

## Review Article

# Molecular mechanisms of the cardiovascular protective effects of polyphenols

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### Abstract

Epidemiological studies have reported a greater reduction in cardiovascular risk and metabolic disorders associated with diets rich in polyphenols. The antioxidant effects of polyphenols are attributed to the regulation of redox enzymes by reducing reactive oxygen species production from mitochondria, NADPH oxidases and uncoupled endothelial NO synthase in addition to also up-regulating multiple antioxidant enzymes. Although data supporting the effects of polyphenols in reducing oxidative stress are promising, several studies have suggested additional mechanisms in the health benefits of polyphenols. Polyphenols from red wine increase endothelial NO production leading to endothelium-dependent relaxation in conditions such as hypertension, stroke or the metabolic syndrome. Numerous molecules contained in fruits and vegetables can activate sirtuins to increase lifespan and silence metabolic and physiological disturbances associated with endothelial NO dysfunction. Although intracellular pathways involved in the endothelial effects of polyphenols are partially described, the molecular targets of these polyphenols are not completely elucidated. We review the novel aspects of polyphenols on several targets that could trigger the health benefits of polyphenols in conditions such as metabolic and cardiovascular disturbances.

**Key words:** Polyphenols: Cardiovascular system: Nitric oxide: Endothelium: Free radicals: Antioxidants

Polyphenols are found mainly in plant-derived foods and beverages, and provide the tastes and colour of plant foods while also participating in plant defensive responses against stress due to UV radiation, pathogens and physical damage. There are a number of excellent reviews dealing with their protective effect against cancers, cardiovascular, metabolic<sup>(1)</sup> and neurodegenerative diseases<sup>(2)</sup>. The structures of polyphenols vary from a simple phenol core to complex molecules with a high degree of polymerisation. This family can be divided into simple phenols, flavonoids and non-flavonoids such as stilbene (resveratrol), saponin, curcumin and tannins. Flavonoids can be subdivided according to their substituents

into flavanols (catechin and epicatechin), flavonols (quercetin, myricetin and kaempferol), anthocyanidins (cyanidin and delphinidin), flavones (apigenin and diosmin), flavanones (naringenin and hesperetin) and chalcones (phloretin).

Dietary intake of polyphenols is highly variable. In the USA, the intake in 1976 was estimated at 1 g of glycosylated flavonoids per d<sup>(3)</sup>. A Dutch study in 1987–88 established lower amounts of flavanols and flavones of approximately 23 mg/d<sup>(4)</sup>, but of the aglycone forms. In a cohort of US women, the baseline mean intake of flavonols and flavone was 21.2 mg/d, with quercetin (15.4 mg/d) being the major contributor<sup>(5)</sup>. The daily intake of anthocyanins in the USA is

**Abbreviations:** ACE, angiotensin-converting enzyme; AMPK, AMP-activated protein kinase; BH<sub>4</sub>, tetrahydrobiopterin; COX, cyclo-oxygenase; EDHF, endothelium-derived hyperpolarising factor; eNOS, endothelial NO synthase; ER, oestrogen receptor; GPx, glutathione peroxidase; HUVEC, human umbilical vein endothelial cells; KO, knockout; NOX, NADPH oxidase; Nrf2, nuclear factor E<sub>2</sub>-related factor-2; PGC, PPAR $\gamma$  coactivator; ROS, reactive oxygen species; SHR, spontaneously hypertensive rats; siRNA, small-interfering RNA; SIRT1, sirtuin 1; SOD, superoxide dismutase.

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estimated to be 12.5 mg/d per person, with delphinidin contributing approximately 21% of the total anthocyanin intake<sup>(6)</sup>. An accurate estimate of dietary intake of polyphenols is difficult to achieve because of the poor characterisation of polyphenols in foods and the great variability of polyphenol content within foods<sup>(7)</sup>. The cardiovascular effects of polyphenols have mostly been studied using extracts of polyphenols in foods and drinks. Several other studies have used purified resveratrol, quercetin and delphinidin to examine the cardiovascular effects of these components of polyphenols.

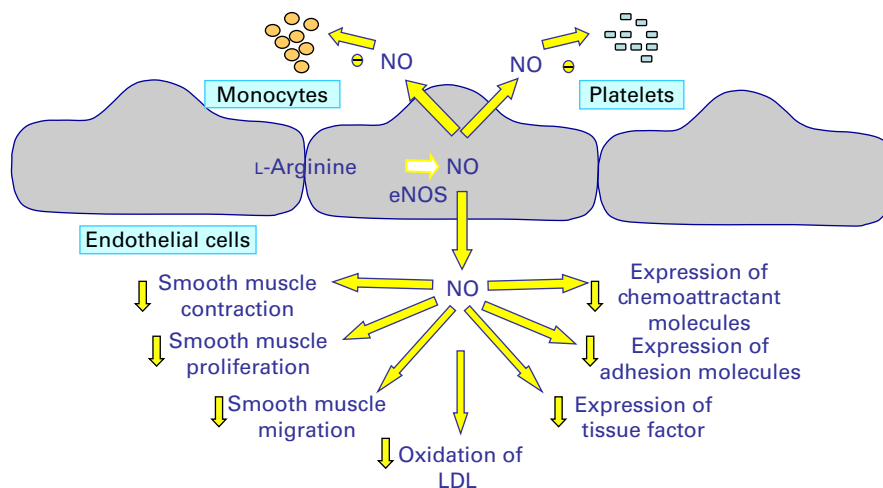
We summarise the cardiovascular effects and the mechanisms implicated in the health benefits associated with resveratrol, quercetin and delphinidin, by comparing their *in vitro* effects on isolated cell systems and their *in vivo* repercussions related to their absorption and bioavailability. It should be noted that most *in vitro* studies have shown health benefits at high concentrations (1–100 μm), with plasma concentrations of polyphenols being approximately 1–20 nM<sup>(8)</sup>. Thus, despite their high absorption, bioavailability is low in humans and precautions concerning the conclusions of published studies are warranted.

### Endothelial cells and the regulation of vascular homeostasis

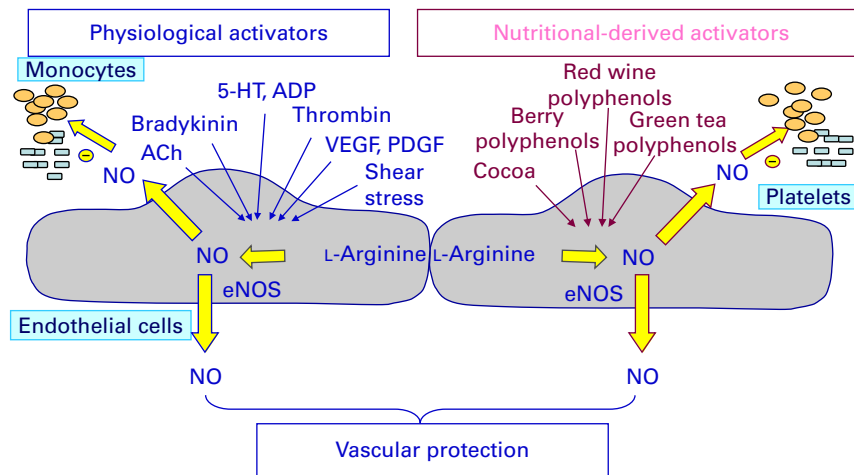
Endothelial cells of healthy blood vessels form a monolayer at the luminal surface to provide chemically mediated control of vascular homeostasis. Due to their strategic localisation, these cells prevent the contact of circulating blood with the underlying prothrombotic arterial wall. Endothelial cells play a critical role in the control of vascular tone via the release of relaxing factors such as NO, endothelium-derived hyperpolarising factor (EDHF) and PGI<sub>2</sub>. The gaseous molecule NO is generated from L-arginine by the enzyme endothelial NO synthase (eNOS) and diffuses towards the underlying

vascular smooth muscle cell layer to dilate blood vessels in a cyclic guanylyl monophosphate-dependent manner (Fig. 1). NO can also diffuse towards the lumen to prevent platelet adhesion and activation, and also monocyte adhesion. In addition, NO prevents the expression of prothrombotic and proatherosclerotic mediators including tissue factor, the physiological activator of the coagulation cascade, adhesion molecules, chemoattractant factors and the oxidation of LDL (Fig. 1). A prominent role exists for EDHF in the control of resistance artery tone by hyperpolarising vascular smooth muscle. PGI<sub>2</sub>, generated by the arachidonic acid cascade via cyclo-oxygenases (COX), activates the cyclic AMP pathway during its vasodilator activity. The endothelial formation of vasoprotective factors can be increased within seconds by several stimuli including neurohumoral substances, products released during the degranulation of activated platelets or during the coagulation cascade, and by shear stress at the endothelial cell surface (Figs. 1 and 2)<sup>(9)</sup>. Many CVD such as hypertension, hypercholesterolaemia and the metabolic syndrome are characterised by an endothelial dysfunction as indicated by reduced endothelium-dependent vasodilatation subsequent to a reduced bioavailability of NO. In addition, ageing in humans and animal models is also associated with a progressive decline of endothelium-dependent vasodilatation<sup>(10)</sup>.

