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Endothelial function and cardiovascular disease: Effects of quercetin and wine polyphenols

FRANCISCO PEREZ-VIZCAINO[1], JUAN DUARTE[2] & RAMAROSON ANDRIANTSITOHAINA[3]

[1]*Department of Pharmacology, School of Medicine, Universidad Complutense de Madrid, 28040 Madrid, Spain,* [2]*Department of Pharmacology, School of Pharmacy, Universidad de Granada, 18071 Granada, Spain, and* [3]*Biologie Neuro-Vasculaire Intégrée, UMR CNRS 6214-INSERM 771, School of Medicine, 49045 Anger, France*

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Abstract

Endothelial dysfunction is an early pathophysiological feature and independent predictor of poor prognosis in most forms of cardiovascular diseases. Epidemiological studies report an inverse association between dietary flavonoid consumption and mortality from cardiovascular diseases. In the present paper, we review the effects of flavonoids, especially quercetin and wine polyphenols, on endothelial function and dysfunction and its potential protective role in hypertension, ischemic heart disease and stroke. *In vitro* studies show that flavonoids may exert multiple actions on the NO-guanylyl cyclase pathway, endothelium-derived hyperpolarizing factor(s) and endothelin-1 and protect endothelial cells against apoptosis. *In vivo*, flavonoids prevent endothelial dysfunction and reduce blood pressure, oxidative stress and end-organ damage in hypertensive animals. Moreover, some clinical studies have shown that flavonoid-rich foods can improve endothelial function in patients with hypertension and ischemic heart disease. Altogether, the available evidence indicates that quercetin and wine polyphenols might be of therapeutic benefit in cardiovascular diseases even though prospective controlled clinical studies are still lacking.

Keywords: *Flavonoid, endothelial dysfunction, cardiovascular disease, hyperpolarizing factors*

Introduction

Flavonoids represent the major class of polyphenolics [1]. The term flavonoid comprises several thousand plant-derived compounds sharing a common skeleton of phenylchromane. This basic structure allows a multitude of substitution patterns leading to several flavonoid subclasses such as flavonols, flavones, flavanones, catechins, anthocyanidins, isoflavones, dihydroflavonols and chalcones. They are widely distributed in the plant kingdom, being present in variable amounts in dietary fruits, vegetables, nuts, seeds, herbs, spices, tea and red wine [2]. Human daily intake of flavonols plus flavones has been estimated to be 22–23 mg. However, there are large variations within the population depending on their nutritional habits [3]. The interest in dietary flavonoids has grown in the last fifteen years after the publication of several epidemiological studies showing an inverse correlation between dietary consumption of flavonols and flavones and reduced incidence and mortality from cardiovascular disease and cancer [4,5]. For instance, the meta-analysis of seven prospective cohort studies concluded that the individuals in the top third of dietary flavonol intake are

Correspondence: R. Andriantsitohaina, Biologie Neuro-Vasculaire Intégrée, UMR CNRS 6214-INSERM 771, School of Medicine, 49045 Anger, France. E-mail: ramaroson.andriantsitohaina@univ-angers.fr

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associated with a reduced risk of mortality from coronary heart disease as compared with those in the bottom third, after adjustment for known risk factors and other dietary components [6]. A limited number of intervention studies with flavonoids and flavonoid containing foods and extracts has been performed in several pathological conditions [7].

The first biological property described for flavonoids was related to the barrier function of the endothelium [8]. Due to their effect on capillary permeability, flavonoids were formerly considered vitamins. The term "vitamin P" (for Permeability) [8] was discontinued in the 1950s [9]. In recent years, a large amount of experimental and some clinical data have accumulated regarding the effects of flavonoids on the endothelium under physiological and pathological conditions. In the present paper we review the effects of flavonoids on endothelial function and its potential protective role in cardiovascular diseases. It must be pointed out that flavonoids represent a large group of different compounds and their effects may differ both quantitatively and qualitatively. Most of the available data regard flavonoid-rich foods (cocoa) or beverages (wine, juices and tea), extracts from wine or fruit juices and some isolated compounds such as the flavonols quercetin and kaemfperol, cathechins and the antocyanin delphinidin. The effects of isoflavone phytoestrogens derived from soy, genistein and daidzein, have been also widely studied, but differ from the most classic flavonoids in multiple ways and will not be analyzed in the present paper. The present review mainly focuses on the vascular effects of quercetin (representing a 60–75% of the total dietary flavonols plus flavones intake [4]) and wine polyphenolics (a complex mixture of different compounds), which have been widely studied by several research groups including ours and probably represent the most prototypical examples of a flavonoid and a flavonoid-rich extract, respectively.

