Curr Nutr Rep (2015) 4:290–303
DOI 10.1007/s13668-015-0137-3

The Efficacy of Quercetin in Cardiovascular Health

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Published online: 18 August 2015

Abstract Cardiovascular disease is a major cause of death worldwide despite the majority of its risk factors being preventable and treatable. The results of numerous epidemiological studies suggest that a diet rich in fruits and vegetables affords protection against CVD, and this may be attributed, in part, to the flavonoid quercetin. The aims of this review are to summarise the current knowledge on the bioavailability and metabolism of quercetin as well as discuss the current evidence behind the potential mechanisms by which quercetin exerts its cardioprotective effects. This review summarises key human studies administering quercetin that have been published to date. Although interesting results have been seen in animal and cell culture studies, in general, these have not been replicated in human trials. Several studies have, however, shown that quercetin can reduce blood pressure in hypertensive patients. The exact mechanisms are yet to be elucidated. Further studies are required to investigate the use of quercetin as a cardioprotective treatment, in particular long-term and dose–response studies.

Keywords Cardiovascular disease · Flavonoids · Quercetin · Blood pressure · Flow-mediated dilatation · Nitric oxide · Endothelin-1 · Atherosclerosis · Antioxidant · Oxidative stress · Lipoproteins · Heme oxygenase-1

This article is part of the Topical Collection on *Cardiovascular Disease*

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Introduction

Cardiovascular disease (CVD) is a major cause of death worldwide. There are many risk factors for CVD including hypertension, smoking, hypercholesterolemia, hyperglycaemia, obesity, physical inactivity and an unhealthy diet [1]. Individuals with one CVD risk factor are more likely to have other risk factors, and many cases of CVD can be prevented by identifying a modifiable risk factor and treating it [2]. Although CVD usually affects older adults, the precursor of CVD, particularly atherosclerosis, begins early in life. This makes primary prevention efforts such as healthy eating, exercise and avoidance of smoking necessary from childhood [3]. It is well known that a diet rich in fruits and vegetables is associated with a reduction in CVD [4–6]. Recently, it has been suggested that the beneficial effects of this diet can be attributed, in part, to flavonoids [7]. It is the aims of this review to summarise key human studies with quercetin published to date (Table 1) and to discuss the current evidence behind the potential mechanisms by which quercetin is cardioprotective (summarised in Fig. 1).

Table 1 The cardioprotective effects of quercetin in human interventional studies