Endothelial dysfunction is often associated with pronounced oxidative stress that is due, at least in part, to an increased expression of NADPH oxidase, an enzyme generating superoxide anions in the arterial wall<sup>(11–13)</sup>. Superoxide anions react with NO to reduce its bioavailability and, hence, vascular protective effects. Endothelial dysfunction is frequently associated with the emergence of endothelium-dependent contractile responses involving the unopposed contractile actions of endothelin and vasoconstrictor factors acting on thromboxane receptors<sup>(14)</sup>.



**Fig. 1.** Endothelium-derived NO contributes to the regulation of vascular homeostasis. In healthy blood vessels, endothelial cells release NO, which is produced from L-arginine by endothelial NO synthase (eNOS). NO diffuses towards the underlying vascular smooth muscle to reduce vascular tone and keep smooth muscle cells in a non-migratory and non-proliferative state. NO can also diffuse towards the lumen where at the surface of endothelial cells, it prevents platelet adhesion and aggregation, and adhesion of monocytes. In addition, NO is also a potent inhibitor of the expression of several proatherothrombotic molecules such as tissue factor, chemoattractant molecules such as monocyte chemoattractant protein-1, and adhesion molecules such as vascular cell adhesion molecule-1. Moreover, NO retards the oxidation of LDL, a key step in the development of atherosclerosis. (A colour version of this figure can be found online at <http://www.journals.cambridge.org/bjn>)



**Fig. 2.** Endothelial formation of NO can be increased within seconds in response to numerous physiological activators such as neurohumoral substances, platelet-derived products, products generated by the coagulation cascade, growth factors and shear stress induced by the flowing blood on the endothelial surface. In addition, the endothelial formation of NO can also be stimulated in response to several nutritional-derived products including cocoa, berry polyphenols, red wine polyphenols and green tea polyphenols. eNOS, endothelial NO synthase; ACh, acetylcholine; 5-HT, serotonin; VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor. (A colour version of this figure can be found online at <http://www.journals.cambridge.org/bjn>)

Diets such as the Mediterranean diet are associated with improved cardiovascular health<sup>(15)</sup>, which may be related to the high intake of polyphenol-rich beverages and foods, and fruit and vegetables. The intake of polyphenol-rich sources such as red wine, cocoa, green tea and berries also improves cardiovascular health<sup>(16,17)</sup>. The beneficial effects of polyphenols on the cardiovascular system have been attributed to mechanisms such as improved lipid profiles, anti-atherosclerotic, anti-hypertensive and anti-inflammatory effects, and direct actions on endothelial cells (Fig. 2).

#### Vascular protection by extracts of polyphenols

That polyphenols cause endothelium-dependent relaxations was first observed by Fitzpatrick *et al.*<sup>(18)</sup>, where some wines, grape juices and grape skin extracts caused endothelium-dependent relaxations in aortic rings. Other studies confirmed that polyphenol-rich sources such as extracts from red wines, green and black tea, and several plants caused endothelium-dependent relaxations in large arteries, arterioles and veins that were prevented by competitive inhibitors of eNOS and guanylyl cyclase<sup>(18,19)</sup>. Direct proof that polyphenols (1 µg/ml) stimulate endothelial formation of NO was obtained using electron paramagnetic resonance spectroscopy using rat aortic rings and cultured endothelial cells<sup>(20)</sup>. However, in porcine coronary arteries, red wine extract-induced relaxation was only partially prevented by a competitive inhibitor of eNOS but abolished by the addition of the combination charybdotoxin plus apamin, two inhibitors of the EDHF-mediated relaxation, indicating the involvement of both NO and EDHF<sup>(21)</sup>. The endothelium-dependent relaxation induced by red wine polyphenols is observed at concentrations of 3 µg/ml (or greater) in porcine coronary artery rings<sup>(21)</sup>. Although the concentration of red wine polyphenols in the blood after the intake of red wine remains unknown, estimates are that an intake of 100 ml of

red wine by healthy volunteers increases plasma concentrations of polyphenolic monomers to 2.5 µg/ml<sup>(22)</sup>. Thus, the stimulatory effect of red wine polyphenols on NO levels is observed at plasma concentrations likely to be achieved with the moderate consumption of red wine.

The signal transduction pathway mediating the stimulatory effect of polyphenols on eNOS suggests a key role of an intracellular redox-sensitive mechanism<sup>(23)</sup>. Thus, vasodilation to red wine polyphenols, purple grape juice and grape skin extracts are reduced by membrane-permeant analogues of superoxide dismutase (SOD) and also to some extent by a membrane-permeant analogue of catalase<sup>(24)</sup>. Exposure of cultured endothelial cells to polyphenols increased the intracellular formation of reactive oxygen species (ROS)<sup>(21)</sup>. ROS can trigger the activation of sarcoma oncogene homolog (Src) kinase by phosphorylation, which subsequently leads to a phosphatidylinositol-3-kinase-dependent activation of Akt by phosphorylation, which ultimately causes the phosphorylation of eNOS at Ser1177 to increase its activity in response to polyphenols<sup>(21,25)</sup>. Changes in Ca signalling and oestrogen receptor (ER) function also contribute to eNOS activation caused by some polyphenols<sup>(26)</sup>.

#### Polyphenol extracts reduce CVD

Polyphenols prevent and/or improve endothelial dysfunction and reduce blood pressure in spontaneously hypertensive rats (SHR)<sup>(27,28)</sup>, and in deoxycorticosterone acetate salt<sup>(29)</sup>, the N<sup>ω</sup>-nitro-L-arginine<sup>(30)</sup> and angiotensin II<sup>(31)</sup> hypertension models. In the latter case, ingestion of 150 mg/kg per d of a red wine polyphenol extract in the drinking-water reduced hypertension induced by angiotensin II in rats (0.4 mg/kg per d for 28 d)<sup>(31)</sup>. Angiotensin II-induced hypertension was associated with blunted endothelium-dependent vasodilation that was reversed by the ingestion of red wine polyphenols. Moreover, angiotensin II-induced hypertension also increased

oxidative stress due to the increased formation of ROS in the arterial wall through the up-regulation of NADPH oxidase via angiotensin type 1 receptors<sup>(12,13,31,32)</sup>. Polyphenol-rich red wine extracts abrogate the angiotensin II-stimulated up-regulation of several NADPH oxidase subunits including Nox 1 and p22phox and the associated oxidative stress<sup>(31)</sup>, probably due to the inhibition of the angiotensin II-induced expression of NADPH oxidase by preventing angiotensin type 1 receptor expression<sup>(33)</sup>. Polyphenols also exert antioxidant activities in endothelial cells not only by reducing NADPH oxidase expression but also reducing its activity, and increasing the expression of antioxidant enzymes such as catalase<sup>(34)</sup>. Angiotensin II-induced endothelial dysfunction includes endothelium-dependent contractile responses to acetylcholine<sup>(35)</sup>, which involves COX-dependent formation of endothelium-derived contracting factors that act on thromboxane receptors located on vascular smooth muscle cells. Both the angiotensin II-induced vascular expression of COX and the increased endothelium-derived contracting factors are significantly reduced by red wine polyphenols<sup>(35)</sup>. Thus, polyphenols prevent ROS-mediated degradation of NO, and blunt vasoconstrictor and pro-inflammatory responses (Fig. 3).

