Review

Flavonols and cardiovascular disease

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ABSTRACT

Flavonols, and specially quercetin, are widely distributed in plants and are present in considerable amounts in fruits and vegetables. In addition to their anti-oxidant effect, flavonols interfere with a large number of biochemical signaling pathways and, therefore, physiological and pathological processes. There is solid evidence that, *in vitro*, quercetin and related flavonols exert endothelium-independent vasodilator effects, protective effect on nitric oxide and endothelial function under conditions of oxidative stress, platelet antiaggregant effects, inhibition of LDL oxidation, reduction of adhesion molecules and other inflammatory markers and prevention of neuronal oxidative and inflammatory damage. The metabolites of quercetin show partial protective effects on endothelial function and LDL oxidation. Quercetin produces undisputed antihypertensive and antiatherogenic effects, prevents endothelial dysfunction and protects the myocardium from ischemic damage. It has no clear effects on serum lipid profile and on insulin resistance. Human intervention trials with isolated flavonols demonstrate an antihypertensive effect. The meta-analysis of epidemiological studies show an inverse association between flavonol (together with flavone) intake and coronary heart disease and stroke. Therefore, although there is no solid proof yet, a substantial body of evidence suggests that quercetin may prevent the most common forms of cardiovascular disease contributing to the protective effects afforded by fruits and vegetables.

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1. Introduction

Flavonoids were discovered in the 1930s and were initially considered vitamins ("vitamin P") for their effect on capillary Permeability (Rusznyak and Szent-Györgyi, 1936). The interest in these compounds decayed after they were shown not to be required as micronutrients for human health in the 1940s (Joint-Committee-Nomenclature-ASBC-AIN, 1950). However, a large expansion of the field took place much later, in the 1990s, after the publication of epidemiological studies associating a reduced incidence of cancer and cardiovascular disease with a greater intake of flavonoids (Hertog et al., 1993; Keli et al., 1996). Today above 30,000 studies have been published with flavonoids and above 10,000 with a single one of them: quercetin (Fig. 1). The major advances in the research about flavonols in the cardiovascular field are summarized in Table 1.

Flavonoids constitute a large class of polyphenols found in plants. This group includes several subclasses, such as flavonols, flavones, flavanones, flavanols, anthocyanidins, isoflavones, dihydroflavonols and chalcones (Fig. 2). Among flavonoids, flavonols (together with flavanols) are by far the most abundant and widely distributed in nature. Flavonols are present, usually as diverse glycosides, in considerable amounts in our normal diet. Although flavonols can be found virtually in all
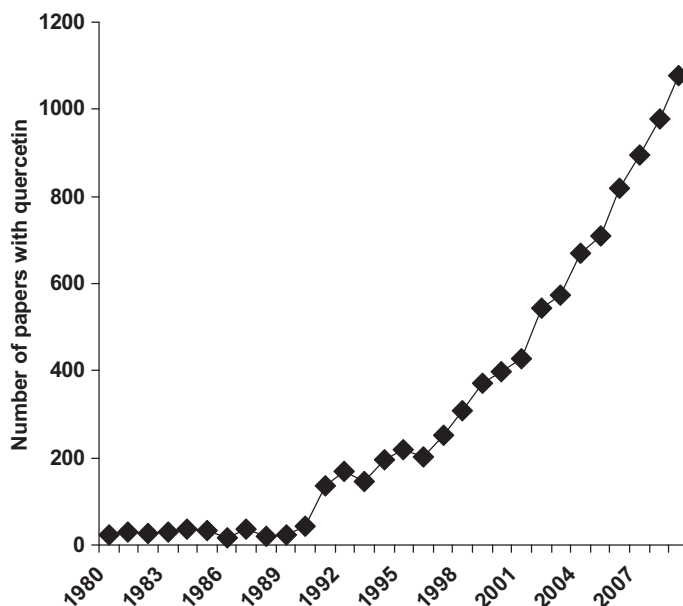

Fig. 1. Trend of publications containing the word "quercetin" in the abstract in the last 30 years analyzed from data in the ISI Web of Science.

Table 1
Major advances in the research of flavonols in the cardiovascular field.

|	References
Discovery of flavonols and effects on capillary permeability	Rusznyak and Szent-Györgyi (1936)
Platelet antiaggregant effects of quercetin	Beretz et al. (1982)
Flavonols inhibit NADPH oxidase and scavenge superoxide	Tauber et al. (1984), Busse et al. (1984)
Quercetin prevents oxidized-LDL cytotoxicity	Negre-Salvayre and Salvayre (1992)
Inverse association between flavonol and flavone intake with coronary heart disease	Hertog et al., (1993)
Quercetin and wine polyphenols inhibit LDL oxidation	Frankel et al. (1993)
Vasodilator effects of quercetin	Duarte et al., (1993a), Fitzpatrick et al. (1993)
Inverse association between flavonol and flavone intake with stroke	Keli et al. (1996)
Antiatherosclerotic effects of wine polyphenols and quercetin	Hayek et al., (1997)
Meta-analysis of epidemiological studies in coronary disease	Huxley and Neil (2003)
Antihypertensive effects of quercetin	Duarte et al. (2001b)
Quercetin and other polyphenolics activate sirtuins	Howitz et al. (2003)
Antihypertensive effects of quercetin in humans	Edwards et al. (2007)
Meta-analysis of epidemiological studies with flavonols and stroke	Hollman et al. (2010)
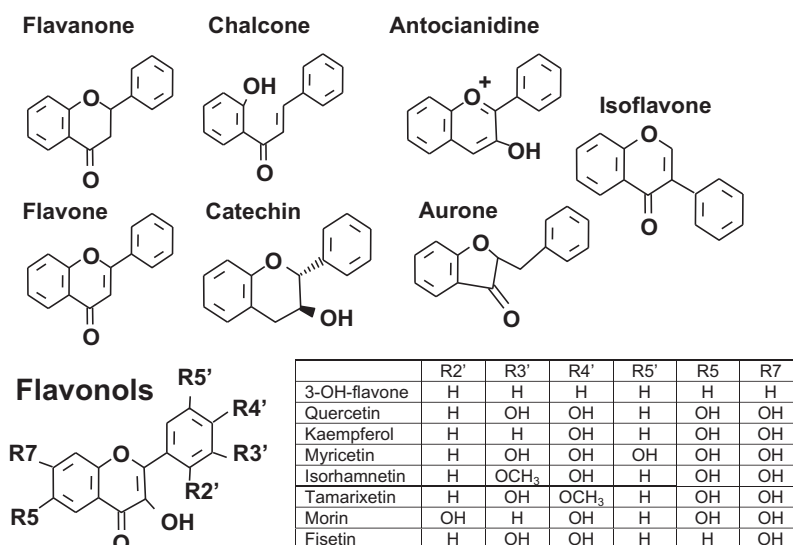
Fig. 2. Chemical structures of the main flavonoid classes and the most common flavonol aglycones.

	R2'	R3'	R4'	R5'	R5	R7
3-OH-flavone	H	H	H	H	H	H
Quercetin	H	OH	OH	H	OH	OH
Kaempferol	H	H	OH	H	OH	OH
Myricetin	H	OH	OH	OH	OH	OH
Isorhamnetin	H	OCH₃	OH	H	OH	OH
Tamarixetin	H	OH	OCH₃	H	OH	OH
Morin	OH	H	OH	H	OH	OH
Fisetin	H	OH	OH	H	H	OH

vegetables and fruits, the richest sources include onions, apples, cider, grapes, wine and tea. They derive from 3-hydroxyflavone, the simplest flavonol. The prototypical flavonol is quercetin, the most abundant in plants and the best studied. Other common flavonols include kaempferol, myricetin, isorhamnetin, tamarixetin, morin and fisetin (Fig. 2). Kaempferol and myricetin are also present in many foods. Isorhamnetin and tamarixetin are methylated metabolites of quercetin and they are usually found in plasma or tissues after quercetin consumption. The daily intake of flavonols has been estimated as 20–35 mg/d, of which more than 65% is quercetin and its glycosides. The bioavailability of quercetin depends on the nature of its attached sugars and the components of the food matrix (ethanol, fat, and emulsifiers) which may affect its solubility. For example, the intake of onions leads to a higher quercetin plasma levels than other dietary sources (Davalos et al., 2006). Glucosides from onions are a better source of bioavailable quercetin than the aglycone given as a pure compound (Hollman et al., 1995). However, when a food source rich in glucosides (shallot flesh) was compared with one rich in the aglycones (the dried shallot skin), the latter led to a higher quercetin plasma levels, indicating that the food matrix is a key factor for quercetin bioavailability (Wiczkowski et al., 2008).

Flavonols show a wide range of biological activities, being the most active compounds within the flavonoids group. Thus, the beneficial effects of diets rich in fruits and vegetables on cardiovascular health have been often attributed to flavonoids in general and more specifically to flavonols. Flavonols are commercialized as dietary supplements either as pure compounds (e.g. quercetin), flavonoids mixtures or extracts, often at doses that exceed by far the dietary intake. Some flavonoids are also used as venotonic drugs for the treatment of several venous diseases (Lyseng-Williamson and Perry, 2003).

In this paper, we will review the continuously growing evidence supporting a beneficial role of flavonols on cardiovascular disease and the potential molecular targets involved. Most of the studies were carried out with quercetin. The available data indicate qualitatively similar biological effects of kaempferol, isorhamnetin and tamarixetin. Myricetin shares with the other flavonols some of their actions but may produce differential and sometimes opposite effects. There is also a vast number of interesting studies using dietary sources, medicinal plants or flavonoid extracts in which the actions may be attributed to flavonols but also to other flavonoids or other substances which will not be addressed herein.

An interesting issue which has been the focus of attention in recent years is whether the observations made *in vitro* with quercetin and other flavonols have any relevance *in vivo*. The biological activity of flavonoids has been analyzed using commercially available aglycones (not glycosylated) compounds which are present at extremely low concentrations in plasma (Kroon et al., 2004). Quercetin is rapidly conjugated with glucuronic acid and/or sulfate during first-pass metabolism (intestine–liver) and a portion of the metabolites are also methylated and, therefore, the major metabolites of quercetin in human plasma are quercetin-3-glucuronide, quercetin-3-sulfate and isorhamnetin-3-glucuronide. The limited information available about the *in vitro* effects of these metabolites will also be reviewed herein. In general, these metabolites are less active than the parent compounds and sometimes totally inactive. However, it is also becoming evident that conjugated metabolites can be deconjugated by beta-glucuronidase and flavonol aglycones can accumulate in tissues (Bieger et al., 2008). There is some indirect evidence that the activity of quercetin glucuronides depends on their deconjugation (Lee-Hilz et al., 2008).

2. Flavonols, the endothelium and the vascular smooth muscle

The vascular endothelium exerts a fine control of cardiovascular homeostasis. The equilibrium between vasodilators and vasoconstrictors, prothrombotic and antithrombotic factors and proliferative and antiproliferative factors is shifted in

cardiovascular diseases leading to hypertension, atherosclerosis, platelet aggregation and ischemia. Thus, endothelial dysfunction is characterized by impaired endothelium-dependent vasodilatation, reduced NO activity 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 (Schachinger et al., 2000; Widlansky et al., 2003). Thus, alterations in endothelial function have been consistently found in hypertension, atherosclerosis, coronary heart disease, diabetes, sepsis, obesity and aging.

The effects of quercetin and related flavonoids modulating endothelial function and dysfunction has been widely studied (Perez-Vizcaino et al., 2006b). Quercetin exerts direct acute vasodilator effects in isolated arteries (Fig. 3) (Duarte et al., 1993a,b; Fitzpatrick et al., 1993). In healthy vessels, these effects are endothelium-independent and occur similarly, albeit with different potency, in arteries constricted by different stimuli. Interestingly, quercetin and its methylated metabolites are more potent in coronary arteries (Ibarra et al., 2002) and in resistance than in conductance vessels (Perez-Vizcaino et al., 2002) and also in vessels from hypertensive animals (Ibarra et al., 2003). Endothelium- and NO-dependent relaxation has been reported for several isolated flavonoids, especially the anthocyanin delphinidin (Andriambeloson et al., 1998) and the flavone chrysin (Duarte et al., 2001a). These effects are related to a pro-oxidant effect because it can be inhibited by superoxide dismutase and catalase and a subsequent increased in endothelial cytosolic Ca^{2+} levels (Andriambeloson et al., 1998). Some groups have also described that the effects of quercetin are partially endothelium-dependent and related to the release of endothelium-derived relaxing factors (Ajay et al., 2003; Khoo et al., 2010). A pro-oxidant mechanism involving the release of H_2O_2 has been proposed (Khoo et al., 2010). We have also observed increased ROS production by quercetin in rat coronary arteries which could be inhibited by catalase (Cogolludo et al., 2007). ROS production may result from the auto-oxidation of quercetin in the incubation medium or by a specific event occurring in vascular cells. However, we have not observed so far endothelium-dependent relaxation by quercetin in many different vessels studied; an observation consistent with recent data from other groups (Suri et al., 2010). In addition, the glucuronidated and sulfated metabolites of quercetin lack a direct vasodilator effect in the rat aorta (Lodi et al., 2009). In the porcine coronary artery, quercetin 3′-sulfate inhibited endothelin-1 and U46619-induced contractions in an endothelium-independent manner, while quercetin 3-glucoronide was inactive (Suri et al., 2010). We have also noted that quercetin 3′-sulfate has acute vasodilator effects in rat mesenteric resistance arteries, but again with a lower potency than quercetin (unpublished observations). In contrast to quercetin and other flavonols, myricetin can induce an endothelium-dependent contractile response via an increase in cyclooxygenase-derived vasoconstrictor metabolites (Jimenez et al., 1999).

