth muscle cells.

The initial demonstration of a participation of EDHF to the endothelium-dependent relaxations to polyphenols has been observed in isolated porcine coronary arteries [78] (Fig. 3). Indeed, RWPs caused concentration-dependent relaxations and hyperpolarizations of vascular smooth muscle cells, at concentrations ranging from 1 to 100 mg/l,

with a maximal effect at 100 mg/l. Thereafter, it was shown that Concord grape juice, a nonalcoholic rich source of grape-derived polyphenols, is also able to induce endothelium-dependent EDHF-mediated relaxations of porcine coronary arteries [5]. EDHF-mediated endothelium-dependent relaxations have also been observed in the isolated, perfused or not, mesenteric arterial bed in response to alcohol-free lyophilized Brazilian red wine [28], an extract of Eucommia bark (a traditional Chinese medicinal herb containing polyphenols) [62], RWPs [26], and açai (Euterpe oleracea Mart.) [89]. Moreover, intercellular communication through gap junctions is involved in the RWP-induced EDHF-mediated relaxation in the rat mesenteric artery [26].

Although resveratrol has been shown to activate IKCa channels (24 pS) in MS1 cell line, a pancreatic islet endothelial cell line, by increasing their open probability [64], other mechanisms have been involved in the polyphenol-induced EDHF-mediated relaxation. Indeed, the RWP-induced EDHF-mediated relaxation and hyperpolarization are strictly dependent on an endothelial redox-sensitive mechanism involving the intracellular formation of superoxide anions in porcine coronary arteries [78] (Fig. 3). EDHF-mediated relaxations are reduced by antioxidants, such as *N*-acetylcysteine, and membrane permeant analogs of superoxide dismutase (SOD), such as Mn(III)tetrakis(1-methyl-4-pyridyl)porphyrin (MnTMPyP) and polyethylene glycol-SOD (PEG-SOD). In addition, exposure of cultured porcine coronary artery endothelial cells to RWPs induced the MnTMPyP-sensitive formation of superoxide anions in the presence of L-NA and indomethacin [78]. Further investigations indicated that the RWP-induced EDHF-mediated relaxation of porcine coronary arteries involves the redox-sensitive activation of PI3-kinase leading to Akt phosphorylation in endothelial cells [79] (Fig. 3). The possibility that the PI3-kinase/Akt pathway modulates myo-endothelial gap junctions and/or potassium channel activity remains to be investigated.

Polyphenols and endothelial function in experimental models of cardiovascular diseases

Since grape-derived products are able to strongly stimulate the endothelial formation of NO and also the EDHF-mediated responses, two potent vasoprotective factors, several studies have assessed their potential to delay the development of major types of cardiovascular diseases characterized by an endothelial dysfunction. These studies have indicated that intake of grape-derived products in the drinking water reduced blood pressure in several experimental models of hypertension including the spontaneously hypertensive rat, the N^G-nitro L-arginine-induced hypertension, the DOCA salt-induced hypertension and the angiotensin II-induced hypertension in rats [16, 53, 85, 90, 93]. Besides normalizing blood pressure in angiotensin II-treated rats, red wine polyphenols prevented the development of an endothelial dysfunction by normalizing the excessive vascular formation of superoxide anions, which react with NO to form peroxynitrites [90]. The protective effect of polyphenols on the endothelial function is