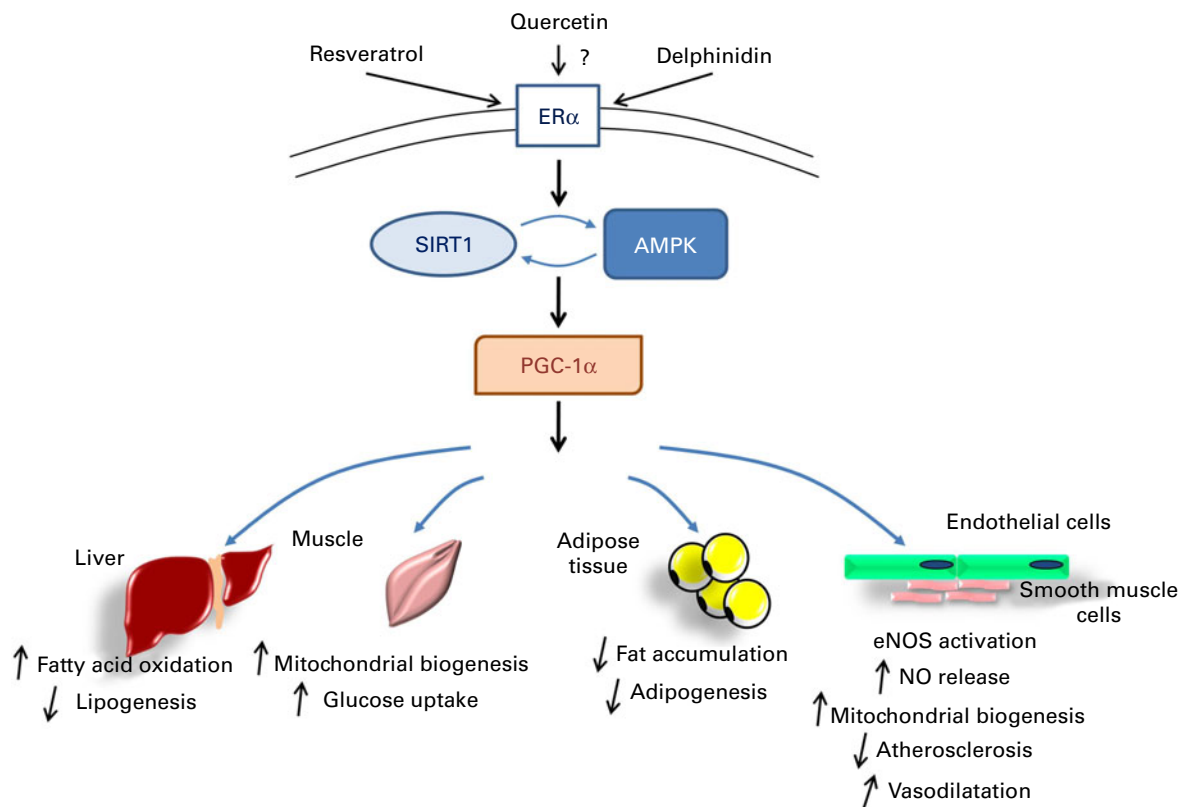
Polyphenol-rich products increase basal flow-mediated dilation in healthy subjects at relatively low doses such as those achieved after the intake of two glasses of red wine<sup>(36)</sup> or 2 weeks of daily consumption of flavonoid-rich dark chocolate bars (46 g)<sup>(37)</sup>. Similar beneficial effects of polyphenol-rich

products on flow-mediated dilation occur in patients with coronary artery disease after consumption of black tea<sup>(38)</sup>, a green tea extract<sup>(39)</sup> or a red grape extract<sup>(40)</sup>. Systolic blood pressure is improved in hypertensive patients by daily ingestion of polyphenol-rich products such as a piece of a sixteen-piece dark chocolate bar<sup>(41)</sup>, two glasses of purple grape juice<sup>(42)</sup> or 50 ml of pomegranate juice<sup>(43)</sup>.

### Resveratrol

Resveratrol is a stilbene identified in 1940 as a component of *Polygonum cuspidatum* (Japanese knotweed) used to treat hyperlipidaemic diseases. This polyphenol phytoalexin is also present in several plant species, including white hellebore (*Veratrum grandiflorum* O. Loes), grapes, peanuts and mulberries<sup>(44–46)</sup>. Many of the cardioprotective effects of red wine could be attributed to resveratrol, and recent studies extend the benefits of resveratrol to the prevention or retardation of cancer<sup>(45)</sup> and also to increasing the lifespan of various organisms from yeast to vertebrates<sup>(44)</sup>.

As a polyphenolic compound, resveratrol is an efficient scavenger of hydroxyl, superoxide and metal-induced radicals<sup>(45,47)</sup>. However, the direct antioxidant effects of resveratrol are weaker than those of ascorbate and cysteine<sup>(45)</sup>. The protective effects of resveratrol against oxidative injury are probably attributed to the up-regulation of the endogenous cellular antioxidant system rather than to its direct ROS-scavenging activity.



**Fig. 3.** Potential mechanism(s) in the cardiovascular and metabolic effects of polyphenols. Polyphenols interact with oestrogen receptor  $\alpha$  (ER $\alpha$ ) to activate the sirtuin-1 (SIRT1)–AMP-activated protein kinase (AMPK) network. Stimulation of SIRT1 and AMPK results in the activation of PPAR $\gamma$  coactivator 1  $\alpha$  (PGC-1 $\alpha$ ), placing mitochondria at the epicentre of targets for polyphenols in CVD and metabolic disorders. (A colour version of this figure can be found online at <http://www.journals.cambridge.org/bjn>)

### Resveratrol and oxidative stress

Resveratrol increases the expression/activity of SOD, catalase and glutathione peroxidase (GPx) in cardiac H9C2 cells<sup>(48)</sup> and aortic smooth muscle cells<sup>(47,49)</sup>. Another study, however, found no changes in the protein levels of SOD1 or SOD2 but an up-regulation of GPx1 and catalase in aortic segments or cultured aortic smooth muscle<sup>(50)</sup>. In a hamster model of dilated cardiomyopathy, treatment with resveratrol increases SOD2 levels, suppresses fibrosis, preserves cardiac function and significantly improves survival<sup>(51)</sup>. Treating hypercholesterolaemic, atherosclerosis-prone apoE-knockout (KO) mice (as a model of oxidative stress) with resveratrol (30–100 mg/kg per d for 7 d) leads to the up-regulation of SOD1, SOD2, SOD3, GPx1 and catalase in the heart<sup>(51)</sup>. The expression of these enzymes is also increased by resveratrol in cultured human endothelial cells<sup>(52,53)</sup>.

The antioxidant transcription factor nuclear factor erythroid 2-related factor-2 (Nrf2) is a recently identified target of resveratrol<sup>(54,55)</sup>. In cultured coronary arterial endothelial cells, resveratrol increases the transcriptional activity of Nrf2 and up-regulates the expression of Nrf2 target genes NAD(P)H:quinone oxidoreductase 1,  $\gamma$ -glutamylcysteine synthetase (glutamate cysteine ligase catalytic subunit, GCLC) and haem oxygenase-1<sup>(55)</sup>. All these enzymes, together with thioredoxin-1<sup>(56)</sup>, could well contribute to the antioxidant actions of resveratrol.

NADPH oxidases (NOX) are major sources of ROS in the cardiovascular system<sup>(57,58)</sup>. Resveratrol reduces the expression of Nox2 and Nox4 in the heart of apoE-KO mice<sup>(52)</sup> and also prevents Nox2 expression in the aorta of diabetic mice<sup>(59)</sup>. In human umbilical vein endothelial cells (HUVEC) and HUVEC-derived EA.hy 926 endothelial cells, resveratrol decreases the expression of Nox4<sup>(53)</sup>, the most predominant Nox isoform in these cell types<sup>(60)</sup>. Small-interfering RNA (siRNA)-mediated knockdown of sirtuin 1 (SIRT1) has no effect on the Nox4 down-regulation by resveratrol, indicating that the effect of resveratrol on Nox4 is likely to be SIRT1-independent<sup>(52)</sup>.

### Resveratrol and endothelial NO synthase uncoupling

Uncoupling of eNOS switches it from a NO-producing enzyme to a superoxide-generating molecule. The major cause of eNOS uncoupling under pathological conditions is a deficiency of the eNOS cofactor tetrahydrobiopterin (BH<sub>4</sub>)<sup>(61,62)</sup>. Tissue levels of BH<sub>4</sub> are a balance of its biosynthesis and degradation/oxidation: synthesis of BH<sub>4</sub> from GTP via a *de novo* pathway, with GTP cyclohydrolase 1 as the rate-limiting enzyme, while rapid oxidation by peroxynitrite makes the cofactor unavailable for eNOS generation of NO.

Untreated apoE-KO mice show increased oxidation of BH<sub>4</sub><sup>(63)</sup> and significant ROS production in their aorta<sup>(63,64)</sup> and heart<sup>(52)</sup>. Both aortic<sup>(63,64)</sup> and cardiac<sup>(52)</sup> superoxide production are reduced by the NOS inhibitor L-N<sup>G</sup>-nitroarginine methyl ester (L-NAME), indicating that eNOS is in an uncoupled state and that it produces ROS in this pathological model. Resveratrol treatment enhances the expression of GTP

cyclohydrolase 1 and BH<sub>4</sub> biosynthesis. In addition, resveratrol decreases the cardiac content of superoxide and peroxynitrite, and thereby decreases BH<sub>4</sub> oxidation<sup>(52)</sup>. As a result, the cardiac levels of BH<sub>4</sub> are increased by resveratrol. Cardiac superoxide production in resveratrol-treated mice is markedly reduced to a level that cannot be lowered any further by L-NAME<sup>(52)</sup>, suggesting that eNOS no longer produces superoxide in resveratrol-treated apoE-KO mice, i.e. resveratrol reverses eNOS uncoupling. The expression of GTP cyclohydrolase 1 in cultured human endothelial cells is increased by resveratrol. This up-regulation is reduced by the SIRT1 inhibitor sirtinol or by siRNA-mediated SIRT1 knockdown, indicating SIRT1-dependent mechanisms<sup>(52)</sup>.