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Endothelial function and dysfunction

Endothelial factors and its physiological role

Endothelial cells, strategically located in the interface between the blood and vascular smooth muscle cells, release a number of vasoactive substances and play a major role in the control of vascular structure and function and platelet aggregation [10]. Under physiological conditions, the predominant effect of substances released by the endothelium is vasodilator, antiproliferative and antiaggregant, limiting the raise in blood pressure, regulating tissue blood flow and maintaining blood fluidity. Endothelial-derived substances with vasodilator and antiproliferative effects include NO, prostacyclin and endothelium-derived hyperpolarizing factors (EDHF), and substances with vasoconstrictor and mitogenic effects include endothelin-1 (ET-1) and $PGH_{2\alpha}$.

Endothelial dysfunction

The equilibrium between vasodilator and vasoconstrictors is shifted in cardiovascular diseases, where vasoconstrictor and proliferative effects predominate, leading to hypertension, atherosclerosis, platelet aggregation and ischemia. Thus, endothelial dysfunction is characterized by impaired endothelium-dependent vasodilatation and a prothrombotic and proinflammatory state of endothelial cells. Endothelial dysfunction is an early and independent predictor of poor prognosis in most forms of cardiovascular diseases [11–13]. Thus, alterations in endothelial function have been consistently found in hypertension, atherosclerosis, coronary heart disease, diabetes, sepsis, obesity and aging [11–14].

Nitric oxide

NO is formed from L-arginine by the endothelial NO synthase (eNOS) which requires Ca^{2+}/calmodulin, FAD, FMN and tetrahydrobiopterin (BH4) as cofactors [15]. eNOS is activated upon increases in cytosolic Ca^{2+} which occurs in response to multiple stimuli such as shear stress, hormones, platelet derived substances and several drugs. NO diffuses to the adjacent smooth muscle cells where it activates soluble guanylyl cyclase and induces vasodilatation [16]. It also exerts antiaggregant effects in platelets. NO plays a fundamental role in controlling blood pressure, tissue flow and blood fluidity.

The most characteristic pathophysiological feature of endothelial dysfunction is a diminished bioactivity of endothelium-derived NO resulting in impaired vascular homeostasis [11–14]. The classical approach to analyze endothelial function both *in vitro* and *in vivo* is to stimulate endothelial NO release with agonists such as acetylcholine. The mechanisms involved in the decreased endothelial-derived NO responses have been extensively studied and include [11,17,18] : (1) inhibition of the signal transduction from receptor activation to eNOS activation, (2) changes in the activity and/or expression of eNOS, (3) changes in the vascular levels of superoxide anion $\left(O_2^-\right)$ and, thus, O_2^--driven NO inactivation (see below) and, (4) changes in the sensitivity to the NO-cyclic guanosine monophosphate (cGMP) pathway in vascular smooth muscle cells. Reduced NO synthesis associated to endothelial dysfunction may be caused by reduced expression of eNOS, postransductional modification of the enzyme (e.g. phosphorylation or fatty acid modifications), interactions with heat shock protein 90 (hsp90), calmodulin or caveolin, suboptimal concentrations of the substrate L-arginine or the cofactor tetrahydrobiopterin (BH4), or the presence of endogenous NOS inhibitors such as asymmetric dimethylarginine and N-monometylarginine [11]. The isoprenoid geranylgeranyl pyrophosphate,

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an intermediate factor in the cholesterol synthesis pathway, also inhibits the activity of eNOS. In addition, hyperhomocysteinemia, which is associated with increased risk of stroke, ischemic heart disease, peripheral vascular disease and venous thrombosis, leads to reduced eNOS activity [19].

Oxidative stress

Oxidative stress, characterized by an increased endogenous production of reactive oxygen species such as O_2^- and hydrogen peroxide (H_2O_2), is a central cause of endothelial dysfunction. Potential sources of vascular O_2^- production include membrane NADPH-dependent oxidase, xanthine oxidase, cyclooxygenase, lipoxygenase, the mitochondrial respiratory chain and eNOS [18]. NADPH oxidase is a multi-subunit enzymatic complex [20] which comprises membrane-subunits ($p22^{phox}$ and $gp91^{phox}$ [nox 2] or its [nox 1 and nox 4]) and cytoplasmic subunits ($p47^{phox}$, $p67^{phox}$ and $p40^{phox}$). This complex is considered to be the most important source of O_2^- in the vessel wall [21–23]. Interestingly, excess of O_2^- generation synthesized by NADPH oxidase is critically involved in the breakdown of NO associated to endothelial dysfunction in experimental and clinical hypertension [11,18,24]. Under certain circumstances, eNOS becomes dysfunctional ("uncoupled") and electrons are diverted to molecular oxygen rather than to L-arginine, producing O_2^- instead of NO. eNOS uncoupling represents another potential mechanism involved in endothelial dysfunction and it may result from a deficiency of L-arginine, BH_4 or may be promoted by the presence of its endogenous inhibitors. Thus, eNOS uncoupling is also a critical mechanism in experimental and clinical endothelial dysfunction [25].