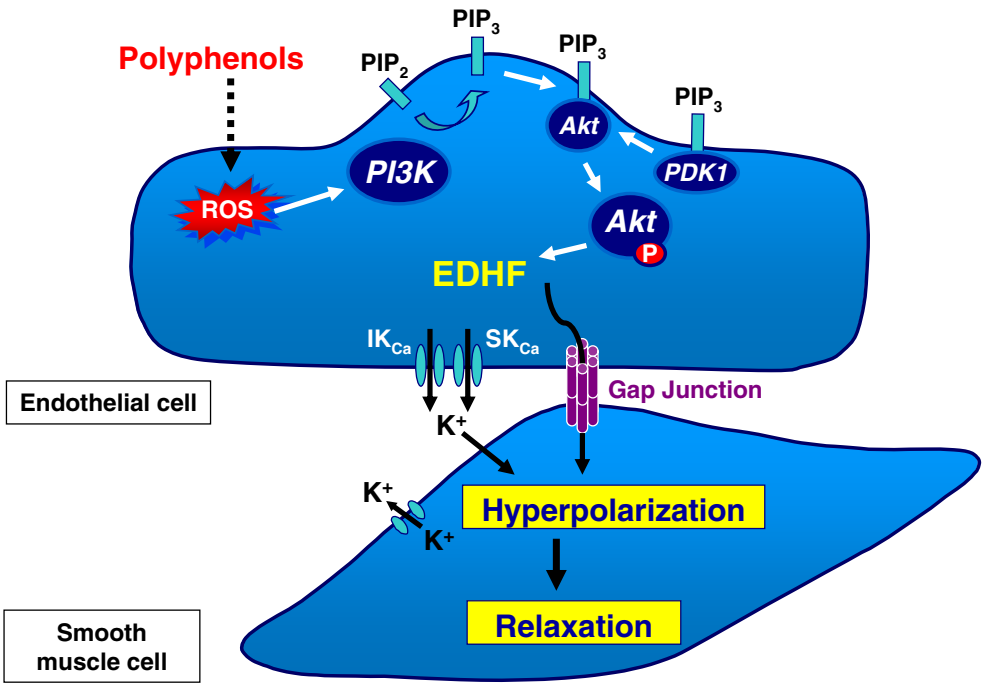

Fig. 3 Schematic indicating that polyphenols are potent inducers of the endothelial formation of EDHF via the PI3-kinase/Akt pathway; *ROS* reactive oxygen species; *PI3K* phosphatidylinositol 3-kinase; *PDK1* phosphoinositide-dependent kinase 1; *EDHF* endothelium-derived hyperpolarizing factor

explained by their ability to prevent the increased vascular expression of NADPH oxidase, a major vascular source of superoxide anions, and the cyclooxygenase-dependent formation of endothelium-derived contracting factors [56, 90] (Fig. 4). Alternatively, the beneficial effect might also be due to the downregulation of angiotensin II type I receptor (AT1) as shown in vascular smooth muscle cells treated with resveratrol [73] (Fig. 4). In the spontaneously hypertensive rat, ingestion of grape-derived polyphenols also reduced blood pressure, and this effect is associated with a reduced vascular formation of superoxide anions, improved cognitive function, and reduced cardiac and ventricular hypertrophies [85]. Moreover, grape-derived extracts prevented the development of hypertension, cardiac hypertrophy, and vascular oxidative stress by reducing NADPH oxidase expression in fructose-fed rats [2, 3]. In addition, intake of grape-derived polyphenols prevented the development of aortic fatty streaks, early lesions of atherosclerosis, in hamsters receiving a high-fat diet, an experimental model of atherosclerosis [11, 12].

A polyphenol-rich cocoa powder (up to 300 mg/kg bodyweight) also reduced blood pressure similarly to 50 mg/kg of captopril, an angiotensin converting enzyme inhibitor, in the spontaneously hypertensive rat [23].

Several studies have also reported an antihypertensive effect in response to several purified polyphenols from fruits and vegetables. Indeed, quercetin, a flavonol found widely in fruits and vegetables, reduced blood pressure in several experimental models of hypertension including spontaneously hypertensive rats, N^G-nitro L-arginine methyl ester-treated rats, two-kidney one clip Goldblatt rats, DOCA-salt, and Dahl salt-sensitive rats [86]. Similarly, genistein, an isoflavone found mainly in vegetables, as well as hesperitin and glucosyl-hesperidin, two flavonones, decreased blood pressure and improved endothelium-dependent relaxations of aortic rings of spontaneously hypertensive rats [104, 112, 113]. Furthermore, catechin prevented endothelial dysfunction in the prediabetic stage of Otsuka Long-Evans Tokushima Fatty rats by reducing vascular NADPH oxidase activity and expression [52].

Altogether, these experimental studies indicate that ingestion of polyphenol-rich products has a beneficial effect in several experimental models of major cardiovascular diseases including hypertension, diabetes, and atherosclerosis. The beneficial effect is associated with the restoration of the vasoprotective effect of endothelial cells most likely by reducing the excessive NADPH oxidase-dependent vascular oxidative stress. In addition, the ability of polyphenols to increase the endothelial formation of NO and the EDHF-mediated responses might also contribute to the vasoprotective effect either directly or indirectly by preventing the oxidative stress-induced inactivation of NO.