### Resveratrol and vasodilation

Resveratrol causes vasodilation by releasing NO from endothelial cells<sup>(65)</sup> and/or improving NO bioavailability<sup>(59)</sup>. Resveratrol increases endothelial eNOS mRNA<sup>(66)</sup> and protein<sup>(67)</sup> expressions, and causes rapid phosphorylation of eNOS at Ser1177 (the activator site of this enzyme), and thereby increasing eNOS enzymatic activity<sup>(68)</sup>. In parallel, resveratrol improves NO bioavailability by decreasing oxidative stress *per se*<sup>(69)</sup>. These actions combine to stimulate cyclic guanylyl monophosphate formation, protein kinase G activation and vasodilation<sup>(70)</sup>. Voltage-gated K<sup>+</sup> channels, large Ca<sup>2+</sup>-activated-K<sup>+</sup> channels or voltage-gated Ca<sup>2+</sup> channels<sup>(71)</sup> mediate the endothelium-independent vasodilation caused by resveratrol. The vasodilator properties of resveratrol offer cardiovascular and vascular protection in several models of CVD.

### Resveratrol and vasoconstriction

The endothelium also releases vasoconstrictor and mitogenic substances such as endothelin-1, which under pathophysiological conditions, counteracts the protective effects of vasodilator products from endothelial cells. Resveratrol is able to reduce endothelial mRNA expression and secretion of endothelin-1<sup>(66)</sup>, inhibit H<sub>2</sub>O<sub>2</sub>-induced endothelin-1 expression in human vascular smooth muscle cells<sup>(72)</sup> and reduce endothelin-1 expression in the ischaemia-reperfused heart<sup>(73)</sup>.

The renin-angiotensin system regulates blood pressure via the release of angiotensin II that interacts with angiotensin type 1 receptors to evoke vasoconstriction<sup>(74)</sup>. Resveratrol suppresses the mRNA and protein expressions of angiotensin type 1 receptors in intact mice and also in isolated vascular smooth muscle cells<sup>(33)</sup>. Resveratrol also possesses a potent *in vitro* angiotensin-converting enzyme (ACE) inhibitory activity<sup>(75)</sup>, which can partially account for resveratrol-induced blood pressure-lowering effects in various animal models of hypertension.

### Resveratrol and inflammation

Resveratrol has *in vitro* and *in vivo* anti-inflammatory effects. Resveratrol treatment decreases the overexpression of

adhesion molecules (vascular cell adhesion molecule-1 and intercellular adhesion molecule-1) by inhibiting the NF- $\kappa$ B pathway in TNF $\alpha$ -activated endothelial cells<sup>(76)</sup>. In intact animal studies, resveratrol inhibits the angiotensin II-induced adhesion of leucocytes to arterioles, partially by reducing cellular adhesion molecule expression and circulating levels of monocyte chemoattractant protein-1 and macrophage inflammatory protein-1 $\alpha$ <sup>(77)</sup>. These effects may partially contribute to the cardiovascular protective activity of resveratrol, especially during the early phase of the atherosclerotic process.

Resveratrol probably improves the pro-inflammatory profile in human obesity by decreasing pro-inflammatory cytokine secretion and increasing adiponectin release from human adipose tissue<sup>(78)</sup>. Resveratrol modulates adipokine expression and improves insulin sensitivity in murine adipocytes, where resveratrol treatment reduces the levels of pro-inflammatory cytokines and adipokines (TNF $\alpha$ , IL-6 and resistin), and increases adiponectin and PPAR $\gamma$  expression and the Ser/Thr phosphorylation state of insulin receptor substrate-1<sup>(79)</sup>. In addition, resveratrol also normalises the levels of pro-inflammatory cytokines (IL-6 and TNF- $\alpha$ ) and COX-2 expression by decreasing NF- $\kappa$ B activation in diabetic rats<sup>(80)</sup>.

#### Resveratrol and platelet function

Resveratrol alters several functions of platelets: adhesion, activation and aggregation of platelets, and thrombus formation<sup>(81)</sup>. Since tissue factor is the major determinant for the extrinsic coagulation pathway, decreases in tissue factor expression can reduce thrombosis risk. Resveratrol attenuates an agonist-induced increase in tissue factor mRNA in endothelial and mononuclear cells, resulting from the inhibition of I $\kappa$ B $\alpha$  (inhibitor of kappa B) degradation, thus decreasing the DNA-binding occupancy by the transcription factor c-Rel/p65<sup>(82)</sup>. Additionally, resveratrol inhibits platelet aggregation induced by collagen, thrombin, ADP or arachidonic acid<sup>(83)</sup>. Resveratrol inhibits COX-1<sup>(84)</sup> and modifies COX metabolite production to modulate platelet activation, and inhibits the arachidonate-dependent synthesis of inflammatory agents such as thromboxane B2, hydroxyheptadecatrienoate and 12-hydroxyeicosatetraenoate<sup>(83)</sup>. Data from molecular modelling studies performed by *in silico* docking show that resveratrol forms stable complexes in platelet COX-1 channels<sup>(83)</sup>.

#### Resveratrol effects and in vivo relevance

Resveratrol at concentrations of up to 100  $\mu$ M is used in many cell-culture studies<sup>(47)</sup>; the molecular mechanisms obtained with such concentrations may not easily extend to understanding the effects of dietary resveratrol. It is unlikely that such high plasma concentrations of resveratrol are achieved, either by drinking red wine or by consuming resveratrol-containing food. However, high doses of resveratrol are well tolerated by animals<sup>(44)</sup> and by humans<sup>(85)</sup>. The low toxicity of resveratrol favours its use as a nutraceutical (to reach higher *in vivo* concentrations).

As much as 70% of orally ingested resveratrol can be absorbed. However, the bioavailability of unchanged resveratrol is very low, due to rapid and extensive metabolism<sup>(85)</sup>. The plasma concentration and the half-life of resveratrol metabolites are much greater than those of resveratrol<sup>(44)</sup>, indicating higher systemic exposure to the modified form than to unchanged resveratrol. It is possible that part of the *in vivo* effects of resveratrol can be attributed to its metabolites.

#### Quercetin

Quercetin is a polyphenol that occurs in abundance in plants and in the diet, and belongs to the flavonoid subclass that is identified by their ketone group<sup>(7)</sup>. The main source of quercetin is black elderberry, but significant quantities are also found in cocoa, Mexican oregano, capers and cloves while smaller concentrations occur in nuts, onions, shallot, cranberry, apple and red wine. Quercetin, present in foods as quercetin glycosides, represents 60–75% of the total dietary flavonols plus flavone intake<sup>(86)</sup>.

#### Quercetin and oxidative stress

Quercetin scavenges free radicals *in vitro* and has epidemiological correlates. Quercetin is a potent scavenger of superoxide anion and peroxyxynitrite, inhibits superoxide anion generation by suppressing xanthine oxidase activity<sup>(87)</sup> and inhibits the mitochondrial NADH/NAD<sup>+</sup> system<sup>(88)</sup>. Important is the finding that the hydroxyl groups of quercetin contribute to the generation of intracellular superoxide, leading to the inhibition of cell proliferation and the induction of apoptosis in leukaemia cells<sup>(89)</sup>.

#### Quercetin and vasodilation

Quercetin causes endothelium-dependent vasodilation through the production of NO<sup>(86,90)</sup> probably by increasing eNOS phosphorylation at 5  $\mu$ M<sup>(91)</sup>. Additionally, 50  $\mu$ M-quercetin is proposed to also increase NO release by causing a hyperpolarisation-dependent capacitative Ca<sup>2+</sup> entry in isolated cultured endothelial cells<sup>(92)</sup>. These effects result in endothelium-dependent vasodilatation that is inhibited by eNOS inhibitors and charybdotoxin, thus demonstrating that the quercetin effect is dependent on both the NO/cyclic guanylyl monophosphate pathway and EDHF<sup>(91)</sup>. Similar to the effects of resveratrol, quercetin, at a physiologically relevant concentration of 0.1  $\mu$ M, also increases eNOS mRNA expression in HUVEC<sup>(66)</sup>. Additionally, quercetin enhancement of cyclic guanylyl monophosphate-dependent relaxation in porcine isolated coronary arteries is insensitive to phosphodiesterase 5 inhibition. Quercetin reduces the development of glyceryl trinitrate-induced tolerance *in vitro* in porcine arteries; these findings can benefit patients with angina pectoris and await confirmation in humans<sup>(93)</sup>.

Other studies report that quercetin treatment (100  $\mu$ M) suppresses eNOS activity in bovine aortic endothelial cells as a result of decreased eNOS phosphorylation<sup>(94)</sup>. This effect is

associated with an *in vitro* disruption of mitotic microtubule polymerisation and an *in vivo* inhibition of angiogenesis, as quercetin inhibits vascular endothelial growth factor-induced endothelial cell function and angiogenesis through the inhibition of ERK1/2 (extracellular signal-regulated kinase 1/2) phosphorylation<sup>(95)</sup>.