The actions of quercetin on NO are very complex and the conditions of oxidative stress strongly influence the outcome. In cell-free systems quercetin can be oxidized by oxygen and generate O_2^- which rapidly reacts and inactivates NO (Lopez-Lopez et al., 2004) an effect which is not shared by the glucuronidated and sulfated metabolites (Lodi et al., 2008). In endothelial cells in the absence of oxidative stress, quercetin has been reported to increase NO when measured by an amperometric electrode (Taubert et al., 2002) and to increase cytosolic Ca^{2+} measured by fura2 via a pro-oxidant mechanism (Khoo et al., 2010). However, some caution should be taken because due to the redox and fluorescent properties of quercetin (Nifli et al., 2007), interferences with the measuring systems cannot be ruled out. In contrast, when NO production was measured by electron paramagnetic resonance spectroscopy, quercetin failed to increase NO in endothelial cells (Stoclet et al., 1999). Inhibitory effects on eNOS activity in bovine endothelial cells have also been reported (Jackson and Venema, 2006). Thus, in the absence of oxidative stress either increases or decreases in NO can be observed.

In contrast, under conditions of high O_2^- and thus accelerated NO metabolism, quercetin can protect NO in a number of ways. First, in cell-free systems when O_2^- is increased enzymatically or chemically, quercetin can scavenge O_2^- and thus
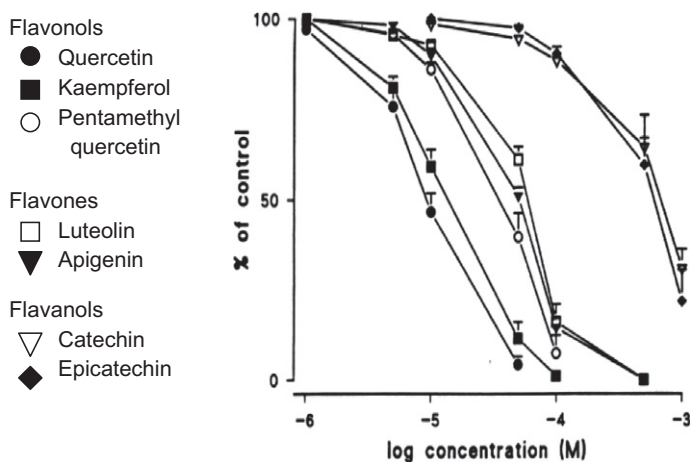

Fig. 3. Vasodilator effects of several representative flavonoids in isolated endothelium-intact rat aorta showing the higher potency of flavonols, followed by flavones and flavanols. Reprinted with permission (Duarte et al., 1993b).

protect NO (Lopez-Lopez et al., 2004) (Fig. 4). Second, in cells, quercetin may not only scavenge O_2^- but also inhibit its enzymatic sources, i.e. xanthine oxidase and NADPH oxidase (Busse et al., 1984; Tauber et al., 1984). The glucuronidated and sulfated metabolites can prevent, albeit less effectively than quercetin, the impaired NO bioavailability under conditions of high oxidative stress (Lodi et al., 2009). Third, due to its anti-oxidant properties, flavonoids can potentially avoid BH_4 oxidation and eNOS uncoupling (Romero et al., 2009). Finally, quercetin can inhibit signaling pathways leading to induction of p47[phox], a NADPH oxidase subunit. Thus, quercetin can prevent the NO impairment induced by angiotensin II (Sanchez et al., 2007) and endothelin-1 (Romero et al., 2009). The preventive effect of quercetin on endothelial dysfunction induced by endothelin-1 seems to be related to down-regulation of p47[phox] through PKC inhibition (Romero et al., 2009). The metabolite quercetin-3-glucuronide also prevented endothelin-1-induced endothelial dysfunction (Lodi et al., 2009). In addition, quercetin *in vitro* restored the impaired endothelial function in arteries from hypertensive (SHR) (Ibarra et al., 2003) and diabetic rats (Ajay et al., 2006).

NO exerts its vasodilator effects by activating soluble guanylyl cyclase in vascular smooth muscle cells and the subsequent increase in cGMP. 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 (Vlachopoulos et al., 2004). Several flavonoids have also been reported to inhibit several PDE isoforms (Picq et al., 1989). Thus, inhibition of PDEs may represent another potential mechanism for flavonoid-induced prevention of endothelial dysfunction. In addition, quercetin and its sulfated metabolite selectively enhanced cyclic-GMP-dependent relaxations by a mechanism not involving phosphodiesterase 5 inhibition (Suri et al., 2010). Interestingly, quercetin and the metabolite also prevented nitroglycerine tolerance *in vitro* (Suri et al., 2010), an effect shared with other O_2^- scavengers (Munzel et al., 1999).

Besides NO, endothelium-derived endothelial factor (EDHF) is another important regulator of vascular function, especially in resistance arteries. The nature of EDHF, depending on the type of artery considered, has been proposed to be epoxyeicosatrienoic acid (EET), potassium or H_2O_2 and smooth muscle cell hyperpolarization can also be transmitted from the endothelial cells via myoendothelial gap junctions (Feletou and Vanhoutte, 2009). The vasodilator effects of quercetin have been recently reported to be prevented by charybdotoxin, a blocker of intermediate (IKCA) and large (BKCA) conductance K^+ channels (Khoo et al., 2010). The authors concluded that quercetin releases endothelium-derived hyperpolarizing factor (EDHF) whose activity is dependent on activation of K^+ channels. We think that this conclusion is not supported by the data. The effect shown by Khoo et al. cannot be attributed to EDHF because (1) charybdotoxin alone does not, or just weakly, inhibit EDHF responses; it must be used in combination with apamin (Corriu et al., 1996) and (2) when arteries are contracted by high concentrations of KCl, as this was the case (70 mM), the membrane potential and the equilibrium potential for potassium approach and therefore, opening or closing K^+ channels would not affect net flow of K^+ across the membrane nor membrane potential (Quast and Cook, 1989). In fact, high KCl is often used to inhibit EDHF responses. However, part of endothelium-independent relaxant responses can be attributed to an activation of BKCA channels directly in the arterial smooth muscle cells (Cogolludo et al., 2007).

Endothelin-1 is a potent vasoconstrictor released by endothelial cells. Endothelial dysfunction is associated with elevation of endothelin-1 (Brunner et al., 2006). Moreover, ET-1 is able to induce endothelial dysfunction. Quercetin is able to
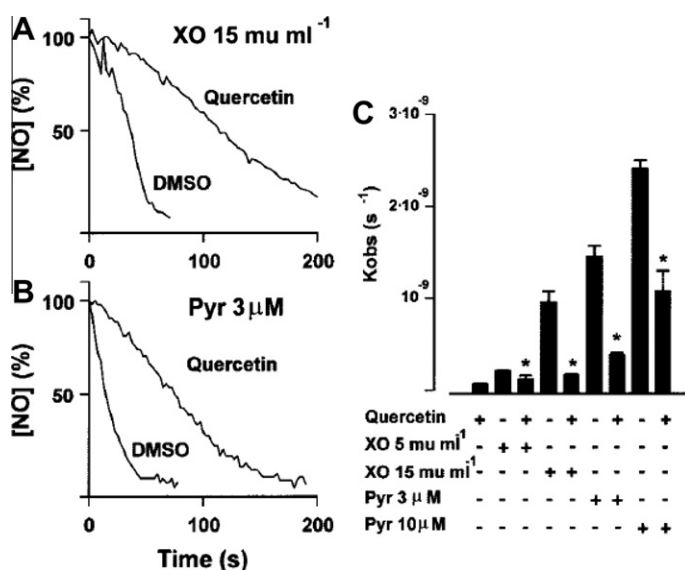

Fig. 4. Quercetin protects NO from superoxide-induced degradation. Superoxide was generated by xanthine oxidase plus hypoxanthine (XO) or by pyrogallol (Pyr). The right panel shows the rate constant (Kobs) for NO dissapearance in the buffer. Reprinted with permission (Lopez-Lopez et al., 2004).

inhibit ET-1 release in human endothelial umbilical vein and in the bovine aortic endothelial cells (Khan et al., 2002; Zhao et al., 1999). *In vivo*, quercetin also reduced urinary ET-1 in the ApoE knockout mice (Loke et al., 2010) and in healthy men (Loke et al., 2008).

In vivo, a high dose of quercetin added to the diet led to increased NOS activity in the aortic wall of healthy rats without changes in the eNOS expression which was accompanied by increased endothelium-dependent relaxation (Benito et al., 2002). We have also observed a weak but occasionally significant increase in endothelium-dependent relaxation in normotensive WKY rats treated with low doses quercetin (Duarte et al., 2001b). In healthy men quercetin increased plasma S-nitrosothiols, plasma nitrite, and urinary nitrate concentrations, indirectly pointing to increased endothelial NO (Loke et al., 2008). However, quercetin can still induce antihypertensive effects *in vivo* when NO synthesis is inhibited (the L-NAME model of hypertension as discussed below).

Hypertensive animals, as well as human essential hypertensive patients, develop reduced endothelium derived NO-dependent vasodilatation. In different experimental rat models of hypertension (SHR, DOCA-salt and Goldblatt rats), chronic quercetin restored the impaired endothelial vasodilator function as measured by the relaxant response to acetylcholine (Duarte et al., 2001b; Galisteo et al., 2004; Garcia-Saura et al., 2005; Sanchez et al., 2006). Increased urinary NO_x (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 (Duarte et al., 2001b; Galisteo et al., 2004; Garcia-Saura et al., 2005; Sanchez et al., 2006). Altogether these results suggest a role for reduced O_2^--driven NO inactivation. Furthermore, in SHR which show upregulated eNOS but with a paradoxical reduction in NOS activity compared to their normotensive WKY controls, quercetin normalized both parameters (Sanchez et al., 2006). In addition, as mentioned above for *in vitro* experiments with angiotensin II and endothelin-1, in SHR quercetin can prevent the upregulation of p47^{phox} (Sanchez et al., 2006). Moreover, chronic quercetin also reduced several markers of endothelial dysfunction in ApoE knockout mice (Loke et al., 2010) and in rats treated with a high-fat high-sucrose diet (Yamamoto and Oue, 2006).

In contrast to the endothelium-dependent vasodilatation to acetylcholine, the endothelium-dependent relaxations to insulin which are also impaired in SHR were unaffected after chronic treatment with quercetin (Romero et al., 2010). The different profile of quercetin against the relaxations induced by these two endothelial NO-releasing agents, despite its protective effect on O_2^--driven NO inactivation, might be related to the different pathways of acetylcholine and insulin to activate eNOS. Acetylcholine is a classic cholinergic agonist that activates eNOS by a calcium-dependent mechanism. However, insulin has calcium-independent vasodilator actions that are mediated by a PI3-K dependent mechanism involving phosphorylation of eNOS by Akt (Montagnani et al., 2001). Insulin-stimulated Akt and eNOS phosphorylations were reduced in aortic rings from SHR and WKY rats treated with quercetin. This effect might be related to a direct inhibitory effect of quercetin on PI3-K (Yoshizumi et al., 2002).

Additionally, apparent changes in endothelial-dependent vasodilatation may occur as a consequence of the opposing effects of the release of endothelium-derived vasoconstrictor prostanoids. Chronic quercetin did not modify the endothelium-dependent vasoconstriction in SHR (Duarte et al., 2001b) but markedly inhibited it in NO-deficient (Duarte et al., 2002) and in Goldblatt rats (Garcia-Saura et al., 2005).

The sirtuins Sir2 and its mammalian analog SIRT1 have been implicated in the extended lifespan induced by caloric restriction (Cohen et al., 2004) and more recently in the prevention of vascular senescence (Ota et al., 2010). Activation of SIRT1 has been shown to promote endothelium-dependent vasodilation (Mattagajasingh et al., 2007) and to downregulate angiotensin II receptor expression (Miyazaki et al., 2008). The discovery of the role of sirtuins in oxidative stress and lifespan has led to a new and fascinating area of research in the field of polyphenols since several polyphenols, including quercetin can activate sirtuins (Howitz et al., 2003). In fact, quercetin extends the lifespan of the yeast *Saccharomyces cerevisiae* and the worm *Caenorhabditis elegans* (Belinha et al., 2007; Pietsch et al., 2009).

It should be also mentioned that quercetin inhibits endothelial proliferation and migration and tube formation (Ahn et al., 2009; Igura et al., 2001; Jackson and Venema, 2006). This effect is associated with decreased VEGF expression and might result in reduced angiogenesis *in vivo* (Luo et al., 2008). Therefore, it might play a role in the chemopreventive effects of quercetin against solid tumors. Several studies have shown that quercetin and its conjugated glucuronide inhibit proliferation and hypertrophy or induce apoptosis in vascular smooth muscle cells in culture (Moon et al., 2003; Perez-Vizcaino et al., 2006a; Yoshizumi et al., 2002). 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, a kinase playing a major role in cell proliferation and differentiation (Moon et al., 2003). In angiotensin II-stimulated vascular smooth muscle cells and in smooth muscle cells with an intimal phenotype (Perez-Vizcaino et al., 2006a), quercetin inhibit hypertrophy via down-regulation of the JNK pathway (Perez-Vizcaino et al., 2006a; Yoshizumi et al., 2002). These effects may also account for the antihypertensive and antiatherosclerotic effect of quercetin.