O_2^- can interfere with NO by a number of mechanisms, both with its synthesis and its activity, ultimately leading to reduced endothelium-dependent vasodilatation [18]. Acutely, O_2^- reacts with and inactivates very rapidly NO, to form peroxynitrite ($ONOO^-$) [26]. O_2^- also oxidizes BH_4, a cofactor required for NO synthesis. Beside, O_2^- and its by-products, $ONOO^-$ and H_2O_2, are involved in the oxidation of LDL cholesterol and in the inflammatory process accompanying atherosclerosis. Oxidized LDL, in turn, increases the synthesis of caveolin-1, which inactivates eNOS. Moreover, O_2^- and H_2O_2 may produce direct vasoconstrictor effects [27].

Cyclooxygenase

Activation of endothelial cyclooxygenase leads to the transformation of arachidonic acid into PGH_2 that is metabolized by several enzymes into different products [28]. In endothelial cells, under physiological conditions, the major metabolite is PGI_2 [29]. The vasodilator activity of PGI_2 is determined by the expression of specific receptors on vascular smooth muscle cells that are coupled to adenylyl cyclase [30]. Cyclooxygenases exist in two isoforms, COX-1 and COX-2, which are constitutively expressed or inducible, respectively [31,32]. Some of the cyclooxygenase by-products are endothelium-derived contracting factors. Indeed, the use of inhibitors of cyclooxygenase, like indomethacin, pointed out an increased participation of endothelial cyclooxygenase-derived vasoconstrictor metabolites in conductance and resistance arteries in animal models of cardiovascular disease and in humans [33–35]. The cyclooxygenase metabolite(s) involved in the increased endothelium-dependent vasoconstriction are those who can activate the thromboxane A_2(TXA_2)/endoperoxide receptor such as PGH_2, TXA_2 or $PGF_{2\alpha}$ [33–37]. Hence, the use of an antagonist of the TXA_2/endoperoxide receptor improves endothelial dysfunction in certain circumstances [33–37]. The increased participation of vasoconstrictor factors derived from cyclooxygenase has been associated with an increased expression of COX-1 and COX-2 proteins in the vessel wall [34]. Regarding the cyclooxygenase isoform responsible for the release of vasoconstrictor prostanoids, no consensus can yet be established. The change in the pattern of products released by cyclooxygenase might be related to an alteration in the lipid substrates. Indeed, an increase in lipid peroxidation was associated with an increased participation of cyclooxygenase-derived vasoconstrictors in endothelium-dependent relaxation in the rat. This may be a consequence of an increased oxidative stress on the cyclooxygenase-dependent response for example in the course of aging. Finally, it should be noted that NO can chemically interact with PGH_2 [38,39] and the observed increased production of cyclooxygenase-derived EDCF may account for the decreased availability of NO.

EDHF

Another important endothelium-derived relaxing factor, especially in resistance arteries, is EDHF. The nature of EDHF, depending on the type of artery considered, has been proposed to be epoxyeicosatrienoic acid (EET), K^+, anandamide and H_2O_2 [40–42]. The responses mediated by EDHF are resistant to NO synthase and cyclooxygenase inhibitors but are sensitive to the combination of the SK_{Ca} and IK_{Ca} channel inhibitors apamin plus charibdotoxin. EDHF-mediated responses are initiated by the activation of endothelial SK_{Ca} and IK_{Ca} channels which leads to the hyperpolarization of endothelial cells [42]. The consecutive hyperpolarization of smooth muscle cells involves the spread of an electric current through myo-endothelial gap junctions [42]. Endothelial dysfunction is associated in some cases with a reduced EDHF-component of the relaxation, independently

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or in addition to NO. Possible reasons to explain the differences observed in the magnitude of the alteration in EDHF-mediated relaxation could be species or anatomical heterogeneity of vasomotor regulation conductance vs. resistance arteries. Also changes might occur downstream of EDHF release such as a decreased expression of voltage- and Ca^{2+}-activated K^+ channels [43]. Under physiological conditions, these channels serve as an hyperpolarizing force that oppose contraction. Thus, their reduced expression could lead to a decreased vasodilatory capacity. However, this change is not uniform among different vascular beds and species.

ET-1

An increased participation of the potent vasoconstrictor ET-1 could also explain reduction of endothelium-dependent vasodilatation. Indeed, endothelial dysfunction is associated with an upregulation of mRNA expression of the precursor of ET-1, prepro-endothelin-1 and ET-1 protein [44,45]. Numerous conditions characterized by an impaired availability of NO have been found to be associated with enhanced synthesis of ET-1 and vice-versa, thereby suggesting that these two factors have a reciprocal regulation [46]. ET-1 was described to exert a bidirectional effect by either enhancing NO production via ET_B receptors located in endothelial cells or blunting its effect via ET_A receptors prevalently located in the vascular smooth muscle cells. Conversely, NO was found to inhibit ET-1 synthesis in different cell types [46]. Several factors affect in opposite direction the transcription of prepro-ET-1 and NOS genes, peroxisome proliferator-activated receptors playing a key role in these regulatory mechanisms [46]. All these data suggest that endothelial dysfunction is also characterized by this dual effect on the NO and ET-1 pathways.