### Quercetin and vasoconstriction

As with resveratrol, quercetin decreases H<sub>2</sub>O<sub>2</sub>-induced endothelin-1 mRNA expression and reduces endothelin-1 release in HUVEC<sup>(66)</sup>. Moreover, quercetin, at 1 μM and more so at 10 μM, prevents endothelin-1-induced endothelial dysfunction and NADPH oxidase subunit p47phox overexpression by inhibiting protein kinase C<sup>(96)</sup>.

The detailed effects of quercetin on the renin–angiotensin system are not known. Treatment of Dahl salt-sensitive hypertensive rats with quercetin (10 mg/kg) for 4 weeks reduces blood pressure along with decreases in angiotensin II type 1 receptor mRNA, suggesting modulation of renal function by quercetin<sup>(97)</sup>. It should be noted that quercetin fails to modify ACE activity either *in vitro* using rat kidney membranes<sup>(98)</sup> or *in vivo* after administration to rats<sup>(99)</sup>, suggesting that the antihypertensive effect of quercetin may be unrelated to actions on the renin–angiotensin system.

### Quercetin and inflammation

Although various mechanisms are involved in the anti-inflammatory properties of quercetin, it mainly targets signalling pathways related to NF-κB activation. Thus, quercetin (10 μM) decreases mRNA and protein levels of TNFα, IL-1β, IL-6, macrophage inflammatory protein-1α and inducible NO synthase in several *in vitro* and *in vivo* studies<sup>(100)</sup>. Quercetin has pleiotropic effects in apoE-KO mice related to the reduction of pro-inflammatory markers (isoprostane, leukotriene B<sub>4</sub> and P-selectin) and the enhancement of anti-inflammatory indicators (eNOS and haem oxygenase-1 expression)<sup>(101)</sup>, suggesting that quercetin at a dose of 1.3 mg/d could delay the atherosclerotic process through its anti-inflammatory properties. Also, adiponectin mRNA levels are enhanced in adipose tissue from rats receiving quercetin fed high-fat diets<sup>(102)</sup>. High concentrations of quercetin (40 μM) suppress the Akt phosphorylation and transactivation of nuclear factor activator protein-1 and NF-κB, resulting in an inhibition of the TNF-α-induced up-regulation of cell migration<sup>(103)</sup>. In addition, by reducing the production of pro-inflammatory cytokines and enzymes, quercetin (50 μM) inhibits mouse dendritic cell activation, suggesting that quercetin could be a potent immunosuppressive agent<sup>(104)</sup>. However, contradictory results have been described in human subjects as no effects on the inflammatory profile were detected in females receiving a 12-week supplementation with quercetin (0.5–1 g/d)<sup>(105)</sup>. Dietary supplementation of quercetin in combination with vitamin C for 4 weeks does not change plasma biomarkers of inflammation (TNF-α, IL-1β, IL-6 and C-reactive protein) and the disease severity of rheumatoid arthritis patients<sup>(106)</sup>.

### Quercetin and platelet function

Platelet aggregation contributes to both the development of atherosclerosis and to acute platelet thrombus formation, followed by embolisation of stenosed arteries. Quercetin impairs *in vitro* platelet aggregation induced by thrombin by interfering with Ca<sup>2+</sup> mobilisation and serotonin secretion<sup>(107)</sup>, and inhibiting platelet kinases such as phosphatidylinositol-3-kinase and Src kinases<sup>(108)</sup>. These results were obtained with concentrations that exceed those attained after standard consumption of flavonoid-rich foods. Quercetin inhibits platelet aggregation independently of the agonist used (arachidonic acid or ADP)<sup>(83)</sup>. Quercetin inhibits platelet activation through the blockade of activity of the proto-oncogene tyrosine-protein kinase Fyn and the tyrosine phosphorylation of spleen tyrosine kinase (Syk) and phospholipase C gamma 2 (PLCγ2) following quercetin internalisation in platelets<sup>(109)</sup>.

### Limitations of the use of quercetin

Quercetin is absorbed through the gastrointestinal tract and rapidly metabolised by methylation and conjugation with glucuronic acid and/or sulphate in enterocytes and in the liver<sup>(110,111)</sup>. Once conjugated, quercetin is present in plasma after repeated daily dosage<sup>(112)</sup>, and, paradoxically, although the plasma concentrations of free quercetin are very low, it can occur in relatively high concentrations in several tissues indicating that *in situ* deconjugation of quercetin can occur<sup>(113)</sup>.

### Delphinidin

Anthocyanins are the largest group of water-soluble pigments in the plant kingdom and are responsible for most of the red, blue and purple colours of fruits, vegetables, flowers and other plant tissues or products<sup>(2)</sup>. The six anthocyanins commonly found in plants are classified according to the number and position of hydroxyl and methoxyl groups on the flavan nucleus, and are named pelargonidin, cyanidin, delphinidin, peonidin, petunidin and malvidin. The daily intake of anthocyanins in humans is approximately 180–215 mg/d in the USA<sup>(3)</sup>, with the major sources of anthocyanins being blueberries, cherries, raspberries, strawberries, black currants, purple grapes and red wine; a 100 g serving of berries provides up to 500 mg anthocyanins. Various metabolites are formed during the metabolism of anthocyanins and anthocyanidins and include glucuronides and methylated and sulphated derivatives of anthocyanins<sup>(114)</sup>.

Among the different classes of polyphenolic compounds present in red wine, anthocyanins and oligomeric condensed tannins exhibit pharmacological profiles comparable with total red wine extracts in terms of endothelial-dependent NO-mediated vasodilatation<sup>(115)</sup>. Of the different anthocyanins identified in wine, only delphinidin causes endothelium-dependent relaxation, although it is slightly less potent than total red wine extract<sup>(115)</sup>.

### Delphinidin and oxidative stress

Delphinidin possesses antioxidant effects in a wide range of chemical oxidation systems by virtue of two hydroxyl

groups on the phenyl ring<sup>(116)</sup>, and among the anthocyanins, delphinidin has the greatest *in vitro* potency against superoxide anions and peroxynitrite<sup>(117)</sup>. Since this study was performed at neutral pH, it is not clear whether this potency is maintained *in vivo*. Its ability to scavenge ROS protects endothelial cells from LDL-induced lipid oxidation, although it is not clear whether the effects of delphinidin in quenching ROS are by direct actions on LDL. Nevertheless, delphinidin (25–200  $\mu\text{M}$ ) restores SOD activity to a similar extent to that produced by vitamin C, suggesting that delphinidin maintains endothelial cell function by preserving endogenous antioxidants and by attenuating lipid peroxidation<sup>(118)</sup>. Treatment of  $\text{CCl}_4$ -intoxicated mice with delphinidin (25 mg/kg, once daily for 2 weeks) decreases oxidative stress in the liver as reflected by the recovery of GPx activity and the ratio reduced glutathione:oxidised glutathione. These antioxidant effects of delphinidin are associated with antifibrotic activity, indicating that delphinidin possesses a tissue-regenerative capability<sup>(119)</sup>. Cytotoxic effects of delphinidin (100  $\mu\text{M}$  for 24 h) in metastatic cells (but not in cells originating from a primary tumour site) are related to cellular free radical accumulation, inhibition of glutathione reductase and depletion of glutathione, suggesting that delphinidin could be used as a sensitising agent in metastatic therapy<sup>(120)</sup>.

#### Delphinidin and vasodilation

Delphinidin stimulates NO production independently of its antioxidant property<sup>(20)</sup>. Delphinidin activates NO release by increasing intracellular  $\text{Ca}^{2+}$  concentrations through the release from intracellular stores and the entry from the extracellular space. In bovine aortic endothelial cells, delphinidin-induced increases in intracellular  $\text{Ca}^{2+}$  are accompanied by tyrosine phosphorylation of several intracellular proteins<sup>(121)</sup>. Acute treatment with delphinidin (10 min) enhances NO release and eNOS phosphorylation at Ser1177<sup>(26)</sup>.

The only study of the angiogenic properties of low doses of delphinidin (0.06 mg/kg per d) reports no effects on the recovery of blood flow in ischaemic hindlimbs, while higher doses of delphinidin (0.6 mg/kg per d) have anti-angiogenic effects as characterised by impaired blood flow and decreased vascular density in the ischaemic leg of rats<sup>(122)</sup>. These results are similar to those obtained with the whole extracts from red wine, suggesting that delphinidin could play an important role in the anti-angiogenic effect of red wine.

By targeting STAT1 (a nuclear transcriptional factor of the signal transducers and activators family and which has a critical role in cardiomyocyte apoptosis), delphinidin (more potently than quercetin) provides protection against ischaemia–reperfusion injury in isolated cardiomyocytes and in the Langerdoff-perfused rat heart when used at 10  $\mu\text{M}$  2 h before the onset of the ischaemic insult<sup>(123)</sup>.