Study design	Cohort	Significant effects observed in the treated group	Endpoints with no observed effect	Year	Ref.
Supplement: Q3G 160 mg/day; 4 weeks *Design*: C; DB; R; PC	*N*=37 Age: 66.4±7.9 years *Health status*: healthy (SBP 125–160 mmHg)	↑ in plasma Q after chronic and acute on chronic ingestion	– Change in FMD, SBP, DBP, PWA PWV, body weight, glucose, insulin, insulin resistance, NO, ET-1 or any cholesterols	2015	[36•]
Supplement: Q aglycone 200 and 400 mg *Design*: A; DB; R; PC; CO	*N*=15 *Age*: 25.8±5.2 years *Health status*: healthy	↑ in Q3GA and plasma glutathione at 2 h ↑ in brachial artery diameter at 5 h ↓ in urinary isoprostanes and NOx at 5 h	- change in SBP, DBP, urinary nitrites plus nitrates	2014	[38•]
Supplement: Q3G 0, 50, 100, 200 and 400 mg *Design*: A; DB; R; PC; CO	*N*=15 *Age*: 60.8±9.3 years *Health status*: healthy	– Linear dose–response in plasma Q metabolites ($P<0.001$) 1 h post-intervention	– Change in BP, FMD, plasma NO production	2014	UP
Supplement: Q aglycone 500 or 1000 mg/day; 12 weeks+vitamin C+niacin *Design*: C; DB; R; PC	*N*=100 *Age*: 40–83 years *Health status*: healthy	– A shift in plasma 163 metabolites	– Change in markers of inflammation or oxidative stress	2012	[86]
Supplement: Q aglycone 1095 mg *Design*: A; DB; PC; CO	*N*=5 healthy *Age*: 24±3 years *N*=12 stage 1 hypertensive *Age*: 41±12 years	– Peak in plasma Q at 10 h ↓ in mean SBP, DBP and MAP (in S1 hypertensives only)	– Change in plasma ACE activity, ET-1, nitrites – Change in FMD – Change in BP in normotensives	2012	[37]
Supplement: Q dihydrate 2×500 mg/day; 4 weeks *Design*: C; AM; P	*N*=13 *Age*: 30.1±1.6 years *Health status*: healthy males	↓ in oxLDL ↓ in plasma dendritic cells	– Change in NF-κB gene expression, LDL or HDL	2011	[79]
Supplement: Q dehydrate 150 mg/day; 8 weeks *Design*: C; DB; PC; R; CO	*N*=49 *Age*: 59.4±0.9 years *Health status*: healthy with APOE genotype 3/3, 3/4 or 4/4	↓ in waist circumference ↓ in postprandial SBP and triacylglycerol concentrations ↑ HDL, TNF-α and plasma Q	– Change in endothelial function, glucose, insulin, GSH, CRP, oxLDL inflammation or urinary isoprostanes	2011	[88•]
Supplement: Q aglycone 500 or 1000 mg/day; 12 weeks+vitamin C+niacin *Design*: C; DB; R; PC	*N*=1002 *Age*: 18–85 years *Health status*: varying	↓ in serum creatinine, MAP, HDL and IL-6 ↑ in glomerular filtration rate and plasma Q	– Change in BP, glucose, CRP, LDL, haematocrit, haemoglobin, TNF-α, triglycerides and inflammatory markers	2011	[26]
Supplement: 4×500 mg Q aglycone in 24 h *Design*: A; DB	*N*=18 *Age*: 31–69 years *Health status*: untreated sarcoidosis patients	↑ in plasma Q ↓ in markers of oxidative stress (MDA) and inflammation (TNF-α; IL-10 and IL-8; IL-10) ↑ in plasma oxidative capacity	– Change in plasma GSH – Correlations between plasma Q levels and effects observed	2011	[96]
Supplement: Q aglycone 150 mg/day; 6 weeks *Design*: C; R; DB; PC; CO	*N*=93 *Age*: 45.1±10.5 years *Health status*: features of the metabolic syndrome	↓ in BP in overweight-obese carriers of the apo ∈3/∈3 genotype but not the ∈4 allele ↓ in HDL cholesterol and apoA1 in apo ∈4 carriers ↓ in oxidised LDL and TNF-α in apo ∈3 and apo ∈4 groups	– Change in CRP or nutritional status (body weight, waist circumference, fat mass, fat-free mass)	2010	[41]
Supplement: Q aglycone 500 or 1000 mg/day; 12 weeks+vitamin C+niacin *Design*: C; DB; R; PC	*N*=1002 *Age*: 18–85 years *Health status*: varying	– Dose-dependent increase in plasma quercetin (peaked in first month)	– Change in plasma F_2-isoprostanes, oxLDL, GSH, FRAP or any cholesterols	2009	[85]
Supplement: Q aglycone 150 mg/days; 6 weeks *Design*: C; R; DB; PC; CO	*N*=93 *Age*: 45.1±10.5 years *Health status*: features of the metabolic syndrome	↑ in plasma Q ↓ in SBP ↓ in PP ↓ in serum HDL	– Change in body weight, total cholesterol, TNF-α or CRP	2009	[34]

Table 1 (continued)

Study design	Cohort	Significant effects observed in the treated group	Endpoints with no observed effect	Year	Ref.
		↓ oxidised LDL			
Supplement: Q aglycone 200 mg *Design*: A; R; PC; CO	*N*=12 *Age*: 43.2±4.3 years Health status: healthy males	↑ in plasma S-nitrosothiols. Q metabolites and nitrite ↑ in urinary nitrate ↓ in plasma and urinary ET-1	– Change in plasma nitrate, urinary nitrite and F_2-isoprostanes – Adverse side effects	2008	[56]
Supplement: Q dihydrate 50, 100 or 150 mg/day; 2 weeks *Design*: C; R	*N*=35 *Age*: 26.2±3.7 years *Health status*: healthy	– Dose-dependent increases in plasma Q concentrations	– Change in serum uric acid, lipids and lipoproteins, oxLDL, TNF-α and plasma antioxidative capacity	2008	[87]
Supplement: Q aglycone 1000 mg/day; 3 weeks+vitamin C+niacin (20 mg) *Design*: C; DB; PC	*N*=18 (quercetin) *Age*: 44.2±2 years *N*=21 (placebo) *Age*: 46±2.3 years *Health status*: healthy	↑ in plasma Q	– Difference in plasma antioxidant capacity or F2-isoprostanes – Oxidative damage – Difference in plasma quercetin between the two groups after a race	2008	[97]
Supplement: Q aglycone 730 mg/day; 28 days *Design*: C; R; DB; PC; CO	*N*=19 pre-hypertensive *Age*: 47.8±3.5 years *N*=22 stage 1 hypertensive *Age*: 49.2±2.9 years	↓ in SBP, DBP and MAP (in S1 hypertensives only)	– Change in BP in pre-hypertensives – Change markers of oxidative stress, antioxidant capacity, cholesterols, fasting glucose and triglycerides	2007	[35]
Supplement: Q 1000 mg/day; 28 days *Design*: C	*N*=22 *Age*: 53.1 years *Health status*: patients with interstitial cystitis	– Improvement in cystitis symptoms	– Adverse side effects	2001	[123]
Supplement: rutin 500 mg/day; 6 weeks *Design*: C, SB, PC	*N*=18 *Age*: 18–48 years *Health status*: healthy females	↑ in plasma Q	– Change in plasma antioxidant capacity, phenolic content, liver function indicators, GSH, resistance of lymphocytes to H_2O_2 damage and markers of oxidative stress	2000	[90]
Supplement: Q 30 mg/day; 14 days *Design*: C	*N*=10 *Age*: 33–65 years *Health status*: healthy males	↑ oxidative resistance of LDL	– Change in cholesterol, triglycerides, ascorbic acid and tocopherols	2000	[105]
Supplement: 250 mg Q aglycone+ 50 mg rutin 1 g/day; 28 days *Design*: C; DB; PC	*N*=27 *Age*: 41.5±2.9 years *Health status*: healthy	↑ in plasma Q	– Change in cholesterol, triglycerides, SBP, DBP and HR or thrombogenic risk factors	1998	[39]