In vitro effects of flavonoids

Vascular effects of several flavonoids have been widely studied *in vitro*. However, caution should be made and there are some limitations for the relevance of results from *in vitro* studies because little is known on bioavailability of flavonoids. Quercetin and related flavonoids are absorbed in appreciable amounts in the small intestine. However, they are metabolized both at the enterocytes and in the liver into methylated and glucurono- and sulfo-conjugated derivatives which show a long-lasting presence in plasma [2]. Unconjugated levels of flavonoids in plasma are low and it is currently believed that most of the *in vivo* effects of flavonoids may be due to the their conjugated metabolites. Unfortunately, the literature on the vascular effects of these metabolites is scarce, and it is presently unknown which of the effects obtained *in vitro* reported for the parent compounds are also pertinent to the metabolites.

Direct effect on vascular smooth muscle tone and proliferation

Quercetin and related flavonoids exert direct (endothelium-independent) vasodilator effects in isolated arteries [47–49]. This vasodilator effect occurs similarly in arteries constricted by different stimuli including endogenous vasoconstrictors (noradrenaline, ET-1 or TXA_2), PKC activators (phorbol esters) and depolarizing agents (KCl). The molecular mechanisms of the direct vasodilator effects are not definitely established but might involve multiple actions on protein kinases such as MLCK and PKC. Interestingly, quercetin and its metabolites are more potent in coronary arteries [50] and in resistance than in conductance vessels [51].

Several studies have shown that quercetin and red wine polyphenols inhibit proliferation and hypertrophy of vascular smooth muscle cells in culture [52–54]. The inhibitory effects of quercetin on DNA synthesis of vascular smooth muscle cells stimulated by TNF-α appear to be related to reduced ERK1/2 activity [52], a kinase playing a major role in cell proliferation and differentiation. In contrast, in angiotensin II-stimulated vascular smooth muscle cells, quercetin and its conjugated glucuronide inhibit hypertrophy via down-regulation of the JNK pathway [53]. Quercetin blocks cell-cycle in G1-phase, and subsequently down-regulates cyclins and CDKs and up-regulates the CDK inhibitor p21 expression in vascular smooth muscle cells [53].

Effects on nitric oxide

Flavonoids exert multiple effects on endothelial-derived NO. In addition to its direct vasodilator effects, grape juices and wine or extracts from a variety of vegetables and fruits have been reported to induce endothelium-dependent vasorelaxation [49,55–58]. Endothelium-dependent relaxation has also been reported for several isolated flavonoids, especially the anthocyanin delphinidin [56] and the flavone chrysin [59]. In contrast, for other structurally related anthocyanins (e.g. malvidin and cyanidin) and other flavonols (e.g. quercetin and kaempferol), a possible endothelium-dependent relaxation is masked by endothelium-independent effects [47–49,51,56]. Endothelium-dependent relaxation induced by chrisin, red wine polyphenols and delphinidin is inhibited by NO synthase inhibitors, indicating that it was mediated by the NO-cyclic GMP pathway. Furthermore, this effect does not appear to be related to a protective effect on NO but rather to an increase of NO synthesis. These flavonoids increased endothelial cytosolic Ca^{2+} levels, [55–58] the physiological

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signal for eNOS activation. The increase in Ca^{2+} in endothelial cells was inhibited by a mixture of superoxide dismutase and catalase suggesting the involvement of O_2^- [60].

In addition, by scavenging O_2^- or by inhibiting its synthesis (see below), flavonoids protect NO from O_2^--driven inactivation, increasing its half-life and its biological activity [61]. Due to its antioxidant properties, flavonoids can potentially avoid BH_4 oxidation and eNOS uncoupling. Therefore, under conditions of high O_2^-, flavonoids potentiate NO- and endothelium-dependent relaxation, reverting oxidative stress-induced endothelial dysfunction. However, the chemical relationships between flavonoids and NO is more complex because flavonoids may also scavenge NO [62]. This reaction involved the autoxidation of flavonoids in aqueous buffers producing O_2^- which ultimately inactivates NO [61]. However, the rate of NO scavenging of flavonoids including quercetin is much lower than that of the physiological NO scavenger haemoglobin.

NO exerts its vasodilator effects by activating soluble guanylyl cyclase in vascular smooth muscle cells and the subsequent increase in cGMP [16]. In turn, cGMP is metabolized by cyclic nucleotide phosphodiesterases (PDEs) and thus, NO activity and endothelium-dependent relaxation are strongly dependent on PDE activity. Therefore, PDE inhibitors can prevent endothelial dysfunction in some circumstances [63]. Several flavonoids have also been reported to inhibit several PDE isoforms [64]. In fact, some flavonoids (e.g. kaempferol) potentiate the relaxant response to the guanylyl cyclase activator sodium nitroprusside [47]. Thus, inhibition of PDEs may represent another potential mechanism for flavonoid-induced prevention of endothelial dysfunction.