3. Flavonols and platelets

The platelet antiaggregant effects of flavonols were initially described by Beretz et al. (1982). Several molecular mechanisms have been proposed to be involved. It was initially suggested that these effects were due to their inhibitory effects on cyclic nucleotide phosphodiesterases and therefore to increased cyclic AMP. Gryglewski et al. described the superoxide

scavenging actions of flavonols in platelets which would "resuscitate biosynthesis and action of endothelial prostacyclin and EDRF", accounting for their antithrombotic effects (Gryglewski et al., 1987). Later, it was found that quercetin inhibited PKC-dependent NADPH oxidase activation in platelets (Pignatelli et al., 2006). More recently, the antiaggregant effects were associated to blocking Fyn kinase activity and the tyrosine phosphorylation of Syk and phospholipase C γ (Wright et al., 2010). However, the *in vivo* antiaggregant effects of quercetin have been questioned. In a human study with onions providing 114 mg quercetin per day no changes were found on platelet aggregation, thromboxane B_2 production, factor VII, or other haemostatic variables (Janssen et al., 1998).

4. Flavonols and hypertension

Hypertension is one of the most powerful risk factor for cardiovascular events, including myocardial infarction and stroke. Treatment with any commonly used antihypertensive regimen reduces the risk of total major cardiovascular events; the larger the reductions in blood pressure the larger reductions in risk (Turnbull, 2003). Under rigorously controlled experimental conditions, fruit and vegetable consumption is associated with a decrease in blood pressure (Dauchet et al., 2009). However, the effects of fruit and vegetables on plasma lipid levels, diabetes, and body weight are unclear yet (Dauchet et al., 2009). We have recently reviewed the role of quercetin as antihypertensive (Perez-Vizcaino et al., 2009). Therefore, we will only briefly summarize and update the information with some recent studies.

The first report on the antihypertensive effects of quercetin was carried out in spontaneously hypertensive rats (SHR), a genetic model of multifactorial hypertension (Duarte et al., 1993b) (Fig. 5). This study was confirmed and extended by others (Carlstrom et al., 2007; Machha and Mustafa, 2005; Romero et al., 2010; Sanchez et al., 2006) and followed by other reports in other classical rat models of experimental hypertension such as hypertension induced by inhibition of NO synthase with L-NAME (Duarte et al., 2002), the deoxycorticosterone acetate-salt hypertensive rats (Galisteo et al., 2004), the two-kidney, one-clip (2K1C) Goldblatt hypertensive rats (Garcia-Saura et al., 2005), Dahl salt-sensitive rats (Aoi et al., 2004; Mackraj et al., 2008) and in hypertensive rats with aortic constriction (Jalili et al., 2006). Moreover, quercetin also lowered blood pressure in animal models of insulin resistance and metabolic syndrome such as the Obese Zucker rat (Rivera et al., 2008b) and in rats fed with a high-fat high-sucrose diet (Yamamoto and Oue, 2006). Therefore, quercetin has demonstrated antihypertensive effects when given chronically in the most common rodent models of hypertension. The dose most frequently used is 10 mg/kg per day, but the effective doses used range from 2 to 300 mg/kg per day. The antihypertensive effect was dose-dependent and affects the systolic, the diastolic and the mean blood pressure. This effect usually starts during the first week of treatment and it is sustained during the treatment period. Interestingly, the reduction in blood pressure is long lasting, remaining at least after 48 h of discontinuation of treatment. Remarkably, quercetin was effective in all models of hypertension analyzed, independently of the origin of the hypertension, the status of renin–angiotensin system, oxidative stress, nitric oxide, and other factors. However, quercetin does not exert hypotensive effects, i.e. it has no effect in control normotensive animals.

According to the so-called "Barker hypothesis", strongly supported by animal and human epidemiological studies, many adult chronic diseases, including type 2 diabetes, obesity and hypertension may originate in fetal life due to changes in
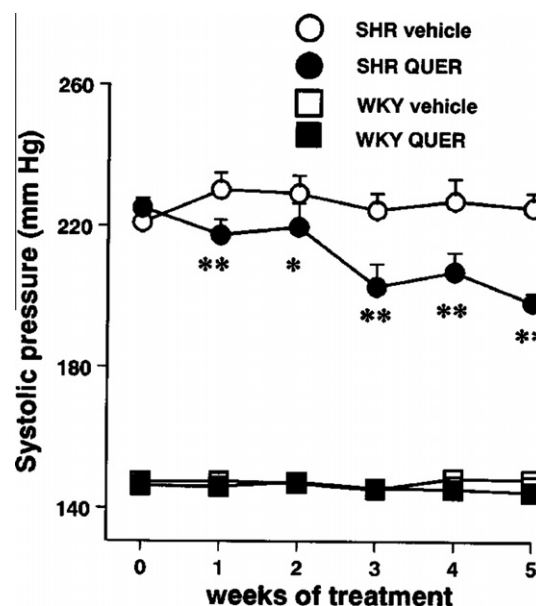

Fig. 5. Antihypertensive effects of quercetin in spontaneously hypertensive rats. Reprinted with permission (Duarte et al., 2001a).
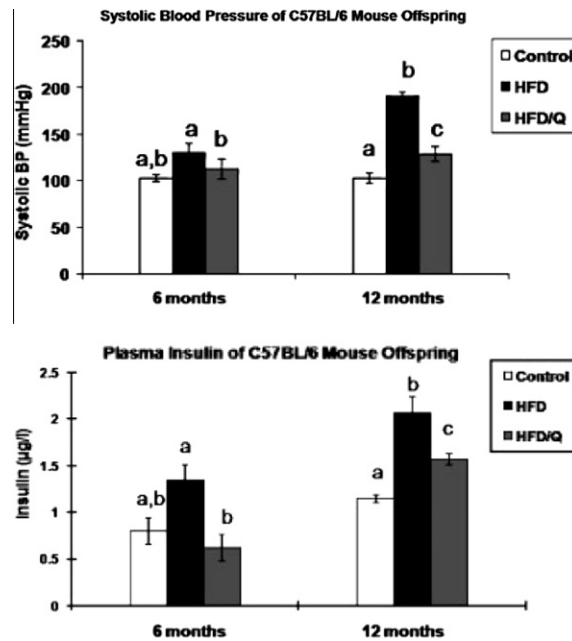
Fig. 6. Systolic blood pressure (upper panel) and plasma insulin (lower panel) in the offspring of mice fed a control diet, a high fat diet (HDF) or a high fat diet supplemented with quercetin (HFD/Q) during pregnancy. Reprinted with permission (Liang et al., 2009b).

genetic programming (Barker, 1998). Thus, adult offspring of rats or mice fed with a high fat diet during pregnancy exhibited adult hyperglycemia, insulin resistance, obesity, and hypertension, despite being fed with a standard diet throughout post-natal life (Buckley et al., 2005). Recently, a very interesting study (Liang et al., 2009b) showed that these effects were reduced by supplementing the mice with quercetin during pregnancy (Fig. 6). These results suggest that quercetin can prevent the epigenetic programming during the pre-natal life.

Sustained high blood pressure is one of the most powerful determinants of the development of cardiac, vascular and renal diseases. Most of the benefits of antihypertensive treatment on end-organ damage are the result of lowered blood pressure *per se* and are largely independent of the drugs or class of drugs employed (Turnbull, 2003). Quercetin has demonstrated a reduction of left ventricular hypertrophy in SHR (Duarte et al., 1993b), the deoxycorticosterone acetate-salt hypertensive rats (Galisteo et al., 2004), the two-kidney, one-clip (2K1C) Goldblatt hypertensive rats (Garcia-Saura et al., 2005), and in hypertensive rats with aortic constriction (Jalili et al., 2006). The flavonol also produced protective effects on renal structure and function in the animal models of hypertension such the NO-deficient rats (Duarte et al., 2002), the deoxycorticosterone acetate-salt hypertensive rats (Galisteo et al., 2004), the two-kidney, one-clip (2K1C) Goldblatt hypertensive rats (Garcia-Saura et al., 2005), and the Dahl salt-sensitive rats (Aoi et al., 2004; Mackraj et al., 2008) The protective effect on endothelial function in these animal models has been mentioned above.

Two randomized, double-blind, placebo-controlled, crossover clinical trials analyzing the effects of quercetin on blood pressure have been recently published. In the study of Edwards et al. (2007), patients with stage 1 hypertension had a reduction in systolic, diastolic and mean arterial pressures after quercetin treatment but the flavonol had no significant effect in pre-hypertensives. In the study of Egert et al. (2010), patients with metabolic syndrome were classified according to their apoE phenotypes. Quercetin decreased systolic blood pressure in the apoE3 group, whereas no significant effect was observed in patients with the apoE4 phenotype. In addition, quercetin had no effect on lipid profile in ApoE3 carriers but exerted a deleterious effect in the ApoE4 subgroup with decreased serum HDL cholesterol and increased the LDL:HDL cholesterol ratio. In another study, carried out in healthy volunteers (Conquer et al., 1998) quercetin intake did not significant modify selected cardiovascular risk factors including blood pressure. Nonetheless, this lack of effect in healthy humans may not be surprising given the lack of effect of quercetin in healthy animals as described above.

5. Flavonols and atherosclerosis

Atherosclerosis is a multifactorial disease characterized by arterial wall thickening caused by deposition of fatty materials over many years. Symptoms become apparent acutely in the late stages of the disease. Alteration in the serum lipid profile is an early and crucial event in the development of atherosclerosis. Oxidative stress, inflammation, and endothelial dysfunction are associated with the pathogenesis of atherosclerosis.

Consumption of low doses of quercetin reduced the progression of atherosclerosis in ApoE-deficient mice (Hayek et al., 1997; Loke et al., 2010). Interestingly, quercetin was more effective than other representative flavonoids [(−)-epicatechin

(flavan-3-ol), theaflavin (dimeric catechin)] and other polyphenols [sesamin (lignan) or chlorogenic acid (phenolic acid)] (Loke et al., 2010). Flavonols may protect against atherosclerosis by preventing one or several processes involved in disease progression, such as oxidative stress, inflammation, and endothelial dysfunction. Most studies have shown that quercetin has no beneficial effect on the plasma lipid profile. Thus, plasma LDL or HDL cholesterol is unchanged in ApoE knockout mice (Hayek et al., 1997), in rats fed with a high-fat high sucrose diet (Yamamoto and Oue, 2006) or in overweight humans with an ApoE3 phenotype and it may even reduce the HDL/LDL ratio in carriers of the ApoE4 (Egert et al., 2010). In contrast, it has been shown to reduce serum triglycerides and cholesterol in high cholesterol-fed rabbits (Kamada et al., 2005). Besides the effects on plasma lipids, quercetin inhibits the crucial steps in the development of atherosclerosis including the susceptibility of LDL to oxidation (Frankel et al., 1993; Hayek et al., 1997), the LDL-induced cytotoxicity (Negre-Salvayre and Salvayre, 1992) and the aortic fatty streak formation (Auger et al., 2005). Interestingly, quercetin metabolites accumulate in the human atherosclerotic lesions, but not in the normal aorta (Kawai et al., 2008). Quercetin also significantly reduced aortic F_2-isoprostane, vascular superoxide, vascular leukotriene B_4, and plasma-sP-selectin concentrations; and augmented NO and heme oxygenase-1 in ApoE$^{-/-}$ mice (Loke et al., 2010). Adhesion molecules and matrix metalloproteinases are key proteins for several processes involved in atherosclerotic plaque formation such as infiltration of inflammatory cells. Quercetin was able to reduce TNF-α-induced upregulation of the adhesion molecules VCAM-1, ICAM-1 and MCP-1 at both the protein and mRNA level in human endothelial and vascular smooth muscle cells. However the quercetin metabolites, quercetin 3′-sulfate, quercetin 3-glucuronide and 3′-methylquercetin 3-glucuronide were almost without effect (Tribolo et al., 2008; Winterbone et al., 2009). The inhibitory effect on ICAM-1 expression occurs through a down-regulation of the JNK/AP-1 pathway (Kobuchi et al., 1999). In addition, quercetin can also preserve human serum paraoxonase (PON1) activity (Aviram et al., 1999) and upregulate its expression (Gong et al., 2009), an additional mechanism by which it can protect LDL from oxidation and play a protective role in atherosclerosis. The effects of isolated flavonols on the development of atherosclerosis in humans have not been addressed.

The underlying molecular mechanisms by which quercetin may antagonize inflammatory gene expression have not yet been fully elucidated. Quercetin and other flavonoids inhibit TNF-α production as well as iNOS expression and NO production in LPS-activated macrophages, an effect that has been associated with the inhibition of the NF-kB pathway, through inhibition of IkB-α phosphorylation (Comalada et al., 2006). Recently, the anti-inflammatory properties of quercetin and isorhamnetin in RAW264.7 macrophages were also accompanied by an increase in hemeoxygenase 1 protein levels, a downstream target of the transcription factor Nrf2, known to antagonize chronic inflammation (Boesch-Saadatmandi et al., 2010). Furthermore, proinflammatory microRNA-155 was down-regulated by quercetin and isorhamnetin but not by quercetin-3-glucuronide.