A acute, *ACE* angiotensin-converting enzyme, *AM* age matched, *APOE* apolipoprotein E, *BP* blood pressure, *C* chronic, *CO* crossover, *CRP* C-reactive protein, *DB* double blind, *DBP* diastolic blood pressure, *FRAP* ferric-reducing ability of plasma, *GSH* glutathione, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *NOx* nitrogen oxides, *oxLDL* oxidised low-density lipoprotein, *P* parallel, *PC* placebo controlled, *PWA* pulse wave analysis, *PWV* pulse wave velocity, *Q* quercetin, *Q3G* quercetin-3-glucoside, *Q3GA* quercetin-3-glucuronide, *R* randomised, *SB* single blind, *SBP* systolic blood pressure, *TNF-α* tumour necrosis factor-α, *UP* unpublished

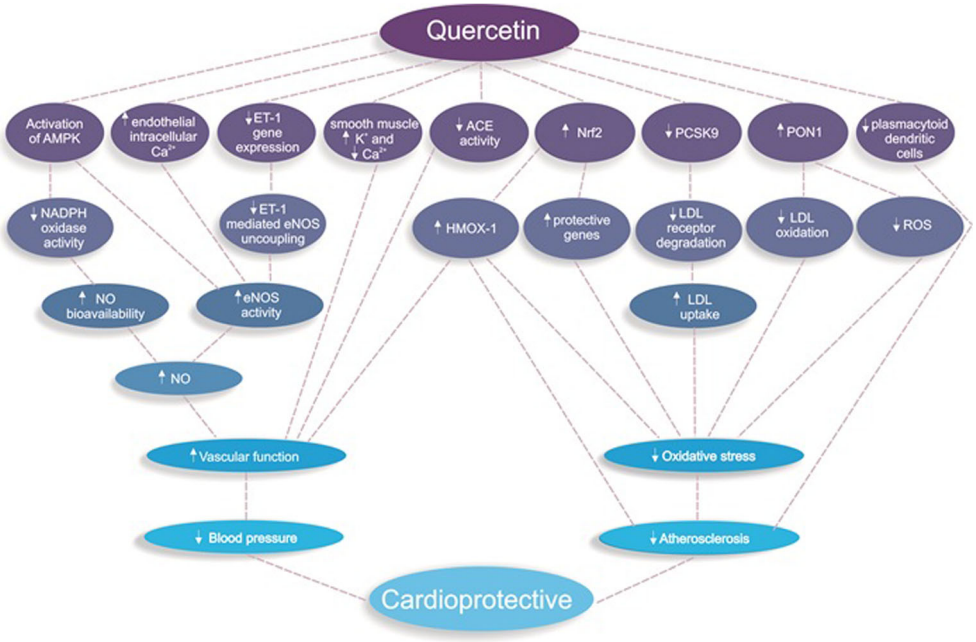

Fig. 1 Potential mechanisms by which quercetin exerts its cardioprotective effects. *ACE* angiotensin-converting enzyme, *AMPK* adenosine monophosphate-activated protein kinase, *eNOS* endothelial nitric oxide synthase, *ET-1* endothelin-1, *HMOX-1* heme oxygenase-1, *LDL* low-density lipoprotein, *NADPH* nicotinamide adenine dinucleotide phosphate, *NO* nitric oxide, *Nrf2* nuclear factor erythroid 2-related factor 2, *PCSK9* proprotein convertase subtilisin/kexin 9, *PON1* paraoxonase 1, *ROS* reactive oxygen species