Flavonoids may also regulate NO activity at the level of eNOS mRNA and/or protein expression. Long-term incubation of endothelial cells with red wine or the anthocyanins delphinidin, malvidin, cyanidin and paeonidin increased eNOS expression while most isolated flavonols were without effect [65–66].

Antioxidant effects

The health-promoting effects of flavonoids are usually attributed to their powerful antioxidant activity. Quercetin and related flavonoids, in the low micromolar range, are effective scavengers of several reactive oxygen species including O_2^-, singlet oxygen and hydroxyl and peroxyl radicals [67–68]. Furthermore, the glucuronized metabolites of quercetin also behave as antioxidants [69]. The copper chelating properties of flavonoids have also been suggested to contribute to its antioxidant effect [70]. Besides these ROS scavenging effects, and probably more importantly, flavonoids inhibit several ROS generating enzymes including xanthine oxidase [71] and the membrane NADPH oxidase complex in neutrophils [72]. As mentioned above, by reducing cellular O_2^- concentrations flavonoids protect NO and increase its bioactivity. In addition, they are powerful inhibitors of LDL oxidation, a key event in the process of atherosclerotic plaque genesis. The hydrophilic properties of flavonoids facilitate its localization at the water phase-lipid bilayer interface and thereby protect lipids and vitamin E in phospholipid bilayers and in LDL from the initial attack by aqueous radicals. In addition, flavonoids prevent cytotoxicity of oxidized LDL.

On the other hand, flavonoids are not devoid of pro-oxidant properties. For example, quercetin can autoxidize in aqueous solutions to generate free radicals [61] and may also deplete intracellular thiols such as glutathione both *in vitro* and *in vivo*. The pro-oxidant effect of red wine polyphenolics may also play a positive role in endothelial function because O_2^- generation in endothelial cells can stimulate Ca^{2+} signalling and enhance eNOS activity (see above) and increase EDHF release (see below).

Effects on EDHF

An additional mechanism by which flavonoids might induce endothelium-dependent relaxation is by stimulating the release of EDHF. In fact, red wine polyphenols have been shown to produce endothelium-dependent relaxation and hyperpolarization in porcine coronary artery rings in the presence of NOS and cyclooxygenase inhibitors [73]. These responses were inhibited by the combination of charybdotoxin plus apamin and also reduced by antioxidants, membrane permeant analogues of superoxide dismutase, and diphenylene iodonium, an inhibitor of flavin-dependent enzymes. These findings demonstrate that red wine polyphenols cause EDHF-mediated relaxations of coronary arteries involving a pro-oxidant mechanism. The flavone chrysin has also been suggested to release EDHF in addition to NO in the perfused mesenteric bed [74].

Effects on ET-1

The effect of flavonoids on the ET-1 system has been studied by Corder and colleagues [75]. A low concentrations of red wine polyphenols is sufficient to strongly inhibit ET-1 release and transcription of prepro-endothelin-1 in bovine aortic endothelial cells (BAEC). None of the purified compounds (quercetin, resveratrol, catechin, epicathechin, pelargonidin, delphinidin, cyanidin) possess such property at this concentration. The mechanism appears to involve tyrosine kinases. In contrast, other study reports that quercetin (0.5–50 μM) is able to inhibit ET-1 release in human endothelial umbilical vein [76]. No data are available for the *in vivo* effect of flavonoids on ET-1 production.

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Effects on endothelial cell apoptosis and proliferation

Apoptosis of endothelial cells strongly affect endothelium permeability and thus facilitates the development of various pathologies. With regard to the endothelial cells, polyphenols exert a double-edged role in apoptosis by acting on multiple pathways. Polyphenols have been shown to exert protective effects *in vitro* against apoptosis mediated by oxidized LDL and hydrogen peroxide in BAEC and fibroblasts [77]. The inhibition by polyphenols of the apoptosis induced by oxidized LDL in BAEC is related to the modulation of the calcium homeostasis. Recently, we demonstrate that delphinidin is able to protect endothelial cells against apoptosis [78]. Of particular interest is the finding that the antiapoptotic effect of delphinidin results from increased eNOS expression via mitogen-activated protein kinase inhibitor-sensitive pathway. The effect of delphinidin also involves the NO and guanylyl cyclase-dependent pathway and is associated with the maintenance of endothelial cytosolic calcium level in a physiological range and the decrease of cytochrome *c* release from the mitochondria. Flavonoids affect apoptosis partly through caspase pathway. Indeed, polyphenols from oolong tea, theasinensin A, promote apoptosis of tumor cells by activating caspases 3 and 9 [79]. Besides, polyphenols can modulate the level of expression of protective proteins (Bcl-2, Bxl-xL, A1) or pro-apoptotic factors (Bax, Bid, Bak) [80]. Whatever the effects of flavonoids on endothelial, they can either be pro- or anti-apoptotic agents in order to maintain endothelial integrity depending on the aggression.