6. Flavonols, insulin resistance and obesity

Insulin resistance, defined as an attenuated or inadequate response to a given amount of insulin, is associated with a wide variety of conditions including obesity, type 2 diabetes, essential hypertension, cardiovascular disease, polycystic ovary syndrome, non-alcoholic fatty liver, breast cancer and acquired immune deficiency syndrome. Reciprocal relationships between endothelial dysfunction, insulin resistance, obesity and hypertension may help coupling hemodynamic and metabolic abnormalities observed in these important interrelated public health problems (Kim et al., 2006).

6.1. Obesity

Obesity arises from an imbalance in energy intake and energy expenditure that leads to the pathological growth of adipocytes. It is induced by the hypertrophy of adipocytes and the generation of new adipocytes from precursor cells. The exposure of 3T3-L1 preadipocytes to quercetin resulted in attenuated adipogenesis and decreased expression of adipogenesis-related factors and enzymes. Treatment of 3T3-L1 adipocytes with quercetin resulted in the induction of apoptosis and a concomitant decrease in ERK and JNK phosphorylation (Ahn et al., 2008; Yang et al., 2008).

New prevention and treatment options for obesity and type 2 diabetes could be based on strategies to dampen or inhibit fat absorption, cholesterol absorption, and intestinal catabolism of complex carbohydrates. Emerging evidence indicates that apical or luminal facing-facilitated glucose transporter 2 (GLUT2) is a major pathway of sugar absorption, and therefore an attractive target of such potential agents (Kellett and Brot-Laroche, 2005). Robust noncompetitive inhibition of glucose and fructose transport by GLUT2 expressed in *Xenopus laevis* oocytes was produced by the flavonols quercetin, myricetin, fisetin and its glucoside precursor isoquercitrin. The two other major intestinal sugar transporters, GLUT5 and SGLT1, were unaffected by flavonoids. Sugar transport by GLUT2 overexpressed in pituitary cells and naturally present in Caco-2E intestinal cells was similarly inhibited by quercetin (Kwon et al., 2007). Two key benefits could accrue: reduction of postprandial hyperglycemia in diabetic subjects and in subjects with mild glucose intolerance; and reduction of the total amount of glucose absorbed as a caloric and weight reduction strategy. Because quercetin might act as a potent luminal inhibitor of sugar absorption, flavonols might show promise as new pharmacologic agents in obesity.

However, when quercetin was tested in obese rodents and humans, its impact in body weight is unclear. In obese Zucker rats, daily oral administration for 10 weeks of 2 or 10 mg/kg of quercetin decreased body weight gain, only at the higher dose (Rivera et al., 2008b). Similarly, a reduced body weight gain was observed only at high doses of quercetin (0.2% and 0.5% in

the diet) in rats on a high-fat high-sucrose diet (Yamamoto and Oue, 2006). In a model of diet-induced obesity in mice, dietary supplementation with a high dose of quercetin (0.8% of the diet) produces transient increases in energy expenditure that is not detected after 8 weeks on the diet (Stewart et al., 2008). Likewise, overweight-obese subject receiving 150 mg/d of quercetin for 6 weeks had no significant changes in the parameters of nutritional status including body weight, waist circumference, fat mass and fat-free mass (Egert et al., 2010).

6.2. Insulin resistance

Insulin resistance results from a complex interplay between nutrient overload, systemic fatty acids excess, inflammation of the adipose tissue, endoplasmic reticulum and oxidative stress (Hotamisligil, 2006) and hypoxia of the adipose tissue (Regazzetti et al., 2009). The action of insulin is initiated by its binding to the insulin receptor, followed by the autophosphorylation and phosphorylation of insulin receptor substrates (IRS) and a complex cascade of kinases and mediators (Sesti, 2006). These include the p85 regulatory subunit of phosphatidylinositol 3-kinase (PI3-K), phosphatidylinositol 3,4,5-trisphosphate (PIP3), phosphoinositide-dependent protein kinase and downstream protein kinase B/Akt as well as the atypical protein kinase C isoforms ζ and λ (PKC)(Stump et al., 2006). Both atypical PKCζ/λ and Akt are important in mediating glucose transport in muscle by allowing translocation of GLUT4 to the sarcolemma to facilitate glucose entry into the cell (Ishiki and Klip, 2005). Accumulation of lipid metabolites as a consequence of alterations in fatty acid metabolism can have a profound impact on insulin signaling. Accumulation of lipid intermediates such as triacylglycerol, diacylglycerol, and ceramide is known to activate kinases such as JNK-AP-1, IKK-NF-κB and PKC inhibiting insulin action which serves as a negative feedback mechanism (Yu et al., 2002). Current pharmacological approaches to treat insulin resistance and type 2 diabetes include glitazones (agonists of peroxisome proliferator-agonist receptor gamma, PPARγ), metformin (an activator of AMP-activated protein kinase, AMPK), sulphonylureas (ATP-dependent potassium channel blockers), acarbose (α-glucosidase inhibitor), amylin analogs, incretin mimetics and dipeptidyl-peptidase 4 inhibitors (Rodbard et al., 2007).

As mentioned above, a potential mechanism by which quercetin may be beneficial in insulin resistance and type 2 diabetes is by inhibition of intestinal glucose transporters. In addition, quercetin also inhibits alpha-glucosidase (Li et al., 2009) preventing the intestinal digestion of carbohydrates more potently than acarbose which may also help to reduce postprandial hyperglycemia.

Quercetin and related flavonols have been reported to inhibit (Nomura et al., 2008; Strobel et al., 2005) or to increase (Fang et al., 2008) insulin-induced glucose uptake in adipocytes. These opposed actions may be explained because flavonols, as wide spectrum protein kinase inhibitors, have been described to inhibit the activity of both the kinases involved in insulin signaling and the kinases involved in the development of insulin resistance in several tissues (Dias et al., 2005; Granado-Serrano et al., 2010; Perez-Vizcaino et al., 2006a). In addition, the inhibitory effect of quercetin and myricetin on the insulin-stimulated uptake of methylglucose by adipocytes has been related to a transport inhibition mechanism in which flavonoids interact directly and GLUT4, rather than by a mechanism related to protein-kinases and insulin signaling inhibition (Strobel et al., 2005). On the other side, increased glucose uptake may also be related to an interaction with PPARγ receptors. Kaempferol and quercetin served as weak partial agonists in the PPARγ reporter gene assay, but could not induce differentiation of 3T3-L1 preadipocytes as traditional PPARγ agonists (Fang et al., 2008). When added together with the PPARγ agonist rosiglitazone to 3T3-L1 preadipocytes, they could inhibit 3T3-L1 differentiation. Competitive ligand-binding assay confirmed that kaempferol and quercetin could compete with rosiglitazone at the same binding pocket site as PPARγ.

AMP-activated protein kinase (AMPK), a member of a metabolite-sensing protein kinase family, also regulates glucose transport in skeletal muscle (Bergeron et al., 1999; Fryer et al., 2002). Since skeletal muscle accounts for disposal of approximately 80% of an oral glucose load and type 2 diabetes is associated with reduced muscle glucose disposal, AMPK may be critical in the control of whole body glucose homeostasis and perhaps exercise capacity. Quercetin exposure up-regulates the levels of phosphorylated AMPK and its substrate, acetyl-CoA carboxylase (ACC). Quercetin and other flavonoids can activate AMPK in HepG2cells through the activation of the sirtuin analog SIRT1 (Suchankova et al., 2009). Moreover, in C2C12 skeletal muscle cells, quercetin enhanced glucose uptake in the absence of insulin which was related to AMPK activation (Eid et al., 2010).

The in vivo studies with flavonoids on insulin resistance also yielded conflicting results. In the obese Zucker rats, daily oral administration for 10 weeks of 2 or 10 mg/kg of quercetin, reduced dyslipidemia, insulin resistance and hypertension (Rivera et al., 2008b). The release of adipocytokines (leptin, resistin, plasminogen activator inhibitor type 1, and adiponectin) as well as inflammatory cytokines (TNF-α, interleukin-1 and 6 and MCP-1), critically involved in insulin resistance and chronic inflammation, were also measured. Quercetin decreased both production of TNF-α and proinflammatory iNOS expression by the visceral adipose tissue, and increased the plasma concentration of adiponectin (Rivera et al., 2008b). Moreover, dietary quercetin also reduced circulating markers of inflammation (interferon-γ, interleukin-1α, and interleukin-4) in diet-induced models of obesity (Stewart et al., 2008). Stewart et al. (2008), in contrast, show that diet-induced insulin resistance in mice was not ameliorated by dietary quercetin. Similarly, in SHR rats which are not only genetically hypertensive but also show insulin resistance (Reaven and Chang, 1991), we found that the oral glucose tolerance test, the homeostatic model assessment of insulin resistance, the total cholesterol and triglycerides were unaffected by quercetin (Romero et al., 2010).

Oxidative stress generation impairs pancreatic β-cell insulin secretion (Bast et al., 2002). Flavonols may preserve β-cell function by reducing oxidative stress-induced tissue damage and therefore protect against the progression of insulin resistance to type 2 diabetes. In fact, quercetin, the main dietary flavonol, prevents and protects streptozotocin-induced oxidative

stress and β-cell damage in rat pancreas (Coskun et al., 2005) and decreased blood glucose concentration in both alloxan- and streptozotocin-induced diabetic rats (Coskun et al., 2005; Kobori et al., 2009; Nuralievlu and Avezov, 1992), two models of type 1 diabetes.

A recent study (Liang et al., 2009a) showed that the gestational diabetes mellitus-induced placental oxidative stress in mice were significantly mitigated by quercetin supplementation. Results from this report also revealed that quercetin partially decreased maternal hyperglycemia and hyperinsulinemia, and offer additional information about preventive and therapeutic management of gestational diabetes mellitus, a commonly encountered disorder of mid to late pregnancy.

Despite the crucial role of insulin resistance in the development of atherosclerosis and cardiovascular risk, there are not human studies addressing the role of chronic flavonols supplementation as a therapeutic approach to treat insulin resistance. There are two epidemiological studies analyzing the relationship between flavonol intake and type 2 diabetes which again yielded conflicting results. A trend toward a reduction in risk of type 2 diabetes was associated with higher quercetin and myricetin intakes in the Finish study (Knekt et al., 2002) while in a large study of American women free of cardiovascular disease, high intake of flavonols and flavones was not significantly associated with risk of type 2 diabetes and insulin resistance (Song et al., 2005).

7. Flavonols and myocardial ischemia

Chronic coronary disease and the acute coronary syndromes involve multiple alterations in vascular reactivity, vascular structure, and interactions of the vessel wall with circulating blood elements.

Hypertension and atherosclerosis are the main risk factors for myocardial infarction and an immense body of evidence indicates that reduction of LDL-cholesterol and atherosclerotic lesions and blood pressure produces a dramatic decline in the risk of coronary disease (Turnbull, 2003). Additionally, endothelial dysfunction is an independent prognostic factor for myocardial infarction (Schachinger et al., 2000; Widlansky et al., 2003). Flavonols by preventing hypertension, atherosclerosis and endothelial dysfunction, as reviewed above, may protect the coronary vessels in the long term.

Most acute coronary events result from a rupture in the atherosclerotic plaque, thrombus formation and the subsequent myocardial ischemia. Degradation of the interstitial collagen conferring biomechanical strength to the plaque fibrous cap by matrix metalloproteinases (MMPs) appears to be involved in the plaque instability and its rupture. Quercetin reduces the expression of matrix metalloproteinases (MMP-2 and MMP-9) (Huang et al., 1999) and may help in stabilizing the atherosclerotic plaque (Motoyama et al., 2009). Coronary vasospasm may also contribute to acute impaired arterial flow. Flavonols by their platelet antiaggregant and vasodilator effects, as reviewed above, may also provide additional protective benefit in the acute phase. During the ischemic event and the eventual post-ischemic reperfusion there is an acute inflammatory process with the release of multiple cytokines and reactive oxygen species. 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. Experimental studies in animal models in which ischemia is acutely induced by coronary artery ligation or infusion of isoproterenol have shown that quercetin reduces the contractile dysfunction of the heart, the infarct size and the pattern of protein expression changes (including iNOS and NOX2) induced by cardiac ischemia (Annapurna et al., 2009; Brookes et al., 2002; Punithavathi and Prince, 2010; Wan et al., 2009). These effects may be observed following the oral intake of quercetin *in vivo* and *ex vivo*. Most reports associate the protective effect on the heart to the anti-oxidants effects of quercetin. The capacity of flavonoids to protect NO probably plays a crucial role to prevent ischemia.

In the seminal epidemiological report (Hertog et al., 1993), flavonoid intake (analyzed in tertiles) was strongly inversely associated with mortality from coronary heart disease [relative risk 0.42 (95% CI 0.20–0.88)] after adjustment for known risk factors and other dietary components. An inverse relation with incidence of myocardial infarction, which was of borderline
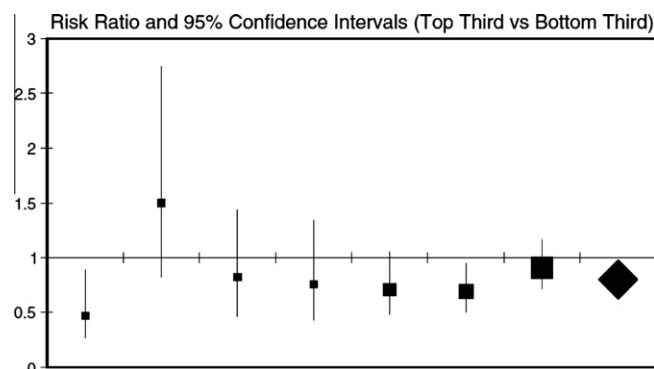
Fig. 7. Prospective cohort studies of flavonoid and coronary heart disease. Risk ratios compare top and bottom thirds of baseline measurements. Black squares indicate the risk ratio in each study, with the square size proportional to the number of CHD fatal events and the horizontal lines representing 95% CI. The combined risk ratio and its 95% CI is denoted by the black diamond. Reprinted with permission (Huxley and Neil, 2003).

significance was also noted. A number of similar epidemiological studies followed. The meta-analysis of this prospective cohort studies concluded that the individuals in the top third of dietary flavonol intake are associated with a reduced risk [0.80 (95% CI 0.69–0.93)] of mortality from coronary heart disease as compared with those in the bottom third (Fig. 7) (Huxley and Neil, 2003).