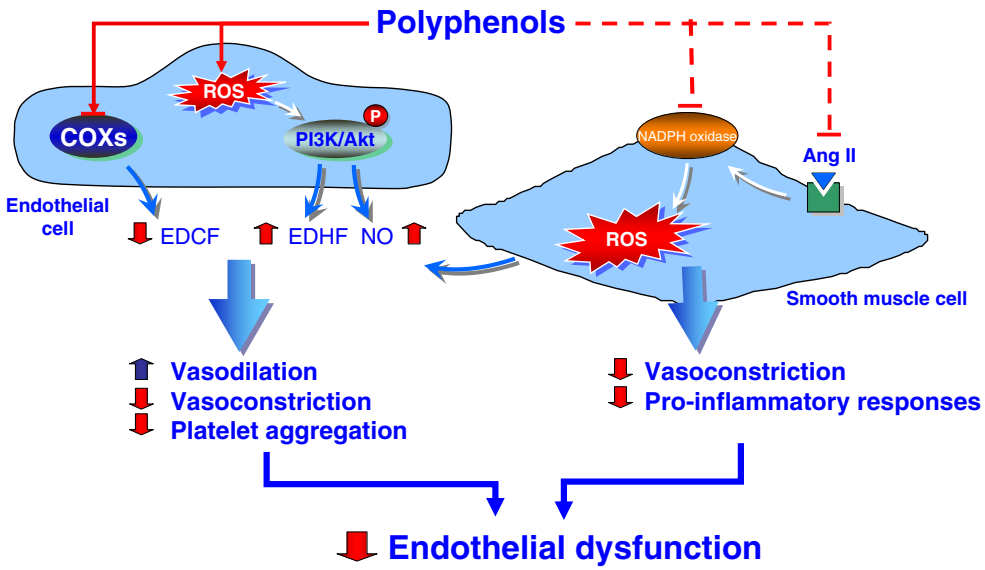

Fig. 4 Schematic summarizing the protective effect of polyphenols on the arterial wall in major types of cardiovascular diseases; polyphenols are able to strongly stimulate the redox-sensitive endothelial formation of NO and EDHF, two potent vasoprotective factors, and to prevent the endothelial formation of cyclooxygenase-derived metabolites of arachidonic acid that act on thromboxan-protanoid receptors at the vascular smooth muscle to cause vasoconstriction. In addition, polyphenols also prevent the excessive vascular formation of reactive oxygen species mostly by normalizing the expression of NADPH oxidase and the angiotensin AT1 receptor in the arterial wall. Both the effect at the endothelial cell and the vascular smooth muscle cell will promote vasodilation and prevent inflammatory responses and activation of platelets. *ROS* reactive oxygen species; *COXs* cyclooxygenases; *PI3K* phosphatidylinositol 3-kinase; *EDCF* endothelium-derived contracting factors; *EDHF* endothelium-derived hyperpolarizing factor; *NO* nitric oxide; *Ang II* angiotensin II

Polyphenols and endothelial function in healthy and diseased humans

In healthy subjects, ingestion of 250 to 500 ml of red wine or de-alcoholized red wine increased, in some but not all studies, flow-mediated vasodilatation as assessed in the brachial artery by plethysmography [1, 31, 47]. Intake of 5–7 ml/kg of purple grape juice by healthy subjects for 7–14 days reduced ex vivo platelet aggregation and increased platelet-derived NO formation [44, 58]. In patients with coronary artery diseases, flow-mediated dilatation was improved after intake of 4 ml/kg of either white or red wine during a light meal [109] and also after intake of about 8 ml/kg of purple grape juice [22, 97]. The beneficial effect of purple grape juice on the endothelial function was associated with a reduced susceptibility of LDL particles to oxidation [97]. In addition, intake of 5.5 ml/kg of purple grape juice daily by hypertensive Korean patients for 8 weeks reduced both systolic and diastolic blood pressure by, respectively, 7.2 and 6.2 mmHg [84]. Besides grape-derived products, an improved endothelial function in patients with coronary artery diseases has been observed in response to intake of short- and long-term black tea consumption [34] and after dietary supplementation with epigallocatechin gallate, a major catechin in tea (300 mg) [110]. In addition, consumption of a flavonoid-rich dark chocolates and cocoa beverages improved endothelial function in healthy subjects, hypercholesterolemic postmenopausal women, and in diabetic patients associated to an increased circulating level of NO species [13, 37, 92, 108]. Moreover, an improved endothelial function was observed after administration of (–)-epicatechin (1 mg/kg, a major flavanol of cocoa) to healthy humans [92]. Chronic intake of flavanol-rich dark chocolate reduced blood pressure in patients with upper-range hypertension or stage 1 hypertension without concomitant risk factors [46, 100].

Conclusion

Altogether, these experimental and clinical studies highlight the potential of polyphenol-rich sources to ameliorate or restore vascular protection in health and disease by enhancing the two major endothelial vasoprotective mechanisms, i.e., the formation of NO and EDHF-mediated responses and also by reducing oxidative stress in the arterial wall which promotes pro-inflammatory and pro-thrombotic responses (Fig. 4).