Few studies were conducted on the effect of flavonoids on endothelial proliferation but in general they are reported as inhibitors of this process [81]. We recently reported that endothelial cell migration and proliferation are prevented by delphinidin through the involvement of cyclin D1- and A-dependent pathway [78,82]. Surprisingly, the effect of delphinidin does not involve the NO pathway in contrast to its action on endothelial cell apoptosis.

Ex vivo and *in vivo* effects of flavonoids: Experimental and clinical

Endothelial function in hypertension

Blood pressure elevation is a common and powerful contributor to all of the major cardiovascular diseases, including coronary disease, stroke, peripheral artery disease, renal disease and heart failure [83]. The blood pressure lowering effects of vegetables and fruit-rich diet in hypertensive patients is well known [84]. It has been mainly attributed to the presence of multiple antioxidants present in these foodstuffs. The effects of the flavonol quercetin and red wine polyphenols have been analyzed in detail in several animal models of hypertension.

Chronic quercetin treatment (5 or 10 mg Kg^{-1} day^{-1}) lowered blood pressure or prevented the development of hypertension in all experimental models tested including spontaneously hypertensive rats (SHR), nitric oxide deficient rats (chronically treated with L-NAME), desoxycorticosterone acetate-(DOCA) salt hypertensive rats, Dahl salt-sensitive hypertensive rats and two-kidney, one-clip Goldblatt hypertensive rats [85–89]. Therefore, regardless of the status of the renin-angiotensin system, volume-expansion, nitric oxide, renal injury or oxidative stress, quercetin produced consistent antihypertensive effects. Quercetin also exerted end-organ protection in hypertensive animals, reducing the cardiac and renal hypertrophy, the proteinuria and the vascular remodelling associated with elevated blood pressure. These preventive effects on structural changes may result from the reduction in blood pressure and the subsequent reduced trophic effect of haemodynamic forces but also by a direct antiproliferative effect of quercetin as discussed above. These experimental models, as well as human essential hypertension, are associated with reduced endothelium derived NO-dependent vasodilatation. In SHR, DOCA-salt and Goldblatt rats, chronic quercetin restored the impaired endothelial vasodilator function as measured by the relaxant response to acetylcholine. Increased urinary NOx (nitrites + nitrates, main NO metabolites) was also found. All these models were associated with increased plasma, vascular and hepatic oxidative status as measured by plasma, tissue and urinary levels of either malonyldialdehyde or isoprostanes, and quercetin consistently reduced these parameters [85–87,89]. Altogether these results suggest a role for reduced O_2^--driven NO inactivation. Furthermore, in a recent study, quercetin has been shown to downregulate the expression of SHR aortic $p47^{phox}$, a key regulatory subunit of NADPH oxidase which was accompanied by a reduction in vascular NADPH oxidase derived O_2^- production [90]. Additionally, apparent changes in endothelial-dependent vasodilatation may occur as a consequence of the opposing effects of the release of endothelium-derived vasoconstrictor prostanoids [91,92]. Chronic quercetin did not modify the endothelium-dependent vasoconstriction in SHR [85,90] but markedly inhibited the later in NO-deficient [86] and in Goldblatt rats [89]. In addition, aortic TXA_2 production was increased in SHR [89], in NO-deficient [86] and in Goldblatt rats [89] as compared to their normotensive controls. Again, quercetin reduced aortic TXA_2 levels in NO-deficient and in Goldblatt rats but not in SHR. Other isolated flavonoids such as baicalein and flavone have been reported to prevent the development of endothelial dysfunction in SHR [93]. Moreover, baicalein

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reduced blood pressure in hypertensive animals via an increase in prostacyclin production [94].

It must be noted that quercetin had minimal, if any, effects on blood pressure or endothelial function in normotensive animals. This is consistent with the lack of effect on blood pressure of a high dose of quercetin in normotensive subjects [95] or the minimal effect of a fruit and vegetables rich diet in the subgroup of normotensive subjects [84]. Thus, quercetin and related flavonoids may exert their effect on endothelial function specifically when endothelial function is impaired and blood pressure is elevated.

Administration of Provinols™ (a standardized red wine polyphenolics extract) reduced the increase in both blood pressure and protein synthesis in heart and aorta caused by chronic inhibition of NO synthesis [96]. This was accompanied by reduced end-organ damage such as myocardial fibrosis and aortic thickening. Provinols™ also prevented endothelium-dysfunction that was associated with an increase of NOS activity, a moderate enhancement of endothelial NOS expression and a reduction of oxidative stress in the left ventricle and aorta. In addition, an alcohol-free grape skin extract also significantly reduced systolic, mean and diastolic arterial pressure in DOCA-salt and L-NAME-induced experimental hypertension in rats [97].