8. Flavonols and stroke

The primary cause of stroke is an interruption of cerebral blood flow occurring during vascular obstruction by thrombo-embolism or local thrombosis. The pathophysiological processes in stroke are complex and depend on the severity, duration and localization of the ischemic damage in the brain. The main risk factors that lead to occurrence of stroke are hypertension, atherosclerosis, LDL-cholesterol, diabetes and atrial fibrillation. Flavonoids have been proposed to be effective both as preventive agents and as treatment options in the acute phase of stroke (Simonyi et al., 2005). As described above, flavonols prevent endothelial dysfunction, atherosclerosis, hypertension and possibly thrombosis, all potential mechanism to prevent strokes. With regard to acute treatment, flavonols may act on different phases of stroke. For the acute phase, flavonols improve cerebral blood flow, prevent platelet aggregation and thrombosis, reduce excitotoxicity and inhibit oxidative stress. For the intermediate phase, flavonols reduce inflammation and protect endothelial integrity. For the late phase, flavonols interfere with ischemia induced cell death mechanisms such as apoptosis and necrosis.

Quercetin and kaempferol, in *in vitro* conditions, inhibit excitotoxicity. In fact, both agents significantly reduced neuronal death caused by kainate plus N-methyl-D-aspartate (Silva et al., 2008). The observed neuroprotection was correlated with prevention of delayed calcium deregulation and with the maintenance of mitochondrial transmembrane electric potential. These flavonols reduced mitochondrial lipid peroxidation and loss of mitochondrial transmembrane electric potential caused by oxidative stress induced by ADP plus iron. Thus, the neuroprotective action induced by quercetin and kaempferol was mainly attributed to its anti-oxidant effects (Silva et al., 2008). Moreover, quercetin abolished hypoxia-induced increase in types 1 and 2 IP3 receptors on cerebellar granular cells of rat, and regulates intracellular calcium (Jurkovicova et al., 2007). Quercetin also effectively protected cerebellar granule neurons or mesencephalic dopamine neurons from death induced by oxidative stress (Echeverry et al., 2010; Mercer et al., 2005).

In vivo, quercetin has been also reported to be able to scavenge superoxide anions released during reperfusion after forebrain ischemia using a four-vessel occlusion model in rats (Shutenko et al., 1999). In another study, the reduction in global ischemia-induced neuronal damage was attributed to inhibition of MMP-9 activity (Cho et al., 2006). However, in a model of neuronal oxidative damage *in vivo* by unilateral infusion of 6-OHDA into the medial forebrain bundle (Zbarsky et al., 2005), quercetin or fisetin had no effect on the loss of tyrosine hydroxylase-positive cells in the substantia nigra. The lack of ability of quercetin in some *in vivo* models despite the *in vitro* neuroprotective effect on damage induced by different stimuli, is probably due to difficulties in crossing the blood–brain barrier and to penetrate into the brain. Moreover, quercetin metabolites seem to be less neuroprotective and penetrate the blood–brain barrier less efficiently than the aglycone. However, increased blood–brain barrier permeability may occur under inflammatory conditions as occur in stroke which would facilitate quercetin brain penetration. When flavonoids were administered in lecithin preparation to facilitate the crossing of the blood–brain barrier, treatment of permanent focal ischemia with this lecithin/quercetin preparation decreased lesion volume (Dajas et al., 2003). The protective effect against ischemic lesion was demonstrated by a significant increase in numbers of cells in striatum and cortex, together with a partial reversal of motor deficits. Moreover, reduced glutation (GSH) levels decreased after ischemia in the ipsilateral striatum and cortex, and the liposomal quercetin preparation reversed these effects 24 h after the permanent middle cerebral arterial occlusion (Rivera et al., 2008a).

Chronic administration of quercetin has anti-inflammatory properties in the brain. In fact, in lipopolysaccharide (LPS)-treated mice, quercetin inhibits the expression of proinflammatory enzymes COX2 and iNOS, reversing LPS-induced memory deficits (Patil et al., 2003). In BV-2 microglial cells stimulated by LPS/interferon-gamma, quercetin produces an inhibitory effect on iNOS and NO production. The anti-inflammatory action of quercetin may be attributable to its raft disrupting and anti-oxidant effects. These distinct mechanisms work in synergy to down-regulate iNOS expression and NO production (Kao et al., 2010). Sharma et al. (2007) showed that flavonoids confer protection against IL-1beta induced astrocyte mediated neuronal damage by: (i) enhancing the potential of activated astrocytes to detoxify free radical (superoxide dismutase-1 and thioredoxin-mediators), (ii) reducing the expression of proinflammatory cytokines (IL-6) and chemokines (IL-8, IP-10, MCP-1 and RANTES), and (iii) modulating expression of mediators associated with enhanced physiological activity of astrocyte in response to injury.

There are several prospective epidemiological studies analyzing the relationship between flavonol intake and stroke. The Zutphen study reported an inverse association of stroke with increasing dietary flavonoid consumption (mainly quercetin) after adjustment for several confounders, including vitamin intake (Keli et al., 1996). Later studies (Knekt et al., 2000; Yochum et al., 1999) carried out in large cohorts in Finland and USA, respectively, showed that the relative risk of cerebrovascular disease was similar among subjects with high or low intake of flavonols. Thus, the authors concluded that quercetin intake is not associated with cerebrovascular disease. However, very recently, a meta-analysis of six prospective cohort studies (Hollman et al., 2010) found that a high intake of flavonols compared with a low intake was inversely associated with nonfatal and fatal stroke with a pooled relative risk of 0.80 (95% CI: 0.65, 0.98). It was concluded that flavonols may reduce stroke risk, even when publication bias was suspected.
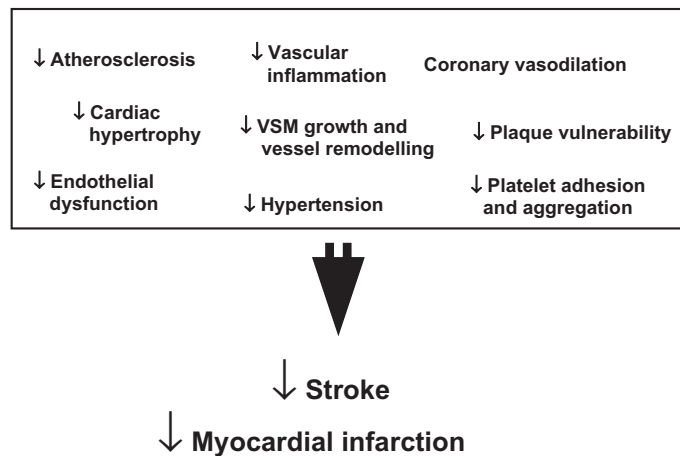
Fig. 8. Scheme showing the potential mechanism by which quercetin may interfere in the reduction of stroke and myocardial infarction.

9. Conclusions

Flavonols, and specially quercetin, are widely distributed in plants and are present in considerable amounts in fruits and vegetables. In addition to its anti-oxidant effect, there is an impressive number of enzymes whose activity is modulated (mostly inhibited) by quercetin. Thus, it can be predicted that a huge number of biochemical signaling pathways and, therefore, physiological and pathological processes, can be affected by this flavonol. It is surprising that quercetin is still widely regarded just as an anti-oxidant and even more astonishing that it is often used as a pharmacological tool to "specifically" inhibit a given enzyme. It can potentially interact with many of the molecular targets known to be involved in the pathophysiology of ischemic heart disease and stroke. Thus, it may act by multiple mechanisms operating both in the long term prevention and in the acute phase of cardiovascular events (Fig. 8). These multiple interactions often go in the right direction and explain the protective effects in cardiovascular disease but occasionally result in detrimental effects.

To summarize, there is solid evidence that, *in vitro*, quercetin and related flavonols exert: (1) endothelium-independent vasodilator effects, (2) protective effect on nitric oxide and endothelial function under conditions of oxidative stress, (3) platelet antiaggregant effects, (4) inhibition of LDL oxidation, (5) reduction of adhesion molecules and other inflammatory markers and (6) prevention of neuronal oxidative and inflammatory damage. The *in vitro* effects regarding NO production in healthy vessels and glucose uptake in adipocytes are controversial. The metabolites of quercetin show partial protective effects on endothelial function and LDL oxidation. In animal models of disease, quercetin produces undisputed antihypertensive and antiatherogenic effects, prevents endothelial dysfunction and protects the myocardium from ischemic damage. It has no clear effects on serum lipid profile and on insulin resistance and at high doses it may also reduce obesity. Human intervention trials with isolated flavonols demonstrate an antihypertensive effect while no data is available on endothelial function, insulin resistance or atherosclerosis. Some evidence also points to differential effects depending on the genetic background of the patients. The meta-analysis of epidemiological studies show an inverse association between flavonol (together with flavone) intake and coronary heart disease and stroke. Therefore, although there is still no solid proof, a substantial body of evidence suggests that quercetin may prevent the most common forms of cardiovascular disease contributing to the protective effects afforded by fruits and vegetables.

References

Ahn, J., Lee, H., Kim, S., Park, J., Ha, T., 2008. The anti-obesity effect of quercetin is mediated by the AMPK and MAPK signaling pathways. Biochem. Biophys. Res. Commun. 373, 545–549.

Ahn, M.R., Kunimasa, K., Kumazawa, S., Nakayama, T., Kaji, K., Uto, Y., Hori, H., Nagasawa, H., Ohta, T., 2009. Correlation between antiangiogenic activity and antioxidant activity of various components from propolis. Mol. Nutr. Food Res. 53, 643–651.

Ajay, M., Achike, F.I., Mustafa, A.M., Mustafa, M.R., 2006. Effect of quercetin on altered vascular reactivity in aortas isolated from streptozotocin-induced diabetic rats. Diabetes Res. Clin. Pract. 73, 1–7.

Ajay, M., Gilani, A.U., Mustafa, M.R., 2003. Effects of flavonoids on vascular smooth muscle of the isolated rat thoracic aorta. Life Sci. 74, 603–612.

Andriambeloson, E., Magnier, C., Haan-Archipoff, G., Lobstein, A., Anton, R., Beretz, A., Stoclet, J.C., Andriantsitohaina, R., 1998. Natural dietary polyphenolic compounds cause endothelium-dependent vasorelaxation in rat thoracic aorta. J. Nutr. 128, 2324–2333.

Annapurna, A., Reddy, C.S., Akondi, R.B., Rao, S.R., 2009. Cardioprotective actions of two bioflavonoids, quercetin and rutin, in experimental myocardial infarction in both normal and streptozotocin-induced type I diabetic rats. J. Pharm. Pharmacol. 61, 1365–1374.

Aoi, W., Niisato, N., Miyazaki, H., Marunaka, Y., 2004. Flavonoid-induced reduction of ENaC expression in the kidney of Dahl salt-sensitive hypertensive rat. Biochem. Biophys. Res. Commun. 315, 892–896.

Auger, C., Teissedre, P.L., Gerain, P., Lequeux, N., Bornet, A., Serisier, S., Besancon, P., Caporiccio, B., Cristol, J.P., Rouanet, J.M., 2005. Dietary wine phenolics catechin, quercetin, and resveratrol efficiently protect hypercholesterolemic hamsters against aortic fatty streak accumulation. J. Agric. Food Chem. 53, 2015–2021.

Aviram, M., Rosenblat, M., Billecke, S., Erogul, J., Sorenson, R., Bisgaier, C.L., Newton, R.S., La Du, B., 1999. Human serum paraoxonase (PON 1) is inactivated by oxidized low density lipoprotein and preserved by antioxidants. Free Radic. Biol. Med. 26, 892–904.

Barker, D.J., 1998. In utero programming of chronic disease. Clin. Sci. (Lond.) 95, 115–128.

Bast, A., Wolf, G., Oberbaumer, I., Walther, R., 2002. Oxidative and nitrosative stress induces peroxiredoxins in pancreatic beta cells. Diabetologia 45, 867–876.

Belinha, I., Amorim, M.A., Rodrigues, P., de Freitas, V., Moradas-Ferreira, P., Mateus, N., Costa, V., 2007. Quercetin increases oxidative stress resistance and longevity in Saccharomyces cerevisiae. J. Agric. Food Chem. 55, 2446–2451.

Benito, S., Lopez, D., Saiz, M.P., Buxaderas, S., Sanchez, J., Puig-Parellada, P., Mitjavila, M.T., 2002. A flavonoid-rich diet increases nitric oxide production in rat aorta. Br. J. Pharmacol. 135, 910–916.