#### Delphinidin and vasoconstriction

Delphinidin reduces both mRNA and protein levels of endothelin-1 in cultured HUVEC<sup>(124)</sup>. While resveratrol and quercetin (30  $\mu\text{M}$ ) reduce endothelin-1 production by

only 20%, similar concentrations of delphinidin lower endothelin-1 production by approximately 75%<sup>(125)</sup>. Although the inhibition of purified ACE by oligomeric procyanidins (mainly oligomeric epicatechins) is well established<sup>(126)</sup>, the effects of delphinidin-3-O-sambubioside on ACE have only been recently reported. This compound inhibits ACE by competing with the active site of the enzyme, with a half-maximal inhibitory concentration value similar to that obtained with quercetin<sup>(127)</sup>.

#### Delphinidin and inflammation

Several *in vitro* studies report that delphinidin interacts directly with kinases; however, it is not established whether delphinidin also has similar effects *in vivo*. Delphinidin (5–20  $\mu\text{M}$ ) suppresses COX-2 promoter activity and COX-2 expression in mouse epidermal cells by inhibiting activator protein-1 and NF- $\kappa\text{B}$  pathways; these effects result from the direct binding of delphinidin to the ATP-binding site in the kinase domain of mitogen-activated protein kinase kinase 4 and to the ATP-binding site of the catalytic domain of phosphatidylinositol-3-kinase<sup>(128)</sup>. Delphinidin (10–40  $\mu\text{M}$ ) inhibits phosphorylations of c-Jun N-terminal kinases, p38 mitogen-activated protein kinase, Akt and ERK as well as Fyn kinase in mouse epidermal cells, and directly binds with Fyn kinase in a non-competitive manner with ATP<sup>(129)</sup>. Additionally, delphinidin also inhibits a broad spectrum of receptor tyrosine kinases of the epidermal growth factor receptor B (ErbB) and vascular endothelial growth factor receptor families in both cell-free assays and intact cell systems<sup>(130)</sup>. Other enzymes potentially playing a role in inflammation are also inhibited by delphinidin: for example, a mixed competitive and non-competitive phospholipase A2 inhibition has been described for delphinidin in a cell-free assay<sup>(131)</sup>, while delphinidin also weakly inhibits proteasome activity<sup>(132)</sup>. These data highlight the ability of delphinidin to interfere with pro-inflammatory pathways, although no evidence of *in vivo* effects on these enzyme systems is available.

#### Delphinidin and platelet function

Although aqueous residues containing the anthocyanic fraction from red wine suppressed ADP-induced platelet aggregation<sup>(133)</sup>, delphinidin was unable to inhibit collagen-induced platelet aggregation *in vitro*<sup>(133)</sup>. Delphinidin containing fractions from purple grapes inhibits whole-blood aggregation, suggesting a potential mechanism for the beneficial effects of polyphenols on the suppression of platelet-mediated thrombosis<sup>(134)</sup>.

#### Limitations on the use of delphinidin

Although abundant in the diet, anthocyanins, in general, and delphinidin in particular, are either poorly absorbed or not absorbed at all. One consequence of the poor bioavailability of anthocyanins is that many effects observed *in vitro* (e.g. inhibition of COX-2) are unlikely to occur *in vivo*. The measurement in plasma or urine of the original anthocyanins



and their conjugated metabolites (glucuronidated and sulphated anthocyanins) indicates their very low bioavailability<sup>(7,135)</sup>. In addition, intestinal microflora play an important role in the metabolism of anthocyanins<sup>(136)</sup>. Clearly, additional *in vivo* studies on the effects of delphinidin are needed to establish the beneficial effects of concentrations used in *in vitro* studies, which generally tend to be higher than those attained physiologically.

### Molecular targets of polyphenols

The ability of polyphenols to target transcriptional networks that modulate gene expression favouring NO production, anti-inflammatory mediators and energy expenses provides an attractive pharmacological approach to treat cardiovascular and metabolic diseases (Fig. 3). Some molecular targets of polyphenols are discussed below.

#### AMP-activated protein kinase

AMP-activated protein kinase (AMPK) is a Ser/Thr protein kinase involved in ATP production in mammalian cells<sup>(137)</sup>. The AMPK cascade may have an important role in preventing diseases since AMPK inhibits fat accumulation, reduces cholesterol synthesis and modulates inflammatory cytokines. Polyphenols found in natural products can target and activate AMPK leading to numerous beneficial effects in cardiovascular and metabolic diseases, as shown by the finding that activation of AMPK by resveratrol is SIRT1-independent<sup>(138)</sup> (see below). By increasing AMPK phosphorylation, resveratrol prevents the development of hyperlipidaemia and atherosclerosis in diabetic mice<sup>(139)</sup>. These effects may be related to reduced fat accumulation<sup>(140)</sup>, enhanced glucose transporter GLUT4 translocation and increases in glucose uptake by diabetic rat cardiomyocytes<sup>(141)</sup>. Resveratrol increases physical endurance and mitochondrial biogenesis as revealed by increases in the expressions of PPAR $\gamma$  coactivator (PGC)-1 $\alpha$ , PGC-1 $\beta$ , oestrogen-related receptor  $\alpha$  and nuclear respiratory factor (NRF) in AMPK-deficient mice, leading to improved glucose homeostasis through mechanisms dependent on AMPK activation<sup>(140)</sup>.

Quercetin also activates the AMP–AMPK pathway via down-regulation of protein phosphatase 2C in the brains of old mice fed a cholesterol-rich diet, indicating that quercetin enhances the resistance of neurons to age-related diseases via AMPK pathway activation<sup>(142)</sup>. Furthermore, quercetin inhibits adipocyte 3T3-L1 differentiation by decreasing adipogenic transcription factors such as PPAR $\gamma$  and CCAAT/enhancer-binding protein via the phosphorylation of mitogen-activated protein kinase, suggesting that quercetin can regulate the adipocyte life cycle<sup>(143)</sup>. Dietary bilberry extracts rich in anthocyanidins ameliorate hyperglycaemia and insulin sensitivity in diabetic mice by activating AMPK in the adipose tissue, skeletal muscle and liver<sup>(144)</sup>.

#### Sirtuin 1

Polyphenols such as resveratrol activate a NAD<sup>+</sup>-dependent protein deacetylase, silent information regulator orthologue 1

(SIRT1)<sup>(145)</sup>, which regulates a variety of cellular functions such as genome maintenance, longevity and metabolism<sup>(146,147)</sup>. Resveratrol increases the lifespan in animals partially via the stimulation of SIRT1, in a manner similar to energy restriction<sup>(148)</sup>. Resveratrol augments exercise endurance in mice through the deacetylation of PGC-1 $\alpha$  (a mitochondrial biogenesis factor) by SIRT1 to stimulate mitochondrial function in muscle and brown adipose tissue<sup>(149)</sup>. The pleiotropic effects of resveratrol, which occur by the activation of SIRT1, could protect animals from obesity and diabetes by shifting the energy balance towards energy consumption rather than storage<sup>(149)</sup>.

Small-interfering RNA against SOD2 or SIRT1 reduce the cell-protective effects of resveratrol<sup>(51)</sup>. Although a recent study using cell-free assays questions the ability of resveratrol to activate SIRT1 directly<sup>(150)</sup>, it is highly likely that resveratrol (or its metabolites) can promote SIRT1 activation *in vivo*. Moreover, resveratrol also enhances the expression levels of SIRT1. In an attempt to address this, Li's group reported that inhibition of SIRT1 activity with sirtinol or knockdown of SIRT1 expression with siRNA both reduced the effects of resveratrol on SOD1, SOD2 and GPx1, but not those on SOD3 and catalase<sup>(52)</sup>. This finding is consistent with the findings that resveratrol up-regulates SOD2 in C2C12 myoblasts in a SIRT1-dependent manner<sup>(51)</sup>.

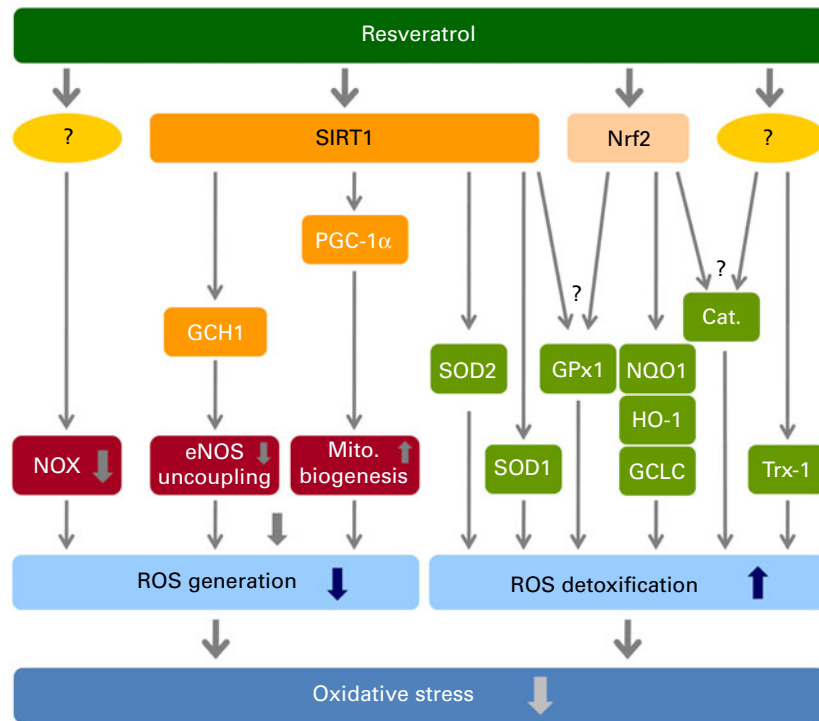
Of note is the report that SIRT1 also activates the transcriptional activity of PGC-1 $\alpha$ , and subsequently induces mitochondrial biogenesis and lipolysis, and so inhibits the generation of ROS from the mitochondria<sup>(151)</sup>. The activation of SIRT1 is related to both lipid and glucose homeostasis; thus, SIRT1 inhibits adipogenesis, reduces fat storage in adipose tissue<sup>(152)</sup> and increases insulin secretion and sensitivity<sup>(153)</sup>.