References

1. Agewall S, Wright S, Doughty RN et al (2000) Does a glass of red wine improve endothelial function? Eur Heart J 21:74–78
2. Al-Awwadi NA, Araiz C, Bornet A et al (2005) Extracts enriched in different polyphenolic families normalize increased cardiac NADPH oxidase expression while having differential effects on insulin resistance, hypertension, and cardiac hypertrophy in high-fructose-fed rats. J Agric Food Chem 53:151–157
3. Al-Awwadi NA, Bornet A, Azay J et al (2004) Red wine polyphenols alone or in association with ethanol prevent hypertension, cardiac hypertrophy, and production of reactive oxygen species in the insulin-resistant fructose-fed rat. J Agric Food Chem 52:5593–5597
4. Andriambeloson E, Kleschyov AL, Muller B et al (1997) Nitric oxide production and endothelium-dependent vasorelaxation induced by wine polyphenols in rat aorta. Br J Pharmacol 120:1053–1058
5. Anselm E, Chataigneau M, Ndiaye M et al (2007) Grape juice causes endothelium-dependent relaxation via a redox-sensitive Src- and Akt-dependent activation of eNOS. Cardiovasc Res 73:404–413
6. Anselm E, Socorro VF, Dal-Ros S et al (2009) Crataegus special extract WS 1442 causes endothelium-dependent relaxation via a redox-sensitive Src- and Akt-dependent activation of endothelial NO synthase but not via activation of estrogen receptors. J Cardiovasc Pharmacol 53:253–260
7. Anter E, Chen K, Shapira OM et al (2005) 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 96:1072–1078
8. Anter E, Thomas SR, Schulz E et al (2004) Activation of endothelial nitric-oxide synthase by the p38 MAPK in response to black tea polyphenols. J Biol Chem 279:46637–46643
9. Appeldoorn MM, Venema DP, Peters TH et al (2009) Some phenolic compounds increase the nitric oxide level in endothelial cells in vitro. J Agric Food Chem 57:7693–7699
10. Arts IC, Hollman PC (2005) Polyphenols and disease risk in epidemiologic studies. Am J Clin Nutr 81:317S–325S
11. Auger C, Caporiccio B, Landrault N et al (2002) Red wine phenolic compounds reduce plasma lipids and apolipoprotein B and prevent early aortic atherosclerosis in hypercholesterolemic golden Syrian hamsters (Mesocricetus auratus). J Nutr 132:1207–1213
12. Auger C, Gerain P, Laurent-Bichon F et al (2004) Phenolics from commercialized grape extracts prevent early atherosclerotic lesions in hamsters by mechanisms other than antioxidant effect. J Agric Food Chem 52:5297–5302
13. Balzer J, Rassaf T, Heiss C et al (2008) Sustained benefits in vascular function through flavanol-containing cocoa in medicated diabetic patients a double-masked, randomized, controlled trial. J Am Coll Cardiol 51:2141–2149
14. Baraboi VA, Stoian OP (1968) Effect of natrium gallate and some other polyphenols on systemic arterial pressure and tonus of vessels of the skeletal muscles in cats. Fiziol Zh 14:782–790
15. Bell DR, Gochenaur K (2006) Direct vasoactive and vasoprotective properties of anthocyanin-rich extracts. J Appl Physiol 100:1164–1170
16. Bernatova I, Pechanova O, Babal P et al (2002) Wine polyphenols improve cardiovascular remodeling and vascular function in NO-deficient hypertension. Am J Physiol Heart Circ Physiol 282:H942–H948
17. Burns J, Gardner PT, O'Neil J et al (2000) Relationship among antioxidant activity, vasodilation capacity, and phenolic content of red wines. J Agric Food Chem 48:220–230
18. Caulin-Glaser T, Garcia-Cardena G, Sarrel P et al (1997) 17 beta-estradiol regulation of human endothelial cell basal nitric oxide release, independent of cytosolic Ca2+ mobilization. Circ Res 81:885–892
19. Chen G, Suzuki H, Weston AH (1988) Acetylcholine releases endothelium-derived hyperpolarizing factor and EDRF from rat blood vessels. Br J Pharmacol 95:1165–1174