To the best of our knowledge, there are no studies published in hypertensive patients with isolated flavonoids. However, there are some reports on the effects of flavonoid-rich foods such as cocoa and fruit juices compared to similar foods or beverages devoid or poor in flavonoids. The effects of flavonoid-rich dark chocolate compared to white chocolate on blood pressure and endothelial function in essential hypertension have been analyzed in a double-blind cross-over study [98]. Dark chocolate significantly reduced blood pressure and insulin resistance and improved flow-dilated (endothelium-dependent) vasodilatation. Another double-blind, cross-over study conducted in 12 hypertensive patients which received alternately high-flavonoid sweetie juice and low-flavonoid sweetie juice for 5 weeks [99] showed that the flavonoid rich juice significantly reduced blood pressure. Even when concerns can be raised about which specific components are responsible for these effects, these well controlled trials suggest that flavonoid-rich foods exert a beneficial effect on blood pressure and endothelial function in hypertensive patients. Interestingly, in healthy male adults the ingestion of flavanol-rich cocoa was associated with increased levels of circulating NO species and enhanced endothelium-dependent vasodilation and these effects were mimicked by (-)-epicatechin [100].

LDL oxidation and atherosclerosis

As indicated above, one of the mechanisms responsible for endothelial dysfunction is the increased LDL oxidation and flavonoids possess the ability to reduce lipid sensitivity to oxidation. In animals, Vinson et al. [101] reported that polyphenols from red wine or grape juice reduce the plasmatic concentration of lipids in hamsters. This is consistent with studies in humans. Red, but not white, wine consumption for 2 weeks resulted in a 20% reduction in the propensity of plasma to undergo lipid peroxidation in healthy subjects [102]. In addition, short-term ingestion of purple grape juice has been shown to reduce LDL susceptibility to oxidation in patients with coronary artery disease [103]. This is also in harmony with the recent study in hypercholesterolemic postmenopausal women with red wine complementation [104]. However, in several studies, the LDL oxidation was not modified by flavonoid intake in human. de Rijke et al. [105] did not observe a modification of the LDL oxidizability in healthy volunteers after red wine consumption (550 ml during 4 weeks). O'Reilly et al. [106] have published that the intake of flavonoids from onions and green tea does not modify the level of LDL oxidation in human. Moreover, a study in postmenopausal women have recently determined that the intake of lyophilized grape powder (rich in flavans, anthocyanins, quercetin, myricetin, kaempferol, and resveratrol) during 4 weeks does not reduce the LDL oxidation, even when plasma triglycerides, plasma LDL-cholesterol and apolipoproteins B and E concentrations, are lowered by the treatment [107]. Therefore, the effect of flavonoids on LDL oxidation may vary depending on their structure, the type of natural diet they were originated and the dose used. In addition, several flavonoids have been reported to prevent the development of atherosclerosis lesions in ApoE deficient mice without effects on plasma LDL or HDL cholesterol levels [108].

Coronary disease

The pathophysiology of ischemic heart disease and acute coronary syndromes involves multiple alterations in vascular reactivity, vascular structure, and interactions of the vessel wall with circulating blood elements [109]. Alterations in the serum lipid profile and the development of atherosclerosis in the coronary arteries is an early and crucial event in ischemic heart disease. Furthermore, blood pressure elevation is a powerful risk factor for coronary disease. Controlled trials indicate that a reduction of LDL cholesterol and atherosclerotic lesions and blood pressure produces a dramatic decline in the risk of coronary disease [83,109,110]. Therefore, the antiatherogenic and antihypertensive effects of flavonoids (as discussed above) might prevent coronary disease. Most acute coronary events result from a rupture in the atherosclerotic plaque and the subsequent thrombus formation. Degradation of the interstitial collagen conferring biomechanical strength to the plaque fibrous cap by matrix metalloproteinases

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(MMPs) appears to be involved in the plaque instability and its rupture. Coronary vasospasm may also contribute to acute impaired arterial flow. Flavonoids exert platelet antiaggregant properties [111,112], reduce the expression of matrix metalloproteinases (MMP-2 and MMP-9) [113] and induce a coronary vasodilator effect [50,114] providing additional potential mechanisms for the prevention of the acute coronary events.

Endothelial dysfunction in coronary artery disease was reported as early as 1986 [115]. Reduced bioavailability of endothelial NO is involved in the genesis progression and complications of coronary atherosclerosis. An increase in endothelium-dependent vasodilatation has been observed in patients with coronary disease treated with grape purple juice or tea [103,116]. Post-ischemic reperfusion occurring in coronary diseases is generally associated with a reduction of endogenous NO production resulting from endothelial dysfunction and tissue damage linked to neutrophil infiltration. The capacity of flavonoids to enhance NO generation probably plays a crucial role to prevent ischemia. These observations have been reinforced by the abrogation of the protective effect of wine polyphenols after blockade of NO production with NO synthase inhibitor. Experimental studies in animal models have shown that grape juice, red wine or isolated polyphenols including flavone and quercetin reduce the contractile dysfunction of the heart and protect against the cellular lesions induced by cardiac ischemia [117–120]. These effects may be observed following the oral intake of these substances or after their perfusion in an isolated heart before the induction of an ischemia. Using an *ex vivo* rat model of cardiac ischemia/reperfusion, we have recently published that short-term red wine polyphenol extract treatment protects against post-ischemic infarction via decreased oxidative stress and implies an involvement of NO-dependent pathway [121].