Flavonoids

Polyphenols, compounds that are found in high concentrations in some fruits and vegetables, are produced as secondary plant metabolites and are usually involved in the defence of the plant against stress such as UV radiation and invading pathogens [8]. It has been suggested that molecules used by plants as a protection against stress may work similarly in animals that consume these plants as food [9]. The main classes of polyphenols include phenolic acids, flavonoids, stilbenes and lignans. Flavonoids are the largest and most researched subclass of polyphenols. All flavonoids have a C6-C3-C6 structure made up of two aromatic rings, linked by a 3-carbon bridge. In the past decade, many studies have tried to find an association between flavonoid intake and prevalence of CVD. However, not all flavonoid-rich foods improve cardiovascular risk factors, and it is likely that certain flavonoids are more bioactive than others [10].

Quercetin

Quercetin (5,7,3′,4′-hydroxyflavonol) is the most ubiquitous of the dietary flavonoids. The richest sources are onions, curly kale, leeks, broccoli, apples, tea, capers and blueberries, with onion often being the biggest contributor to total quercetin intake containing 300 mg/kg fresh onion [11]. Quercetin and other flavonols are mainly found in the outer and aerial tissue of the fruit or vegetable, such as the skin and leaves, as their biosynthesis is stimulated by light [12]. Quercetin in food is usually in its glycosylated form with the sugar moiety often being glucose or rhamnose, but other sugars such as galactose, arabinose and xylose may be involved. Quercetin can be bought over the counter in capsules that contain between 250 and 1500 mg quercetin, which are purported to be beneficial for a range of ailments including allergies, asthma, bacterial infections, arthritis, gout, eye disorders, hypertension and neurodegenerative disorders [13].

Absorption and Bioavailability of Quercetin

The absorption of quercetin depends on the food matrix in which it is found and whether it is ingested as an aglycone or in its glycosylated form. The presence and type of sugar attached determines the site and extent of absorption, while the position of the sugar defines the mechanisms of intestinal uptake [14]. Absorption of glycosides in humans occurs in the small intestine via diffusion after a crucial deglycosylation step [15]. Absorption of quercetin aglycone can also occur in the small intestine via diffusion [16]; however, it is not absorbed as readily as quercetin glycosides. This may be because it is chemically unstable in the pH and temperature conditions of the small intestine, and the intestinal mucus layer provides a barrier for lipophilic substances such as the quercetin aglycone. Flavonoid glycosides that are not absorbed in the small intestine, such as rutin (quercetin-3-O-rutinoside), pass into the colon where they are acted on by enterobacterial β-glucosidases and microfloral rhamnosidases [14]. Although the product that enters the epithelial cells is the same, absorption is not as efficient due to the smaller exchange area and lower density of transporters in the colon. This explains the lower bioavailability and longer time to peak for quercetin rhamnoglucosides when compared to quercetin aglycone or quercetin glucosides. There is higher inter-

individual variability after ingestion of rutin, most likely due to the large diversity of colonic microflora [17].

Metabolism and Safety of Quercetin

Following absorption, quercetin undergoes three main types of conjugation: sulphation, methylation and glucuronidation. This occurs in the enterocyte by the action of sulfotransferases, catechol-*O*-methyl transferases and UDP-glucuronosyltransferases [8]. This conjugation process is very efficient and no aglycones or quercetin glycosides are found in the plasma [18]. The main quercetin metabolites found in the plasma are quercetin-3′-sulphate, quercetin-3-glucuronide, isorhamnetin-3-glucuronide, quercetin diglucuronide and quercetin glucuronide sulphate [19]. Ingestion of glycosylated quercetin does not change circulating metabolites [8].

Metabolites follow two different methods of excretion: via the urine or as part of biliary secretions back into the small intestine [8]. Any quercetin not absorbed in the small intestine, together with that secreted in the bile, is degraded by colonic microflora with the resultant aglycone undergoing ring fission, leading to the production of phenolic acids and hydroxycinnamates [20]. The elimination of quercetin metabolites is slow with reported half-lives ranging from 11 to 28 h [8]. Studies suggest that lower doses of quercetin are more methylated than higher doses in humans [21]. Additionally, sulphation is generally a higher affinity, lower capacity pathway than glucuronidation; an increase in the amount of quercetin ingested may lead to a shift from sulphation towards glucuronidation. A substantial amount of research using quercetin aglycone in vitro has been questioned due to the very low concentrations of aglycone found in the plasma. Furthermore, recent research has shown that the major metabolites of quercetin found in the plasma show weaker bioactivity in vitro than the aglycone [22, 23]. It has been hypothesised that quercetin metabolites are deconjugated in the tissue, by β-glucuronidase, releasing quercetin aglycone which acts as the final effector, a concept that has been discussed in greater detail by Perez-Vizcaino et al. [24].