Beretz, A., Cazenave, J.P., Anton, R., 1982. Inhibition of aggregation and secretion of human platelets by quercetin and other flavonoids: structure–activity relationships. Agents Actions 12, 382–387.

Bergeron, R., Russell 3rd, R.R., Young, L.H., Ren, J.M., Marcucci, M., Lee, A., Shulman, G.I., 1999. Effect of AMPK activation on muscle glucose metabolism in conscious rats. Am. J. Physiol. 276, E938–E944.

Bieger, J., Cermak, R., Blank, R., de Boer, V.C., Hollman, P.C., Kamphues, J., Wolffram, S., 2008. Tissue distribution of quercetin in pigs after long-term dietary supplementation. J. Nutr. 138, 1417–1420.

Boesch-Saadatmandi, C., Loboda, A., Wagner, A.E., Stachurska, A., Jozkowicz, A., Dulak, J., Doring, F., Wolffram, S., Rimbach, G., 2010. Effect of quercetin and its metabolites isorhamnetin and quercetin-3-glucuronide on inflammatory gene expression: role of miR-155. J. Nutr. Biochem. doi:10.1016/j.jnutbio.2010.02.008.

Brookes, P.S., Digerness, S.B., Parks, D.A., Darley-Usmar, V., 2002. Mitochondrial function in response to cardiac ischemia–reperfusion after oral treatment with quercetin. Free Radic. Biol. Med. 32, 1220–1228.

Brunner, F., Bras-Silva, C., Cerdeira, A.S., Leite-Moreira, A.F., 2006. Cardiovascular endothelins: essential regulators of cardiovascular homeostasis. Pharmacol. Ther. 111, 508–531.

Buckley, A.J., Keseru, B., Briody, J., Thompson, M., Ozanne, S.E., Thompson, C.H., 2005. Altered body composition and metabolism in the male offspring of high fat-fed rats. Metabolism 54, 500–507.

Busse, W.W., Kopp, D.E., Middleton Jr., E., 1984. Flavonoid modulation of human neutrophil function. J. Allergy Clin. Immunol. 73, 801–809.

Carlstrom, J., Symons, J.D., Wu, T.C., Bruno, R.S., Litwin, S.E., Jalili, T., 2007. A quercetin supplemented diet does not prevent cardiovascular complications in spontaneously hypertensive rats. J. Nutr. 137, 628–633.

Cogolludo, A., Frazziano, G., Briones, A.M., Cobeno, L., Moreno, L., Lodi, F., Salaices, M., Tamargo, J., Perez-Vizcaino, F., 2007. The dietary flavonoid quercetin activates BKCa currents in coronary arteries via production of H_2O_2. Role in vasodilatation. Cardiovasc. Res. 73, 424–431.

Cohen, H.Y., Miller, C., Bitterman, K.J., Wall, N.R., Hekking, B., Kessler, B., Howitz, K.T., Gorospe, M., de Cabo, R., Sinclair, D.A., 2004. Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. Science 305, 390–392.

Comalada, M., Ballester, I., Bailon, E., Sierra, S., Xaus, J., Galvez, J., de Medina, F.S., Zarzuelo, A., 2006. Inhibition of pro-inflammatory markers in primary bone marrow-derived mouse macrophages by naturally occurring flavonoids: analysis of the structure–activity relationship. Biochem. Pharmacol. 72, 1010–1021.

Conquer, J.A., Maiani, G., Azzini, E., Raguzzini, A., Holub, B.J., 1998. Supplementation with quercetin markedly increases plasma quercetin concentration without effect on selected risk factors for heart disease in healthy subjects. J. Nutr. 128, 593–597.

Corriu, C., Feletou, M., Canet, E., Vanhoutte, P.M., 1996. Endothelium-derived factors and hyperpolarization of the carotid artery of the guinea-pig. Br. J. Pharmacol. 119, 959–964.

Coskun, O., Kanter, M., Korkmaz, A., Oter, S., 2005. Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and beta-cell damage in rat pancreas. Pharmacol. Res. 51, 117–123.

Cho, J.Y., Kim, I.S., Jang, Y.H., Kim, A.R., Lee, S.R., 2006. Protective effect of quercetin, a natural flavonoid against neuronal damage after transient global cerebral ischemia. Neurosci. Lett. 404, 330–335.

Dajas, F., Rivera, F., Blasina, F., Arredondo, F., Echeverry, C., Lafon, L., Morquio, A., Heizen, H., 2003. Cell culture protection and in vivo neuroprotective capacity of flavonoids. Neurotox. Res. 5, 425–432.

Dauchet, L., Amouyel, P., Dallongeville, J., 2009. Fruits, vegetables and coronary heart disease. Nat. Rev. Cardiol. 6, 599–608.

Davalos, A., Castilla, P., Gomez-Cordoves, C., Bartolome, B., 2006. Quercetin is bioavailable from a single ingestion of grape juice. Int. J. Food Sci. Nutr. 57, 391–398.

Dias, A.S., Porawski, M., Alonso, M., Marroni, N., Collado, P.S., Gonzalez-Gallego, J., 2005. Quercetin decreases oxidative stress, NF-kappaB activation, and iNOS overexpression in liver of streptozotocin-induced diabetic rats. J. Nutr. 135, 2299–2304.

Duarte, J., Jimenez, R., O'Valle, F., Galisteo, M., Perez-Palencia, R., Vargas, F., Perez-Vizcaino, F., Zarzuelo, A., Tamargo, J., 2002. Protective effects of the flavonoid quercetin in chronic nitric oxide deficient rats. J. Hypertens. 20, 1843–1854.

Duarte, J., Jimenez, R., Villar, I.C., Perez-Vizcaino, F., Jimenez, J., Tamargo, J., 2001a. Vasorelaxant effects of the bioflavonoid chrysin in isolated rat aorta. Planta Med. 67, 567–569.

Duarte, J., Perez-Palencia, R., Vargas, F., Ocete, M.A., Perez-Vizcaino, F., Zarzuelo, A., Tamargo, J., 2001b. Antihypertensive effects of the flavonoid quercetin in spontaneously hypertensive rats. Br. J. Pharmacol. 133, 117–124.

Duarte, J., Perez-Vizcaino, F., Zarzuelo, A., Jimenez, J., Tamargo, J., 1993a. Vasodilator effects of quercetin in isolated rat vascular smooth muscle. Eur. J. Pharmacol. 239, 1–7.

Duarte, J., Perez Vizcaino, F., Utrilla, P., Jimenez, J., Tamargo, J., Zarzuelo, A., 1993b. Vasodilatory effects of flavonoids in rat aortic smooth muscle. Structure–activity relationships. Gen. Pharmacol. 24, 857–862.

Echeverry, C., Arredondo, F., Abin-Carriquiry, J.A., Midiwo, J.O., Ochieng, C., Kerubo, L., Dajas, F., 2010. Pretreatment with natural flavones and neuronal cell survival after oxidative stress: a structure–activity relationship study. J. Agric. Food Chem. 58, 2111–2115.

Edwards, R.L., Lyon, T., Litwin, S.E., Rabovsky, A., Symons, J.D., Jalili, T., 2007. Quercetin reduces blood pressure in hypertensive subjects. J. Nutr. 137, 2405–2411.

Egert, S., Boesch-Saadatmandi, C., Wolffram, S., Rimbach, G., Muller, M.J., 2010. Serum lipid and blood pressure responses to quercetin vary in overweight patients by apolipoprotein E genotype. J. Nutr. 140, 278–284.

Eid, H.M., Martineau, L.C., Saleem, A., Muhammad, A., Vallerand, D., Benhaddou-Andaloussi, A., Nistor, L., Afshar, A., Arnason, J.T., Haddad, P.S., 2010. Stimulation of AMP-activated protein kinase and enhancement of basal glucose uptake in muscle cells by quercetin and quercetin glycosides, active principles of the antidiabetic medicinal plant Vaccinium vitis-idaea. Mol. Nutr. Food Res. 54, 991–1003.

Fang, X.K., Gao, J., Zhu, D.N., 2008. Kaempferol and quercetin isolated from Euonymus alatus improve glucose uptake of 3T3-L1 cells without adipogenesis activity. Life Sci. 82, 615–622.

Feletou, M., Vanhoutte, P.M., 2009. EDHF: an update. Clin. Sci. (Lond.) 117, 139–155.

Fitzpatrick, D.F., Hirschfield, S.L., Coffey, R.G., 1993. Endothelium-dependent vasorelaxing activity of wine and other grape products. Am. J. Physiol. 265, H774–H778.

Frankel, E.N., Kanner, J., German, J.B., Parks, E., Kinsella, J.E., 1993. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. Lancet 341, 454–457.

Fryer, L.G., Foufelle, F., Barnes, K., Baldwin, S.A., Woods, A., Carling, D., 2002. Characterization of the role of the AMP-activated protein kinase in the stimulation of glucose transport in skeletal muscle cells. Biochem. J. 363, 167–174.

Galisteo, M., Garcia-Saura, M.F., Jimenez, R., Villar, I.C., Wangensteen, R., Zarzuelo, A., Vargas, F., Duarte, J., 2004. Effects of quercetin treatment on vascular function in deoxycorticosterone acetate-salt hypertensive rats. Comparative study with verapamil. Planta Med. 70, 334–341.

Garcia-Saura, M.F., Galisteo, M., Villar, I.C., Bermejo, A., Zarzuelo, A., Vargas, F., Duarte, J., 2005. Effects of chronic quercetin treatment in experimental renovascular hypertension. Mol. Cell Biochem. 270, 147–155.

Gong, M., Garige, M., Varatharajalu, R., Marmillot, P., Gottipatti, C., Leckey, L.C., Lakshman, R.M., 2009. Quercetin up-regulates paraoxonase 1 gene expression with concomitant protection against LDL oxidation. Biochem. Biophys. Res. Commun. 379, 1001–1004.

Granado-Serrano, A.B., Martin, M.A., Bravo, L., Goya, L., Ramos, S., 2010. Quercetin modulates NF-kappa B and AP-1/JNK pathways to induce cell death in human hepatoma cells. Nutr. Cancer 62, 390–401.

Gryglewski, R.J., Korbut, R., Robak, J., Swies, J., 1987. On the mechanism of antithrombotic action of flavonoids. Biochem. Pharmacol. 36, 317–322.

Hayek, T., Fuhrman, B., Vaya, J., Rosenblat, M., Belinky, P., Coleman, R., Elis, A., Aviram, M., 1997. Reduced progression of atherosclerosis in apolipoprotein E-deficient mice following consumption of red wine, or its polyphenols quercetin or catechin, is associated with reduced susceptibility of LDL to oxidation and aggregation. Arterioscler. Thromb. Vasc. Biol. 17, 2744–2752.

Hertog, M.G., Feskens, E.J., Hollman, P.C., Katan, M.B., Kromhout, D., 1993. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. Lancet 342, 1007–1011.

Hollman, P.C., de Vries, J.H., van Leeuwen, S.D., Mengelers, M.J., Katan, M.B., 1995. Absorption of dietary quercetin glycosides and quercetin in healthy ileostomy volunteers. Am. J. Clin. Nutr. 62, 1276–1282.

Hollman, P.C., Geelen, A., Kromhout, D., 2010. Dietary flavonol intake may lower stroke risk in men and women. J. Nutr. 140, 600–604.

Hotamisligil, G.S., 2006. Inflammation and metabolic disorders. Nature 444, 860–867.

Howitz, K.T., Bitterman, K.J., Cohen, H.Y., Lamming, D.W., Lavu, S., Wood, J.G., Zipkin, R.E., Chung, P., Kisielewski, A., Zhang, L.L., Scherer, B., Sinclair, D.A., 2003. Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan. Nature 425, 191–196.

Huang, Y.T., Hwang, J.J., Lee, P.P., Ke, F.C., Huang, J.H., Huang, C.J., Kandaswami, C., Middleton Jr., E., Lee, M.T., 1999. Effects of luteolin and quercetin, inhibitors of tyrosine kinase, on cell growth and metastasis-associated properties in A431 cells overexpressing epidermal growth factor receptor. Br. J. Pharmacol. 128, 999–1010.

Huxley, R.R., Neil, H.A., 2003. The relation between dietary flavonol intake and coronary heart disease mortality: a meta-analysis of prospective cohort studies. Eur. J. Clin. Nutr. 57, 904–908.

Ibarra, M., Moreno, L., Vera, R., Cogolludo, A., Duarte, J., Tamargo, J., Perez-Vizcaino, F., 2003. Effects of the flavonoid quercetin and its methylated metabolite isorhamnetin in isolated arteries from spontaneously hypertensive rats. Planta Med. 69, 995–1000.

Ibarra, M., Perez-Vizcaino, F., Cogolludo, A., Duarte, J., Zaragoza-Arnaez, F., Lopez-Lopez, J.G., Tamargo, J., 2002. Cardiovascular effects of isorhamnetin and quercetin in isolated rat and porcine vascular smooth muscle and isolated rat atria. Planta Med. 68, 307–310.

Igura, K., Ohta, T., Kuroda, Y., Kaji, K., 2001. Resveratrol and quercetin inhibit angiogenesis in vitro. Cancer Lett. 171, 11–16.

Ishiki, M., Klip, A., 2005. Minireview: recent developments in the regulation of glucose transporter-4 traffic: new signals, locations, and partners. Endocrinology 146, 5071–5078.

Jackson, S.J., Venema, R.C., 2006. Quercetin inhibits eNOS, microtubule polymerization, and mitotic progression in bovine aortic endothelial cells. J. Nutr. 136, 1178–1184.