Stroke

The primary cause of stroke is an interruption of cerebral blood flow, which occurs either during an arterial or venous obstruction (embolus or local thrombosis) or during a cardiac arrest. The pathophysiological processes in stroke are complex and depend on the severity, duration and localization of the ischemic damage in the brain. Flavonoids can be potentially used in both preventive and acute approaches to the treatment of strokes [122]. Indeed, flavonoids prevent endothelial dysfunction responsible for atherosclerosis and thrombosis and lower blood pressure and blood cholesterol. With regard to grape and red wine, the main reported studies examining their *in vivo* effects on cerebral ischemia have been conducted using resveratrol [122]. The beneficial effect of resveratrol on cerebral ischemia is discussed in a recent review and is out of the scoop of the present review which deals mainly on wine and grape extracts [122]. Dietary supplementation of grape powder or an extract of the powder improved ischemia-induced delayed neuron death and glial cell activation. The grape extract showed effective protection even when it was given after ischemia [123] Quercetin has been reported to be able to scavenge superoxide anions released during reperfusion after forebrain ischemia using a four-vessel occlusion model in rats [124]. Consequently, quercetin (30 mg/kg intraperitoneally) given after middle cerebral artery ligation in a permanent middle cerebral artery occlusion model in rats significantly decreased the infarct volume. We have investigated the potential protective effects of wine polyphenols in a rat model of transient cerebral ischemia [125,126]. A red wine polyphenolic extract was given orally during one week before the induction of the stroke. Microdialysis analysis shows that polyphenols do not modify the energy metabolism and the oxidative stress, but are able to reduce the excitotoxicity by inhibiting the massive release of glutamate and aspartate. The cerebral blood flow has also been monitored and polyphenols can improve the residual blood flow both during occlusion and early reperfusion. Furthermore, polyphenols may induce vascular remodeling, with an increase of the internal diameter of the brain vessels. Most interestingly, wine polyphenols reduce brain infarct size an effect that is associated with inhibition of ischemia-induced excitotoxicity and improvement of cerebral blood flow. Data from this study provide an experimental basis supporting the view that wine polyphenols may be beneficial for stroke protection.

Summary and conclusions

The present review mainly focuses on the vascular effects of quercetin and wine polyphenols. A typical commercial bottle of red wine contains approximately 1.8 g/L of total polyphenols. So the total amount of polyphenols found in a glass of red wine is about 200 mg. Flavonoids constitute the majority of these phenols in red wine and their concentrations are ranged from 1365 to 1500 mg/L [127].

Endothelial dysfunction is a common pathophysiological feature which develops early in the evolution of cardiovascular diseases. The severity of endothelial dysfunction is related to poor prognosis and interventions reducing cardiovascular risk have been shown to improve endothelial function. A large body of evidence supports the hypothesis that flavonoids show protective effects *in vitro* on the vessel wall, particularly on endothelial function, involving multiple mechanisms. In endothelial cells, they induce the release of vasoactive factors such as NO and EDHF and prevent

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the apoptosis induced by oxidative damage. They also inhibit the release or the action of endothelial derived constrictor factors such as ET-1. In vascular smooth muscle cells, flavonoids directly inhibit the contractile and proliferative response induced by several pathological stimuli. In several animal models of endothelial dysfunction associated to hypertension, flavonoids improve endothelial function, reduce blood pressure and the associated cardiac, vascular and renal damage and reduce the expression and the activity of pro-oxidative enzymes such as membrane NADPH oxidase. A limited number of clinical studies have also shown that flavonoid rich foods can improve endothelial function in patients with hypertension, atherosclerosis and coronary heart disease.

Outstandingly, flavonoids can potentially interact with most of the steps involved in the pathophysiology of ischemic heart disease and stroke. Flavonoids might prevent ischemic heart disease by multiple mechanisms operating simultaneously in both the chronic (e.g. by inhibiting the genesis and development of atherogenic plaques, hypertension and endothelial dysfunction) and the acute phase (e.g. by inhibiting coronary vasoconstriction, plaque vulnerability, platelet adhesion and aggregation and myocardial necrosis). Similarly, flavonoids act on different phases of stroke. For the acute phase, flavonoids improve cerebral blood flow, prevent platelet aggregation and thrombosis, reduce excitotoxicity and inhibit oxidative stress. For the intermediate phase, flavonoids reduce inflammation and protect endothelial integrity. For the late phase, flavonoids interfere with ischemia induced cell death mechanisms such as apoptosis and necrosis.

Altogether, the available evidence indicates that flavonoids might be of therapeutic benefit in cardiovascular diseases, which are still a major public health problem. Nevertheless, prospective controlled clinical studies with flavonoids are still lacking. These human intervention studies would be necessary before establishing any recommendations about dietary habits or administration of dietary supplements.

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