Resveratrol stimulates eNOS activity by SIRT1 activation and eNOS deacetylation<sup>(154)</sup>. For example, resveratrol increases mitochondrial mass and up-regulates eNOS by activating SIRT1 in human coronary arterial endothelial cells, where the ability of resveratrol to induce mitochondrial biogenesis is NO-dependent<sup>(155)</sup>. Likewise, SIRT1 activation by other stimuli such as laminar flow and statin treatment also increases eNOS activity and NO production<sup>(156)</sup>. Thus, the interaction between SIRT1 and eNOS contributes to the cardiovascular beneficial effects of resveratrol. The multifaceted molecular mechanisms for the cardiovascular benefits of resveratrol are summarised in Fig. 4.

In a similar manner, treating mice with quercetin enhances mRNA expression of PGC-1 $\alpha$  and SIRT1 to increase both maximal endurance capacity and running activity<sup>(157)</sup>. The possibility of targeting SIRT1 by polyphenols, and thereby co-affecting PGC-1 $\alpha$  signalling, makes endothelial mitochondria important in CVD and metabolic disorders.

#### Oestrogen receptor $\alpha$

Due to the structural similarities with diethylstilbestrol (a synthetic oestrogen), resveratrol has been proposed to activate the ER. Resveratrol binds to and activates gene transcription via the ER in oestrogen-sensitive tissues and cell lines<sup>(158)</sup>. Of interest, resveratrol binds ER $\beta$  with a lower affinity than



**Fig. 4.** Resveratrol reduces oxidative stress by decreasing reactive oxygen species (ROS) production from NADPH oxidases (NOX), uncoupled endothelial NO synthase (eNOS, by up-regulating GTP cyclohydrolase 1, GCH1) and mitochondria (by stimulating mitochondrial (Mito.) biogenesis). In addition, resveratrol enhances the expression of antioxidant enzymes, such as superoxide dismutases (SOD1–3), catalase (Cat.), glutathione peroxidase 1 (GPx1), NAD(P)H:quinone oxidoreductase 1 (NQO1),  $\gamma$ -glutamylcysteine synthetase (glutamate cysteine ligase catalytic subunit, GCLC), haem oxygenase-1 (HO-1) and thioredoxin-1 (Trx-1). SIRT1, sirtuin 1; PGC-1 $\alpha$ , PPAR $\gamma$  coactivator 1- $\alpha$ ; Nrf2, nuclear factor E<sub>2</sub>-related factor-2. (A colour version of this figure can be found online at <http://www.journals.cambridge.org/bjcn>)

at ER $\alpha$ <sup>(159)</sup>. Owing to its properties as an agonist for the ER, resveratrol is able to regulate the transcription of oestrogen-responsive target genes, and possibly has cancer chemopreventive effects<sup>(160)</sup>. Activation of ER is a key step in the effects of resveratrol on glucose uptake by muscles<sup>(161)</sup>. At the molecular level, resveratrol rapidly activates ER $\alpha$  in caveolae, leading to eNOS activation by the stimulation of G-protein G $\alpha$ , caveolin-1 (Cav-1), Src and ERK1/2K; siRNA knockdown of ER $\alpha$ , but not ER $\beta$ , or the presence of ER antagonists inhibits the rapid eNOS activation by resveratrol<sup>(68)</sup>.

As described for resveratrol, quercetin is also able to reduce oestrogen-sensitive tumour growth in mouse models by directly acting on ER<sup>(162)</sup> and by down-regulating cytoplasmic ER levels and promotion of a tighter nuclear association of the ER<sup>(163)</sup>. Quercetin exhibits a similar potency of both ER subtypes<sup>(164)</sup> and stimulates the expression of the proto-oncogene c-fos through ER $\alpha$  activation<sup>(165)</sup>.

Recent data suggest that delphinidin interacts directly with the activator site of ER $\alpha$ , leading to the activation of eNOS. Thus, the ability of delphinidin (and of total polyphenolic extract from red wine) to induce NO production and endothelium-dependent vasorelaxation data is lost in ER $\alpha$ -deficient mice or after using siRNA for this receptor<sup>(26)</sup>. Silencing the effects of ER $\alpha$  completely prevents delphinidin activation of Src, ERK1/2 and eNOS, while binding assay and docking experiments indicate a direct interaction between delphinidin and the ER $\alpha$  activator site. Oral

administration of total polyphenolic extracts from red wine increases the sensitivity of endothelium-dependent relaxation to acetylcholine and is associated with increased NO production and decreased superoxide anions in control mice but absent in ER $\alpha$ -deficient mice<sup>(26)</sup>.

#### Interaction between molecular targets of polyphenols

It is likely that there is an intracellular crosstalk of signalling cascades activated by molecular targets of polyphenols. For example, resveratrol modulates tumour cell proliferation and protein translation via SIRT1-dependent AMPK activation in ER-positive breast cancer cells, highlighting the interactions of ER, SIRT1 and AMPK<sup>(166)</sup>. Resveratrol induces deacetylation of AMPK in the liver to promote fatty acid oxidation and inhibit lipogenesis<sup>(167)</sup>. It is likely that SIRT1 may be upstream of AMPK, since SIRT1 activation increases AMPK activity<sup>(168)</sup>, probably by SIRT1 deacetylation/activation of the upstream AMPK kinase liver kinase B1 (LKB1)<sup>(169)</sup>. Finally, eNOS acetylation is higher in AMPK $\alpha$ 2-deficient mice, suggesting that AMPK phosphorylation of eNOS is required for SIRT1 deacetylation of eNOS<sup>(156)</sup>. These findings suggest that the improvement of cell function produced by the polyphenols resveratrol, quercetin and delphinidin occurs by the activation of several signalling mechanisms in addition to the transcriptional and post-translational effects.

## Polyphenols and CVD

Cardiovascular mortality exceeds cancers as the leading cause of death in the world. CVD include CHD, stroke, hypertension, peripheral artery disease and heart failure. The major causes of CVD are tobacco use, physical inactivity and hyperenergetic diets.

### Hypertension

Hypertension causes modifications of the vascular walls that lead to hypertensive cardiomyopathy and heart failure<sup>(170)</sup>. Changes in the mechanical properties of arteries affect vascular resistance by altering the pressure–lumen diameter relationship of small arteries<sup>(74)</sup>. Part of the cardioprotective actions of polyphenols is by lowering blood pressure. However, contradictory data are available: for instance, an antihypertensive effect of resveratrol was reported in partially nephrectomised rats<sup>(171)</sup>, while in double transgenic rats harbouring human renin and angiotensinogen genes, resveratrol reduces blood pressure, ameliorates cardiac hypertrophy and prevents angiotensin II-induced mortality, probably by increasing mitochondrial biogenesis and SIRT1 activity<sup>(172)</sup>. Resveratrol probably suppresses angiotensin II type 1 receptor expression through SIRT1 activation, suggesting that the inhibition of the renin–angiotensin system may contribute, at least in part, to the resveratrol-induced cardioprotective effects<sup>(33)</sup>. Other studies report that resveratrol does not affect established hypertension in SHR<sup>(173)</sup>, although it attenuates the compliance of arteries from SHR without changes in wall stiffness by reducing eutrophic remodelling<sup>(174)</sup>.

Chronic treatment with quercetin (10 mg/kg) reduces systolic blood pressure and significantly reduces left ventricular and renal hypertrophy in SHR<sup>(175)</sup>, hypertension induced by the inhibition of NOS<sup>(86)</sup> and in deoxycorticosterone acetate-salt hypertensive rats<sup>(176)</sup>. It appears that quercetin is effective in all animal models of hypertension studied, and acts independently of the status of renin–angiotensin system, oxidative stress, NO, etc.<sup>(86)</sup>.