20. Chen LG, Liu YC, Hsieh CW et al (2008) Tannin 1-alpha-O-galloylpunicalagin induces the calcium-dependent activation of endothelial nitric-oxide synthase via the phosphatidylinositol 3-kinase/Akt pathway in endothelial cells. Mol Nutr Food Res 52:1162–1171
21. Chen ZY, Zhang ZS, Kwan KY et al (1998) Endothelium-dependent relaxation induced by hawthorn extract in rat mesenteric artery. Life Sci 63:1983–1991
22. Chou EJ, Keevil JG, Aeschlimann S et al (2001) Effect of ingestion of purple grape juice on endothelial function in patients with coronary heart disease. Am J Cardiol 88:553–555
23. Cienfuegos-Jovellanos E, Quinones MM, Muguerza B et al (2009) Antihypertensive effect of a polyphenol-rich cocoa powder industrially processed to preserve the original flavonoids of the cocoa beans. J Agric Food Chem 57:6156–6162
24. Cishek MB, Galloway MT, Karim M et al (1997) Effect of red wine on endothelium-dependent relaxation in rabbits. Clin Sci (Lond) 93:507–511
25. Csiszar A, Labinskyy N, Pinto JT et al (2009) Resveratrol induces mitochondrial biogenesis in endothelial cells. Am J Physiol Heart Circ Physiol 297:H13–H20
26. Dal-Ros S, Bronner C, Schott C et al (2009) Angiotensin II-induced hypertension is associated with a selective inhibition of endothelium-derived hyperpolarizing factor-mediated responses in the rat mesenteric artery. J Pharmacol Exp Ther 328:478–486
27. David-Dufilho M, Privat C, Brunet A et al (2001) Transition metals and nitric oxide production in human endothelial cells. C R Acad Sci III 324:13–21
28. de Moura RS, Miranda DZ, Pinto AC et al (2004) Mechanism of the endothelium-dependent vasodilation and the antihypertensive effect of Brazilian red wine. J Cardiovasc Pharmacol 44:302–309
29. Di CA, Rotondo S, Iacoviello L et al (2002) Meta-analysis of wine and beer consumption in relation to vascular risk. Circulation 105:2836–2844
30. Dimmeler S, Fleming I, Fisslthaler B et al (1999) Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. Nature 399:601–605
31. Djousse L, Ellison RC, McLennan CE et al (1999) Acute effects of a high-fat meal with and without red wine on endothelial function in healthy subjects. Am J Cardiol 84:660–664
32. Dresse A, Lecomte J (1960) Action of polyphenols and of metanephrine on the myocardial effects of adrenalin. C R Seances Soc Biol Fil 154:851–854
33. Duarte J, Jimenez R, Villar IC et al (2001) Vasorelaxant effects of the bioflavonoid chrysin in isolated rat aorta. Planta Med 67:567–569
34. Duffy SJ, Vita JA, Holbrook M et al (2001) Effect of acute and chronic tea consumption on platelet aggregation in patients with coronary artery disease. Arterioscler Thromb Vasc Biol 21:1084–1089
35. Edirisinghe I, Burton-Freeman B, Tissa KC (2008) Mechanism of the endothelium-dependent relaxation evoked by a grape seed extract. Clin Sci (Lond) 114:331–337
36. Edirisinghe I, Burton-Freeman B, Varelis P et al (2008) Strawberry extract caused endothelium-dependent relaxation through the activation of PI3 kinase/Akt. J Agric Food Chem 56:9383–9390
37. Engler MB, Engler MM, Chen CY et al (2004) Flavonoid-rich dark chocolate improves endothelial function and increases plasma epicatechin concentrations in healthy adults. J Am Coll Nutr 23:197–204
38. Feletou M, Vanhoutte PM (1988) Endothelium-dependent hyperpolarization of canine coronary smooth muscle. Br J Pharmacol 93:515–524