Jalili, T., Carlstrom, J., Kim, S., Freeman, D., Jin, H., Wu, T.C., Litwin, S.E., David Symons, J., 2006. Quercetin-supplemented diets lower blood pressure and attenuate cardiac hypertrophy in rats with aortic constriction. J. Cardiovasc. Pharmacol. 47, 531–541.

Janssen, K., Mensink, R.P., Cox, F.J., Harryvan, J.L., Hovenier, R., Hollman, P.C., Katan, M.B., 1998. Effects of the flavonoids quercetin and apigenin on hemostasis in healthy volunteers: results from an in vitro and a dietary supplement study. Am. J. Clin. Nutr. 67, 255–262.

Jimenez, R., Andriambeloson, E., Duarte, J., Andriantsitohaina, R., Jimenez, J., Perez-Vizcaino, F., Zarzuelo, A., Tamargo, J., 1999. Involvement of thromboxane A2 in the endothelium-dependent contractions induced by myricetin in rat isolated aorta. Br. J. Pharmacol. 127, 1539–1544.

Joint-Committee-Nomenclature-ASBC-AIN, 1950. Term "vitamin P" recommended to be discontinued. Science 112, 268.

Jurkovicova, D., Kopacek, J., Stefanik, P., Kubovcakova, L., Zahradnikova Jr., A., Zahradnikova, A., Pastorekova, S., Krizanova, O., 2007. Hypoxia modulates gene expression of IP3 receptors in rodent cerebellum. Pflugers Arch. 454, 415–425.

Kamada, C., da Silva, E.L., Ohnishi-Kameyama, M., Moon, J.H., Terao, J., 2005. Attenuation of lipid peroxidation and hyperlipidemia by quercetin glucoside in the aorta of high cholesterol-fed rabbit. Free Radic. Res. 39, 185–194.

Kao, T.K., Ou, Y.C., Raung, S.L., Lai, C.Y., Liao, S.L., Chen, C.J., 2010. Inhibition of nitric oxide production by quercetin in endotoxin/cytokine-stimulated microglia. Life Sci. 86, 315–321.

Kawai, Y., Nishikawa, T., Shiba, Y., Saito, S., Murota, K., Shibata, N., Kobayashi, M., Kanayama, M., Uchida, K., Terao, J., 2008. Macrophage as a target of quercetin glucuronides in human atherosclerotic arteries: implication in the anti-atherosclerotic mechanism of dietary flavonoids. J. Biol. Chem. 283, 9424–9434.

Keli, S.O., Hertog, M.G., Feskens, E.J., Kromhout, D., 1996. Dietary flavonoids, antioxidant vitamins, and incidence of stroke: the Zutphen study. Arch. Intern. Med. 156, 637–642.

Kellett, G.L., Brot-Laroche, E., 2005. Apical GLUT2: a major pathway of intestinal sugar absorption. Diabetes 54, 3056–3062.

Khan, N.Q., Lees, D.M., Douthwaite, J.A., Carrier, M.J., Corder, R., 2002. Comparison of red wine extract and polyphenol constituents on endothelin-1 synthesis by cultured endothelial cells. Clin. Sci. (Lond.) 103 (Suppl. 48), 72S–75S.

Khoo, N.K., White, C.R., Pozzo-Miller, L., Zhou, F., Constance, C., Inoue, T., Patel, R.P., Parks, D.A., 2010. Dietary flavonoid quercetin stimulates vasorelaxation in aortic vessels. Free Radic. Biol. Med. 49, 339–347.

Kim, J.A., Montagnani, M., Koh, K.K., Quon, M.J., 2006. Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. Circulation 113, 1888–1904.

Knekt, P., Isotupa, S., Rissanen, H., Heliovaara, M., Jarvinen, R., Hakkinen, S., Aromaa, A., Reunanen, A., 2000. Quercetin intake and the incidence of cerebrovascular disease. Eur. J. Clin. Nutr. 54, 415–417.

Knekt, P., Kumpulainen, J., Jarvinen, R., Rissanen, H., Heliovaara, M., Reunanen, A., Hakulinen, T., Aromaa, A., 2002. Flavonoid intake and risk of chronic diseases. Am. J. Clin. Nutr. 76, 560–568.

Kobori, M., Masumoto, S., Akimoto, Y., Takahashi, Y., 2009. Dietary quercetin alleviates diabetic symptoms and reduces streptozotocin-induced disturbance of hepatic gene expression in mice. Mol. Nutr. Food Res. 53, 859–868.

Kobuchi, H., Roy, S., Sen, C.K., Nguyen, H.G., Packer, L., 1999. Quercetin inhibits inducible ICAM-1 expression in human endothelial cells through the JNK pathway. Am. J. Physiol. 277, C403–411.

Kroon, P.A., Clifford, M.N., Crozier, A., Day, A.J., Donovan, J.L., Manach, C., Williamson, G., 2004. How should we assess the effects of exposure to dietary polyphenols in vitro? Am. J. Clin. Nutr. 80, 15–21.

Kwon, O., Eck, P., Chen, S., Corpe, C.P., Lee, J.H., Kruhlak, M., Levine, M., 2007. Inhibition of the intestinal glucose transporter GLUT2 by flavonoids. FASEB J. 21, 366–377.

Lee-Hilz, Y.Y., Stolaki, M., van Berkel, W.J., Aarts, J.M., Rietjens, I.M., 2008. Activation of EpRE-mediated gene transcription by quercetin glucuronides depends on their deconjugation. Food Chem. Toxicol. 46, 2128–2134.

Li, Y.Q., Zhou, F.C., Gao, F., Bian, J.S., Shan, F., 2009. Comparative evaluation of quercetin, isoquercetin and rutin as inhibitors of alpha-glucosidase. J. Agric. Food Chem. 57, 11463–11468.

Liang, C., Decourcy, K., Prater, M.R., 2009a. High-saturated-fat diet induces gestational diabetes and placental vasculopathy in C57BL/6 mice. Metabolism 59, 943–950.

Liang, C., Oest, M.E., Prater, M.R., 2009. Intrauterine exposure to high saturated fat diet elevates risk of adult-onset chronic diseases in C57BL/6 mice. Birth Defects Res. B Dev. Reprod. Toxicol. 86, 377–384.

Lodi, F., Jimenez, R., Menendez, C., Needs, P.W., Duarte, J., Perez-Vizcaino, F., 2008. Glucuronidated metabolites of the flavonoid quercetin do not auto-oxidise, do not generate free radicals and do not decrease nitric oxide bioavailability. Planta Med. 74, 741–746.

Lodi, F., Jimenez, R., Moreno, L., Kroon, P.A., Needs, P.W., Hughes, D.A., Santos-Buelga, C., Gonzalez-Paramas, A., Cogolludo, A., Lopez-Sepulveda, R., Duarte, J., Perez-Vizcaino, F., 2009. Glucuronidated and sulfated metabolites of the flavonoid quercetin prevent endothelial dysfunction but lack direct vasorelaxant effects in rat aorta. Atherosclerosis 204, 34–39.

Loke, W.M., Hodgson, J.M., Proudfoot, J.M., McKinley, A.J., Puddey, I.B., Croft, K.D., 2008. Pure dietary flavonoids quercetin and (−)-epicatechin augment nitric oxide products and reduce endothelin-1 acutely in healthy men. Am. J. Clin. Nutr. 88, 1018–1025.

Loke, W.M., Proudfoot, J.M., Hodgson, J.M., McKinley, A.J., Hime, N., Magat, M., Stocker, R., Croft, K.D., 2010. Specific dietary polyphenols attenuate atherosclerosis in apolipoprotein E-knockout mice by alleviating inflammation and endothelial dysfunction. Arterioscler Thromb Vasc Biol. 30, 749–757.

Lopez-Lopez, G., Moreno, L., Cogolludo, A., Galisteo, M., Ibarra, M., Duarte, J., Lodi, F., Tamargo, J., Perez-Vizcaino, F., 2004. Nitric oxide (NO) scavenging and NO protecting effects of quercetin and their biological significance in vascular smooth muscle. Mol. Pharmacol. 65, 851–859.

Luo, H., Jiang, B.H., King, S.M., Chen, Y.C., 2008. Inhibition of cell growth and VEGF expression in ovarian cancer cells by flavonoids. Nutr. Cancer 60, 800–809.

Lyseng-Williamson, K.A., Perry, C.M., 2003. Micronised purified flavonoid fraction: a review of its use in chronic venous insufficiency, venous ulcers and haemorrhoids. Drugs 63, 71–100.

Mackraj, I., Govender, T., Ramesar, S., 2008. The antihypertensive effects of quercetin in a salt-sensitive model of hypertension. J. Cardiovasc. Pharmacol. 51, 239–245.

Machha, A., Mustafa, M.R., 2005. Chronic treatment with flavonoids prevents endothelial dysfunction in spontaneously hypertensive rat aorta. J. Cardiovasc. Pharmacol. 46, 36–40.

Mattagajasingh, I., Kim, C.S., Naqvi, A., Yamamori, T., Hoffman, T.A., Jung, S.B., DeRicco, J., Kasuno, K., Irani, K., 2007. SIRT1 promotes endothelium-dependent vascular relaxation by activating endothelial nitric oxide synthase. Proc. Natl. Acad. Sci. USA 104, 14855–14860.

Mercer, L.D., Kelly, B.L., Horne, M.K., Beart, P.M., 2005. Dietary polyphenols protect dopamine neurons from oxidative insults and apoptosis: investigations in primary rat mesencephalic cultures. Biochem. Pharmacol. 69, 339–345.

Miyazaki, R., Ichiki, T., Hashimoto, T., Inanaga, K., Imayama, I., Sadoshima, J., Sunagawa, K., 2008. SIRT1, a longevity gene, downregulates angiotensin II type 1 receptor expression in vascular smooth muscle cells. Arterioscler. Thromb. Vasc. Biol. 28, 1263–1269.

Montagnani, M., Chen, H., Barr, V.A., Quon, M.J., 2001. Insulin-stimulated activation of eNOS is independent of Ca^{2+} but requires phosphorylation by Akt at Ser(1179). J. Biol. Chem. 276, 30392–30398.

Moon, S.K., Cho, G.O., Jung, S.Y., Gal, S.W., Kwon, T.K., Lee, Y.C., Madamanchi, N.R., Kim, C.H., 2003. Quercetin exerts multiple inhibitory effects on vascular smooth muscle cells: role of ERK1/2, cell-cycle regulation, and matrix metalloproteinase-9. Biochem. Biophys. Res. Commun. 301, 1069–1078.

Motoyama, K., Koyama, H., Moriwaki, M., Emura, K., Okuyama, S., Sato, E., Inoue, M., Shioi, A., Nishizawa, Y., 2009. Atheroprotective and plaque-stabilizing effects of enzymatically modified isoquercitrin in atherogenic apoE-deficient mice. Nutrition 25, 421–427.

Munzel, T., Hink, U., Yigit, H., Macharzina, R., Harrison, D.G., Mulsch, A., 1999. Role of superoxide dismutase in in vivo and in vitro nitrate tolerance. Br. J. Pharmacol. 127, 1224–1230.

Negre-Salvayre, A., Salvayre, R., 1992. Quercetin prevents the cytotoxicity of oxidized LDL on lymphoid cell lines. Free Radic. Biol. Med. 12, 101–106.

Nifli, A.P., Theodoropoulos, P.A., Munier, S., Castagnino, C., Roussakis, E., Katerinopoulos, H.E., Vercauteren, J., Castanas, E., 2007. Quercetin exhibits a specific fluorescence in cellular milieu: a valuable tool for the study of its intracellular distribution. J. Agric. Food Chem. 55, 2873–2878.

Nomura, M., Takahashi, T., Nagata, N., Tsutsumi, K., Kobayashi, S., Akiba, T., Yokogawa, K., Moritani, S., Miyamoto, K., 2008. Inhibitory mechanisms of flavonoids on insulin-stimulated glucose uptake in MC3T3-G2/PA6 adipose cells. Biol. Pharm. Bull. 31, 1403–1409.

Nuralievlu, N., Avezov, G.A., 1992. The efficacy of quercetin in alloxan diabetes. Eksp. Klin. Farmakol. 55, 42–44.

Ota, H., Eto, M., Ogawa, S., Iijima, K., Akishita, M., Ouchi, Y., 2010. SIRT1/eNOS axis as a potential target against vascular senescence, dysfunction and atherosclerosis. J. Atheroscler. Thromb. 17, 431–435.

Patil, C.S., Singh, V.P., Satyanarayan, P.S., Jain, N.K., Singh, A., Kulkarni, S.K., 2003. Protective effect of flavonoids against aging- and lipopolysaccharide-induced cognitive impairment in mice. Pharmacology 69, 59–67.

Perez-Vizcaino, F., Bishop-Bailley, D., Lodi, F., Duarte, J., Cogolludo, A., Moreno, L., Bosca, L., Mitchell, J.A., Warner, T.D., 2006a. The flavonoid quercetin induces apoptosis and inhibits JNK activation in intimal vascular smooth muscle cells. Biochem. Biophys. Res. Commun. 346, 919–925.

Perez-Vizcaino, F., Duarte, J., Andriantsitohaina, R., 2006b. Endothelial function and cardiovascular disease: effects of quercetin and wine polyphenols. Free Radic. Res. 40, 1054–1065.