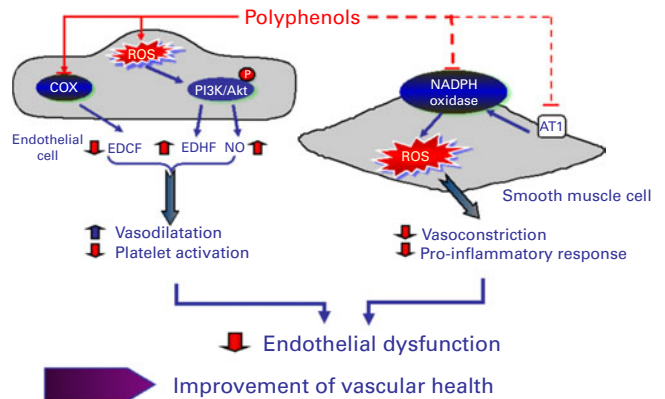
Short-term oral administration of polyphenols from red wine (a rich source of delphinidin) decreases blood pressure in normotensive rats. This haemodynamic effect was associated with an enhanced endothelium-dependent relaxation and an induction of gene expression within the arterial wall, which together maintain unchanged agonist-induced contractility<sup>(177)</sup>. Polyphenols from red wine reduce blood pressure elevations caused by chronic inhibition of NOS, attenuate end-organ damage such as myocardial fibrosis and aortic thickening, and decrease protein synthesis in the heart and aorta<sup>(178,179)</sup>. Polyphenols also prevent endothelium-dysfunction by increasing eNOS activity, moderately enhancing eNOS expression and reducing oxidative stress in the left ventricle and aorta. Endothelial dysfunction associated with excessive NADPH oxidase-dependent vascular formation of ROS in angiotensin II-induced hypertension is prevented by polyphenols<sup>(31)</sup>. Thus, polyphenols from red wine reduce hypertension by modulating the NO and ROS balance in the cardiovascular system.

### Stroke

Cerebral ischaemia is caused by reduced cerebral blood flow. Stroke involves the interaction of neurons, glia, vascular cells and matrix components, all of which participate in the mechanisms of tissue injury and repair. The severe reduction of cerebral blood flow initiates a series of pathophysiological mechanisms such as impaired energy metabolism, loss of ionic homeostasis, excessive release of excitatory amino acids (mainly aspartate and glutamate) and increased oxidative stress. All these processes lead to brain tissue damage and cell death.

Resveratrol reduces infarct volume in various experimental models of stroke<sup>(180)</sup>. The mechanisms involved in neuroprotection are largely by the inhibition of lipid oxidation processes. More recent data indicate that resveratrol significantly restores ATP content and the activity of mitochondrial respiratory complexes in a model of transient rat middle cerebral artery occlusion by decreasing apoptosis, mitochondrial lipid peroxidation, brain infarct volume and oedema<sup>(181)</sup>. In the stroke model, resveratrol improves neurological function by reducing the release of excitatory neurotransmitters (glutamate and aspartate), and increases inhibitory neurotransmitter release ( $\gamma$ -amino-*n*-butyric acid and glycine)<sup>(182)</sup>. It is likely that these effects are mediated through the activation of oestrogen and *N*-methyl-D-aspartate receptors<sup>(183)</sup> or the SIRT1 pathway<sup>(184)</sup>. Resveratrol administration also induces angiogenesis in the cortical area of mice exposed to middle cerebral artery ischaemia<sup>(185)</sup>. These findings highlight the ability of resveratrol to preserve ischaemic neurovascular units in the treatment of ischaemic stroke.

Liposomal preparations of quercetin that enhance neuroprotective capacity reduce cerebral damage provoked by cerebral ischaemia<sup>(186)</sup>. Repeated treatment with quercetin for 15 d before ischaemic surgery in gerbils reduces lipid peroxidation,



**Fig. 5.** The protective effect of polyphenols on blood vessels is due to their ability to act on endothelial cells to increase the formation of the vasoprotective factors NO and endothelium-derived hyperpolarising factor and reduce the endothelial formation of cyclo-oxygenase (COX)-derived vasoconstricting factors, and also on vascular smooth muscle cells to reduce oxidative stress, in part, by decreasing the expression of NADPH oxidase and, possibly, also the angiotensin 1 receptor. ROS, reactive oxygen species; PI3K/AKT, phosphatidylinositol-3-kinase/Akt; EDCF, endothelium-derived contracting factor; EDHF, endothelium-derived hyperpolarising factor; AT1, angiotensin type 1 receptor. (A colour version of this figure can be found online at <http://www.journals.cambridge.org/bjn>)

suggesting that early administration of quercetin could offer protection of neuronal units during cerebral ischaemia<sup>(187)</sup>.

Feeding rats with diets enriched in anthocyanins from blueberries provides neuroprotection after stroke induced by ligation of the left common carotid artery independently of their ability to scavenge oxygen radicals<sup>(188)</sup>. An anthocyanin-rich extract from red wine reduces injury induced by cerebral ischaemia in rats, and protects from ischaemia-induced excitotoxicity (by reducing the release of the excitatory neurotransmitters glutamate and aspartate), energy failure (by increasing glucose concentrations) and oxidative stress (by increasing levels of ascorbic and uric acids)<sup>(189)</sup>. Long-term administration of polyphenols partially restores cerebral blood flow during cerebral artery occlusion and improves flow during reperfusion in the cortex, as measured by increased diameters of the arteries of the cerebral tree, while also causing differential expression of proteins involved in neuroprotection, maintenance of neuronal integrity, oxidative stress, energy metabolism and inflammation (such as neurofilament medium polypeptide (NF-M) or TOAD-64)<sup>(190)</sup>. These experimental data indicate the beneficial effects of polyphenols in stroke protection, or in treatment during different phases of the disease.

### Polyphenols and metabolic diseases

Resveratrol extends the lifespan in mice fed a high-fat diet by reducing fat accumulation and improving glucose tolerance and insulin sensitivity<sup>(167)</sup>. Hypercholesterolaemic swines receiving resveratrol (100 mg/kg per d for 1 month) have reduced BMI, total cholesterol, LDL, blood glucose levels and systolic blood pressure<sup>(191)</sup>, while in Zucker obese rats, resveratrol improves inflammation (by increasing adiponectin and reducing TNF- $\alpha$  production in the visceral adipose tissue) and reduces plasma concentrations of TAG, total cholesterol, NEFA, insulin and leptin<sup>(192)</sup>. At a molecular level, resveratrol inhibits preadipocyte proliferation and adipogenic differentiation in a SIRT1-dependent manner<sup>(193)</sup>. In human adipocytes, resveratrol stimulates basal and insulin-stimulated glucose uptake, while *de novo* lipogenesis is inhibited in parallel with a down-regulation of lipogenic gene expression. Furthermore, resveratrol influences the secretory profile of human preadipocytes in a way that can positively interfere with the development of obesity-associated co-morbidities<sup>(193)</sup>. Other studies implicate ER $\alpha$  in resveratrol-stimulated, insulin-dependent and -independent glucose uptake<sup>(161)</sup>.

Quercetin (2 or 10 mg/kg) improves dyslipidaemia, hypertension and hyperinsulinaemia in obese Zucker rats, but only the higher dose evokes the anti-inflammatory effects in visceral adipose tissue<sup>(194)</sup>. However, quercetin is unable to improve insulin sensitivity in SHR<sup>(175)</sup>. Comparing the same doses of resveratrol and delphinidin (2.1 mg/kg) in a rat model of the metabolic syndrome shows that only delphinidin prevents insulin resistance without reducing high blood pressure<sup>(195)</sup>.

There are beneficial effects of dietary supplementation of red wine polyphenol extracts on obesity-associated alterations with respect to changes in metabolic disturbances and

cardiovascular function in Zucker fatty rats<sup>(196)</sup>. These polyphenols improve glucose metabolism by reducing plasma glucose and fructosamine in Zucker fatty rats. Moreover, polyphenols reduce circulating TAG, total cholesterol as well as LDL-cholesterol in Zucker fatty rats; echocardiography measurements indicate improved cardiac performance associated with decreased peripheral arterial resistance<sup>(196)</sup>. Polyphenol extracts improve vasodilation by enhancing eNOS activity and reducing superoxide anion release via decreased expression of the NADPH oxidase membrane subunit Nox-1<sup>(196)</sup>, suggesting that polyphenol consumption may be helpful in reducing obesity-associated metabolic disorders.

### Conclusions

Several sources of polyphenols including red wines, grape juices and green teas have the potential to improve vascular health, for example, by stimulating the formation of vasoprotective factors such as NO and EDHF to promote vasodilatation and prevent platelet activation. Polyphenols can also improve vascular smooth muscle function, by reducing the excessive vascular oxidative stress of pathological blood vessels. The antioxidant effect probably reflects changes in the expression levels of antioxidant and pro-oxidant enzymes. Polyphenol treatments are associated with a reduced expression of NADPH oxidase, a vascular source of superoxide anions, and a reduced angiotensin system, a strong activator of NADPH oxidase. The reduced oxidative stress will prevent the degradation of NO by superoxide anions and also prevent vasoconstriction and pro-inflammatory responses (Fig. 5). Thus, actions of polyphenols on endothelial and smooth muscle cells can promote vascular health.

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