39. Fitzpatrick DF, Bing B, Rohdewald P (1998) Endothelium-dependent vascular effects of Pycnogenol. J Cardiovasc Pharmacol 32:509–515
40. Fitzpatrick DF, Fleming RC, Bing B et al (2000) Isolation and characterization of endothelium-dependent vasorelaxing compounds from grape seeds. J Agric Food Chem 48:6384–6390
41. Fitzpatrick DF, Hirschfield SL, Coffey RG (1993) Endothelium-dependent vasorelaxing activity of wine and other grape products. Am J Physiol Heart Circ Physiol 265:H774–H778
42. Fitzpatrick DF, Hirschfield SL, Ricci T et al (1995) Endothelium-dependent vasorelaxation caused by various plant extracts. J Cardiovasc Pharmacol 26:90–95
43. Flesch M, Schwarz A, Bohm M (1998) Effects of red and white wine on endothelium-dependent vasorelaxation of rat aorta and human coronary arteries. Am J Physiol 275:H1183–H1190
44. Freedman JE, Parker C III, Li L et al (2001) Select flavonoids and whole juice from purple grapes inhibit platelet function and enhance nitric oxide release. Circulation 103:2792–2798
45. Furchgott RF, Zawadzki JV (1980) The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288:373–376
46. Grassi D, Necozione S, Lippi C et al (2005) Cocoa reduces blood pressure and insulin resistance and improves endothelium-dependent vasodilation in hypertensives. Hypertension 46:398–405
47. Hashimoto M, Kim S, Eto M et al (2001) Effect of acute intake of red wine on flow-mediated vasodilatation of the brachial artery. Am J Cardiol 88:1457–1460
48. Hertog MG, Kromhout D, Aravanis C et al (1995) Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. Arch Intern Med 155:381–386
49. Hsieh TC, Juan G, Darzynkiewicz Z et al (1999) Resveratrol increases nitric oxide synthase, induces accumulation of p53 and p21(WAF1/CIP1), and suppresses cultured bovine pulmonary artery endothelial cell proliferation by perturbing progression through S and G2. Cancer Res 59:2596–2601
50. Hu JP, Calomme M, Lasure A et al (1995) Structure-activity relationship of flavonoids with superoxide scavenging activity. Biol Trace Elem Res 47:327–331
51. Ignarro LJ, Buga GM, Wood KS et al (1987) Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. Proc Natl Acad Sci U S A 84:9265–9269
52. Ihm SH, Lee JO, Kim SJ et al (2009) Catechin prevents endothelial dysfunction in the prediabetic stage of OLETF rats by reducing vascular NADPH oxidase activity and expression. Atherosclerosis 206:47–53
53. Jimenez R, Lopez-Sepulveda R, Kadmiri M et al (2007) Polyphenols restore endothelial function in DOCA-salt hypertension: role of endothelin-1 and NADPH oxidase. Free Radic Biol Med 43:462–473
54. Jin BH, Qian LB, Chen S et al (2009) Apigenin protects endothelium-dependent relaxation of rat aorta against oxidative stress. Eur J Pharmacol 616:200–205
55. Kane MO, Anselm E, Rattmann YD et al (2009) Role of gender and estrogen receptors in the rat aorta endothelium-dependent relaxation to red wine polyphenols. Vascul Pharmacol 51:140–146
56. Kane M, Etienne-Selloum N, Madeira S et al (2010) Endothelium-derived contracting factors mediate the Ang II-induced endothelial dysfunction in the rat aorta: preventive effect of red wine polyphenols. Pflügers Arch, in press. doi:10.1007/s00424-009-0759-7
57. Karim M, McCormick K, Kappagoda CT (2000) Effects of cocoa extracts on endothelium-dependent relaxation. J Nutr 130:2105S–2108S
58. Keevil JG, Osman HE, Reed JD et al (2000) Grape juice, but not orange juice or grapefruit juice, inhibits human platelet aggregation. J Nutr 130:53–56