Perez-Vizcaino, F., Duarte, J., Jimenez, R., Santos-Buelga, C., Osuna, A., 2009. Antihypertensive effects of the flavonoid quercetin. Pharmacol. Rep. 61, 67–75.

Perez-Vizcaino, F., Ibarra, M., Cogolludo, A.L., Duarte, J., Zaragoza-Arnaez, F., Moreno, L., Lopez-Lopez, G., Tamargo, J., 2002. Endothelium-independent vasodilator effects of the flavonoid quercetin and its methylated metabolites in rat conductance and resistance arteries. J. Pharmacol. Exp. Ther. 302, 66–72.

Picq, M., Dubois, M., Prigent, A.F., Nemoz, G., Pacheco, H., 1989. Inhibition of the different cyclic nucleotide phosphodiesterase isoforms separated from rat brain by flavonoid compounds. Biochem. Int. 18, 47–57.

Pietsch, K., Saul, N., Menzel, R., Sturzenbaum, S.R., Steinberg, C.E., 2009. Quercetin mediated lifespan extension in *Caenorhabditis elegans* is modulated by age-1, daf-2, sek-1 and unc-43. Biogerontology 10, 565–578.

Pignatelli, P., Di Santo, S., Buchetti, B., Sanguigni, V., Brunelli, A., Violi, F., 2006. Polyphenols enhance platelet nitric oxide by inhibiting protein kinase C-dependent NADPH oxidase activation: effect on platelet recruitment. FASEB J. 20, 1082–1089.

Punithavathi, V.R., Prince, P.S., 2010. Pretreatment with a combination of quercetin and alpha-tocopherol ameliorates adenosine triphosphatases and lysosomal enzymes in myocardial infarcted rats. Life Sci. 86, 178–184.

Quast, U., Cook, N.S., 1989. Moving together: K^+ channel openers and ATP-sensitive K^+ channels. Trends Pharmacol. Sci. 10, 431–435.

Reaven, G.M., Chang, H., 1991. Relationship between blood pressure, plasma insulin and triglyceride concentration, and insulin action in spontaneous hypertensive and Wistar-Kyoto rats. Am. J. Hypertens. 4, 34–38.

Regazzetti, C., Peraldi, P., Gremeaux, T., Najem-Lendom, R., Ben-Sahra, I., Cormont, M., Bost, F., Le Marchand-Brustel, Y., Tanti, J.F., Giorgetti-Peraldi, S., 2009. Hypoxia decreases insulin signaling pathways in adipocytes. Diabetes 58, 95–103.

Rivera, F., Costa, G., Abin, A., Urbanavicius, J., Arruti, C., Casanova, G., Dajas, F., 2008a. Reduction of ischemic brain damage and increase of glutathione by a liposomal preparation of quercetin in permanent focal ischemia in rats. Neurotox. Res. 13, 105–114.

Rivera, L., Moron, R., Sanchez, M., Zarzuelo, A., Galisteo, M., 2008b. Quercetin ameliorates metabolic syndrome and improves the inflammatory status in obese Zucker rats. Obesity (Silver Spring) 16, 2081–2087.

Rodbard, H.W., Blonde, L., Braithwaite, S.S., Brett, E.M., Cobin, R.H., Handelsman, Y., Hellman, R., Jellinger, P.S., Jovanovic, L.G., Levy, P., Mechanick, J.I., Zangeneh, F., 2007. American Association of Clinical Endocrinologists medical guidelines for clinical practice for the management of diabetes mellitus. Endocr. Pract. 13 (Suppl. 1), 1–68.

Romero, M., Jimenez, R., Sanchez, M., Lopez-Sepulveda, R., Zarzuelo, M.J., O'Valle, F., Zarzuelo, A., Perez-Vizcaino, F., Duarte, J., 2009. Quercetin inhibits vascular superoxide production induced by endothelin-1: role of NADPH oxidase, uncoupled eNOS and PKC. Atherosclerosis 202, 58–67.

Romero, M., Jimenez, R., Hurtado, B., Moreno, J.M., Rodriguez-Gomez, I., Lopez-Sepulveda, R., Zarzuelo, A., Perez-Vizcaino, F., Tamargo, J., Vargas, F., Duarte, J., 2010. Lack of beneficial metabolic effects of quercetin in adult spontaneously hypertensive rats. Eur. J. Pharmacol. 627, 242–250.

Rusznyak, S., Szent-Györgyi, A., 1936. Vitamin P: flavonols as vitamins. Nature 138, 27–29.

Sanchez, M., Galisteo, M., Vera, R., Villar, I.C., Zarzuelo, A., Tamargo, J., Perez-Vizcaino, F., Duarte, J., 2006. Quercetin downregulates NADPH oxidase, increases eNOS activity and prevents endothelial dysfunction in spontaneously hypertensive rats. J. Hypertens. 24, 75–84.

Sanchez, M., Lodi, F., Vera, R., Villar, I.C., Cogolludo, A., Jimenez, R., Moreno, L., Romero, M., Tamargo, J., Perez-Vizcaino, F., Duarte, J., 2007. Quercetin and isorhamnetin prevent endothelial dysfunction, superoxide production, and overexpression of p47phox induced by angiotensin II in rat aorta. J. Nutr. 137, 910–915.

Schachinger, V., Britten, M.B., Zeiher, A.M., 2000. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. Circulation 101, 1899–1906.

Sesti, G., 2006. Pathophysiology of insulin resistance. Best Pract. Res. Clin. Endocrinol. Metab. 20, 665–679.

Sharma, V., Mishra, M., Ghosh, S., Tewari, R., Basu, A., Seth, P., Sen, E., 2007. Modulation of interleukin-1beta mediated inflammatory response in human astrocytes by flavonoids: implications in neuroprotection. Brain Res. Bull. 73, 55–63.

Shutenko, Z., Henry, Y., Pinard, E., Seylaz, J., Potier, P., Berthet, F., Girard, P., Sercombe, R., 1999. Influence of the antioxidant quercetin in vivo on the level of nitric oxide determined by electron paramagnetic resonance in rat brain during global ischemia and reperfusion. Biochem. Pharmacol. 57, 199–208.

Silva, B., Oliveira, P.J., Dias, A., Malva, J.O., 2008. Quercetin, kaempferol and biapigenin from Hypericum perforatum are neuroprotective against excitotoxic insults. Neurotox. Res. 13, 265–279.

Simonyi, A., Wang, Q., Miller, R.L., Yusof, M., Shelat, P.B., Sun, A.Y., Sun, G.Y., 2005. Polyphenols in cerebral ischemia: novel targets for neuroprotection. Mol. Neurobiol. 31, 135–147.

Song, Y., Manson, J.E., Buring, J.E., Sesso, H.D., Liu, S., 2005. Associations of dietary flavonoids with risk of type 2 diabetes, and markers of insulin resistance and systemic inflammation in women: a prospective study and cross-sectional analysis. J. Am. Coll. Nutr. 24, 376–384.

Stewart, L.K., Soileau, J.L., Ribnicky, D., Wang, Z.Q., Raskin, I., Poulev, A., Majewski, M., Cefalu, W.T., Gettys, T.W., 2008. Quercetin transiently increases energy expenditure but persistently decreases circulating markers of inflammation in C57BL/6J mice fed a high-fat diet. Metabolism 57, S39–46.

Stoclet, J.C., Kleschyov, A., Andriambeloson, E., Diebolt, M., Andriantsitohaina, R., 1999. Endothelial no release caused by red wine polyphenols. J. Physiol. Pharmacol. 50, 535–540.

Strobel, P., Allard, C., Perez-Acle, T., Calderon, R., Aldunate, R., Leighton, F., 2005. Myricetin, quercetin and catechin-gallate inhibit glucose uptake in isolated rat adipocytes. Biochem. J. 386, 471–478.

Stump, C.S., Henriksen, E.J., Wei, Y., Sowers, J.R., 2006. The metabolic syndrome: role of skeletal muscle metabolism. Ann. Med. 38, 389–402.

Suchankova, G., Nelson, L.E., Gerhart-Hines, Z., Kelly, M., Gauthier, M.S., Saha, A.K., Ido, Y., Puigserver, P., Ruderman, N.B., 2009. Concurrent regulation of AMP-activated protein kinase and SIRT1 in mammalian cells. Biochem. Biophys. Res. Commun. 378, 836–841.

Suri, S., Liu, X.H., Rayment, S., Hughes, D.A., Kroon, P.A., Needs, P.W., Taylor, M.A., Tribolo, S., Wilson, V.G., 2010. Quercetin and its major metabolites selectively modulate cyclic GMP-dependent relaxations and associated tolerance in pig isolated coronary artery. Br. J. Pharmacol. 159, 566–575.

Tauber, A.I., Fay, J.R., Marletta, M.A., 1984. Flavonoid inhibition of the human neutrophil NADPH-oxidase. Biochem. Pharmacol. 33, 1367–1369.

Taubert, D., Berkels, R., Klaus, W., Roesen, R., 2002. Nitric oxide formation and corresponding relaxation of porcine coronary arteries induced by plant phenols: essential structural features. J. Cardiovasc. Pharmacol. 40, 701–713.

Tribolo, S., Lodi, F., Connor, C., Suri, S., Wilson, V.G., Taylor, M.A., Needs, P.W., Kroon, P.A., Hughes, D.A., 2008. Comparative effects of quercetin and its predominant human metabolites on adhesion molecule expression in activated human vascular endothelial cells. Atherosclerosis 197, 50–56.

Turnbull, F., 2003. Effects of different blood-pressure-lowering regimens on major cardiovascular events: results of prospectively-designed overviews of randomised trials. Lancet 362, 1527–1535.

Vlachopoulos, C., Tsekoura, D., Alexopoulos, N., Panagiotakos, D., Aznaouridis, K., Stefanadis, C., 2004. Type 5 phosphodiesterase inhibition by sildenafil abrogates acute smoking-induced endothelial dysfunction. Am. J. Hypertens. 17, 1040–1044.

Wan, L.L., Xia, J., Ye, D., Liu, J., Chen, J., Wang, G., 2009. Effects of quercetin on gene and protein expression of NOX and NOS after myocardial ischemia and reperfusion in rabbit. Cardiovasc. Ther. 27, 28–33.

Wiczkowski, W., Romaszko, J., Bucinski, A., Szawara-Nowak, D., Honke, J., Zielinski, H., Piskula, M.K., 2008. Quercetin from shallots (Allium cepa L. var. aggregatum) is more bioavailable than its glucosides. J. Nutr. 138, 885–888.

Widlansky, M.E., Gokce, N., Keaney Jr., J.F., Vita, J.A., 2003. The clinical implications of endothelial dysfunction. J. Am. Coll. Cardiol. 42, 1149–1160.

Winterbone, M.S., Tribolo, S., Needs, P.W., Kroon, P.A., Hughes, D.A., 2009. Physiologically relevant metabolites of quercetin have no effect on adhesion molecule or chemokine expression in human vascular smooth muscle cells. Atherosclerosis 202, 431–438.

Wright, B., Moraes, L.A., Kemp, C.F., Mullen, W., Crozier, A., Lovegrove, J.A., Gibbins, J.M., 2010. A structural basis for the inhibition of collagen-stimulated platelet function by quercetin and structurally related flavonoids. Br. J. Pharmacol. 159, 1312–1325.

Yamamoto, Y., Oue, E., 2006. Antihypertensive effect of quercetin in rats fed with a high-fat high-sucrose diet. Biosci. Biotechnol. Biochem. 70, 933–939.

Yang, J.Y., Della-Fera, M.A., Rayalam, S., Ambati, S., Hartzell, D.L., Park, H.J., Baile, C.A., 2008. Enhanced inhibition of adipogenesis and induction of apoptosis in 3T3-L1 adipocytes with combinations of resveratrol and quercetin. Life Sci. 82, 1032–1039.

Yochum, L., Kushi, L.H., Meyer, K., Folsom, A.R., 1999. Dietary flavonoid intake and risk of cardiovascular disease in postmenopausal women. Am. J. Epidemiol. 149, 943–949.

Yoshizumi, M., Tsuchiya, K., Suzaki, Y., Kirima, K., Kyaw, M., Moon, J.H., Terao, J., Tamaki, T., 2002. Quercetin glucuronide prevents VSMC hypertrophy by angiotensin II via the inhibition of JNK and AP-1 signaling pathway. Biochem. Biophys. Res. Commun. 293, 1458–1465.

Yu, C., Chen, Y., Cline, G.W., Zhang, D., Zong, H., Wang, Y., Bergeron, R., Kim, J.K., Cushman, S.W., Cooney, G.J., Atcheson, B., White, M.F., Kraegen, E.W., Shulman, G.I., 2002. Mechanism by which fatty acids inhibit insulin activation of insulin receptor substrate-1 (IRS-1)-associated phosphatidylinositol 3-kinase activity in muscle. J. Biol. Chem. 277, 50230–50236.

Zbarsky, V., Datla, K.P., Parkar, S., Rai, D.K., Aruoma, O.I., Dexter, D.T., 2005. Neuroprotective properties of the natural phenolic antioxidants curcumin and naringenin but not quercetin and fisetin in a 6-OHDA model of Parkinson's disease. Free Radic. Res. 39, 1119–1125.

Zhao, X., Gu, Z., Attele, A.S., Yuan, C.S., 1999. Effects of quercetin on the release of endothelin, prostacyclin and tissue plasminogen activator from human endothelial cells in culture. J. Ethnopharmacol. 67, 279–285.