59. Kim JA, Formoso G, Li Y et al (2007) Epigallocatechin gallate, a green tea polyphenol, mediates NO-dependent vasodilation using signaling pathways in vascular endothelium requiring reactive oxygen species and Fyn. J Biol Chem 282:13736–13745
60. Kim SH, Kang KW, Kim KW et al (2000) Procyanidins in crataegus extract evoke endothelium-dependent vasorelaxation in rat aorta. Life Sci 67:121–131
61. Klinge CM, Blankenship KA, Risinger KE et al (2005) Resveratrol and estradiol rapidly activate MAPK signaling through estrogen receptors alpha and beta in endothelial cells. J Biol Chem 280:7460–7468
62. Kwan CY, Zhang WB, Deyama T et al (2004) Endothelium-dependent vascular relaxation induced by Eucommia ulmoides Oliv. bark extract is mediated by NO and EDHF in small vessels. Naunyn Schmiedebergs Arch Pharmacol 369:206–211
63. Leikert JF, Rathel TR, Wohlfart P et al (2002) Red wine polyphenols enhance endothelial nitric oxide synthase expression and subsequent nitric oxide release from endothelial cells. Circulation 106:1614–1617
64. Li HF, Chen SA, Wu SN (2000) Evidence for the stimulatory effect of resveratrol on Ca(2+)-activated K+ current in vascular endothelial cells. Cardiovasc Res 45:1035–1045
65. Li HF, Tian ZF, Qiu XQ et al (2006) A study of mechanisms involved in vasodilatation induced by resveratrol in isolated porcine coronary artery. Physiol Res 55:365–372
66. Li Y, Ying C, Zuo X et al (2009) Green tea polyphenols down-regulate caveolin-1 expression via ERK1/2 and p38MAPK in endothelial cells. J Nutr Biochem 20:1021–1027
67. Lorenz M, Wessler S, Follmann E et al (2004) 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 279:6190–6195
68. Madeira SV, Auger C, Anselm E et al (2009) eNOS activation induced by a polyphenol-rich grape skin extract in porcine coronary arteries. J Vasc Res 46:406–416
69. Martin S, Andriambeloson E, Takeda K et al (2002) Red wine polyphenols increase calcium in bovine aortic endothelial cells: a basis to elucidate signalling pathways leading to nitric oxide production. Br J Pharmacol 135:1579–1587
70. Matsui T, Korematsu S, Byun EB et al (2009) Apple procyanidins induced vascular relaxation in isolated rat aorta through NO/cGMP pathway in combination with hyperpolarization by multiple K+ channel activations. Biosci Biotechnol Biochem 73:2246–2251
71. Mattagajasingh I, Kim CS, Naqvi A et al (2007) SIRT1 promotes endothelium-dependent vascular relaxation by activating endothelial nitric oxide synthase. Proc Natl Acad Sci U S A 104:14855–14860
72. Michell BJ, Griffiths JE, Mitchelhill KI et al (1999) The Akt kinase signals directly to endothelial nitric oxide synthase. Curr Biol 9:845–848
73. Miyazaki R, Ichiki T, Hashimoto T et al (2008) SIRT1, a longevity gene, downregulates angiotensin II type 1 receptor expression in vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 28:1263–1269
74. Mombouli JV, Vanhoutte PM (1999) Endothelial dysfunction: from physiology to therapy. J Mol Cell Cardiol 31:61–74
75. Mukamal KJ, Maclure M, Muller JE et al (2002) Tea consumption and mortality after acute myocardial infarction. Circulation 105:2476–2481
76. Mullen W, McGinn J, Lean ME et al (2002) Ellagitannins, flavonoids, and other phenolics in red raspberries and their contribution to antioxidant capacity and vasorelaxant properties. J Agric Food Chem 50:5191–5196
77. Ndiaye M, Chataigneau M, Lobysheva I et al (2005) Red wine polyphenol-induced, endothelium-dependent NO-mediated relaxation is due to the redox-sensitive PI3-kinase/Akt-dependent phosphorylation of endothelial NO-synthase in the isolated porcine coronary artery. FASEB J 19:455–457
78. Ndiaye M, Chataigneau T, Andriantsitohaina R et al (2003) Red wine polyphenols cause endothelium-dependent EDHF-mediated relaxations in porcine coronary arteries via a redox-sensitive mechanism. Biochem Biophys Res Commun 310:371–377
79. Ndiaye M, Chataigneau T, Chataigneau M et al (2004) Red wine polyphenols induce EDHF-mediated relaxations in porcine coronary arteries through the redox-sensitive activation of the PI3-kinase/Akt pathway. Br J Pharmacol 142:1131–1136
80. Nicholson SK, Tucker GA, Brameld JM (2008) Effects of dietary polyphenols on gene expression in human vascular endothelial cells. Proc Nutr Soc 67:42–47
81. Nijveldt RJ, van Noods E, van Hoorn DE et al (2001) Flavonoids: a review of probable mechanisms of action and potential applications. Am J Clin Nutr 74:418–425
82. Orallo F, Alvarez E, Camina M et al (2002) The possible implication of trans-Resveratrol in the cardioprotective effects of long-term moderate wine consumption. Mol Pharmacol 61:294–302
83. Palmer RM, Ferrige AG, Moncada S (1987) Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 327:524–526
84. Park YK, Kim JS, Kang MH (2004) Concord grape juice supplementation reduces blood pressure in Korean hypertensive men: double-blind, placebo controlled intervention trial. Biofactors 22:145–147
85. Peng N, Clark JT, Prasain J et al (2005) Antihypertensive and cognitive effects of grape polyphenols in estrogen-depleted, female, spontaneously hypertensive rats. Am J Physiol Regul Integr Comp Physiol 289:R771–R775
86. Perez-Vizcaino F, Duarte J, Jimenez R et al (2009) Antihypertensive effects of the flavonoid quercetin. Pharmacol Rep 61:67–75
87. Rakici O, Kiziltepe U, Coskun B et al (2005) Effects of resveratrol on vascular tone and endothelial function of human saphenous vein and internal mammary artery. Int J Cardiol 105:209–215
88. Robak J, Gryglewski RJ (1988) Flavonoids are scavengers of superoxide anions. Biochem Pharmacol 37:837–841
89. Rocha AP, Carvalho LC, Sousa MA et al (2007) Endothelium-dependent vasodilator effect of Euterpe oleracea Mart. (Acai) extracts in mesenteric vascular bed of the rat. Vascul Pharmacol 46:97–104
90. Sarr M, Chataigneau M, Martins S et al (2006) Red wine polyphenols prevent angiotensin II-induced hypertension and endothelial dysfunction in rats: role of NADPH oxidase. Cardiovasc Res 71:794–802
91. Schini VB, Boulanger C, Regoli D et al (1990) Bradykinin stimulates the production of cyclic GMP via activation of B2 kinin receptors in cultured porcine aortic endothelial cells. J Pharmacol Exp Ther 252:581–585
92. Schroeter H, Heiss C, Balzer J et al (2006) (–)–Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans. Proc Natl Acad Sci U S A 103:1024–1029
93. Shimokawa H, Yasutake H, Fujii K et al (1996) The importance of the hyperpolarizing mechanism increases as the vessel size decreases in endothelium-dependent relaxations in rat mesenteric circulation. J Cardiovasc Pharmacol 28:703–711
94. De Soares MR, Costa Viana FS, Souza MA et al (2002) Antihypertensive, vasodilator and antioxidant effects of a vinifera grape skin extract. J Pharm Pharmacol 54:1515–1520

95. Sofi F, Cesari F, Abbate R et al (2008) Adherence to Mediterranean diet and health status: meta-analysis. BMJ 337: a1344
96. St Leger AS, Cochrane AL, Moore F (1979) Factors associated with cardiac mortality in developed countries with particular reference to the consumption of wine. Lancet 1:1017–1020
97. Stein JH, Keevil JG, Wiebe DA et al (1999) Purple grape juice improves endothelial function and reduces the susceptibility of LDL cholesterol to oxidation in patients with coronary artery disease. Circulation 100:1050–1055
98. Stoclet JC, Kleschyov A, Andriambeloson E et al (1999) Endothelial no release caused by red wine polyphenols. J Physiol Pharmacol 50:535–540
99. Taubert D, Berkels R, Klaus W et al (2002) Nitric oxide formation and corresponding relaxation of porcine coronary arteries induced by plant phenols: essential structural features. J Cardiovasc Pharmacol 40:701–713
100. Taubert D, Berkels R, Roesen R et al (2003) Chocolate and blood pressure in elderly individuals with isolated systolic hypertension. JAMA 290:1029–1030
101. Taylor SG, Weston AH (1988) Endothelium-derived hyperpolarizing factor: a new endogenous inhibitor from the vascular endothelium. Trends Pharmacol Sci 9:272–274
102. Torel J, Cillard J, Cillard P (1986) Antioxidant activity of flavonoids and reactivity with peroxy radical. Phytochemistry 25:383–385
103. Vera R, Galisteo M, Villar IC et al (2005) Soy isoflavones improve endothelial function in spontaneously hypertensive rats in an estrogen-independent manner: role of nitric-oxide synthase, superoxide, and cyclooxygenase metabolites. J Pharmacol Exp Ther 314:1300–1309
104. Vera R, Sanchez M, Galisteo M et al (2007) Chronic administration of genistein improves endothelial dysfunction in spontaneously hypertensive rats: involvement of eNOS, caveolin and calmodulin expression and NADPH oxidase activity. Clin Sci (Lond) 112:183–191
105. Wallerath T, Deckert G, Ternes T et al (2002) Resveratrol, a polyphenolic phytoalexin present in red wine, enhances expression and activity of endothelial nitric oxide synthase. Circulation 106:1652–1658
106. Wallerath T, Li H, Godtel-Ambrust U et al (2005) A blend of polyphenolic compounds explains the stimulatory effect of red wine on human endothelial NO synthase. Nitric Oxide 12:97–104
107. Wallerath T, Poleo D, Li H et al (2003) Red wine increases the expression of human endothelial nitric oxide synthase: a mechanism that may contribute to its beneficial cardiovascular effects. J Am Coll Cardiol 41:471–478
108. Wang-Polagruto JF, Villablanca AC, Polagruto JA et al (2006) Chronic consumption of flavanol-rich cocoa improves endothelial function and decreases vascular cell adhesion molecule in hypercholesterolemic postmenopausal women. J Cardiovasc Pharmacol 47(Suppl 2):S177–S186
109. Whelan AP, Sutherland WH, McCormick MP et al (2004) Effects of white and red wine on endothelial function in subjects with coronary artery disease. Intern Med J 34:224–228
110. Widlansky ME, Hamburg NM, Anter E et al (2007) Acute EGCG supplementation reverses endothelial dysfunction in patients with coronary artery disease. J Am Coll Nutr 26:95–102
111. Xu PH, Long Y, Dai F et al (2007) The relaxant effect of curcumin on porcine coronary arterial ring segments. Vascul Pharmacol 47:25–30
112. Yamamoto M, Suzuki A, Hase T (2008) Short-term effects of glucosyl hesperidin and hesperetin on blood pressure and vascular endothelial function in spontaneously hypertensive rats. J Nutr Sci Vitaminol (Tokyo) 54:95–98
113. Yamamoto M, Suzuki A, Jokura H et al (2008) Glucosyl hesperidin prevents endothelial dysfunction and oxidative stress in spontaneously hypertensive rats. Nutrition 24:470–476