The safety of quercetin has been extensively reviewed by Harwood et al. [25]. In brief, quercetin is not classified as carcinogenic or mutagenic in vivo. Recent studies looking at both acute and chronic supplementation with high doses of quercetin up to 1000 mg/day for 12 weeks have not reported any adverse side effects [26]. It is important to note that quercetin inhibits CYP3A4, an enzyme that breaks down several commonly prescribed drugs [27]. Quercetin should not be taken in conjunction with drugs such as alprazolam (Xanax) and colchicine, which rely on this pathway for metabolism.

Epidemiology

The correlation between flavonoid intake and risk of CVD has been investigated in several epidemiological studies. Most but not all suggest an inverse association between flavonoid intake and CVD. A recent prospective cohort study found that elderly women with higher total flavonoid consumption were at a lower risk of all-cause mortality [28•]. High total flavonoid consumers had a 40–50 % reduced risk of CVD compared to those with the lowest intake. In the same cohort, women in the highest tertile of flavonol intake had a lower risk of atherosclerotic vascular disease death compared with women in the lowest tertile [29]. To date, few epidemiological studies have looked at quercetin intake; in the Finnish Mobile Clinic Health Examination Survey (n=10,054), it was found that high quercetin intake was associated with lower mortality from ischaemic heart disease. In this study, quercetin from the diet was predominantly from apples and onions. The relative risk (RR) between the highest and lowest quartiles was 0.79 (95 % CI 0.63, 0.99: P for trend=0.02) [7]. Although epidemiological studies suggest a correlation between increased flavonoid consumption and lower risk of CVD, these studies have a number of limitations such as the use of food frequency questionnaires, confounding and publication bias. Thus, we must be careful when inferring causality using traditional epidemiological methods. Plasma biomarkers are a promising new technique for determining flavonoid consumption; however, the methods need further refinement. Currently, there are no validated biomarkers of quercetin intake, although it has been suggested that urinary 4-ethylphenol, benzoic acid and 4-ethylbenzoic acid may be potential markers of quercetin intake [30]. Problems with this method arise due to large inter-subject variability, which can result from differences in gut microflora, as well as the food matrix.

Blood Pressure

Hypertension is a major risk factor for CVD [31] and there is a strong relationship between arterial pressure and death from stroke and ischaemic heart disease [32]. A diet high in fruits and vegetables is known to reduce blood pressure, an effect also attributed to high flavonoid content. Recent studies have shown a decrease in systolic BP following quercetin supplementation, ranging from 2.9 to 7 mmHg in hypertensive individuals [33–35] but not in pre-hypertensives or normotensives [36•, 37, 35, 38•, 40•]. Interestingly, in a study by Egert et al., 6 weeks of 150 mg/day quercetin supplementation led to a significant decrease in BP (3.4 mmHg, P<0.01) in overweight-obese carriers of the ApoE3 (Apolipoprotein E3) gene but not in carriers of the ApoE4 gene [41]. Although the BP reductions observed in these studies are low, the impact on CVD for a population would be significant with a 2–3 % reduction in risk expected for each mmHg reduction in BP

[42]. Long-term regular consumption of foods high in specific flavonoids such as quercetin may be useful dietary lifestyle changes that could help to decrease the use of pharmaceuticals. The mechanisms behind the beneficial decreases in BP remain to be elucidated; potential pathways include improvement in endothelial function, increases in nitric oxide bioavailability, decreases in the vasoconstrictor endothelin-1, direct action on vascular smooth muscle and inhibition of angiotensin-converting enzyme activity.

Endothelial Function and Dysfunction

The endothelium is a monolayer of cells that lines the lumen of the heart and blood and lymphatic vessels. It responds to both physical and chemical stimuli to regulate vascular tone, inflammation, permeability and growth, as well as blood fluidity and coagulation [43]. Interestingly, the endothelium secretes both powerful vasorelaxing (e.g. nitric oxide) and vasoconstricting substances (e.g. endothelin-1). Endothelial dysfunction has been defined as an impairment in endothelium-dependent relaxation, with a tendency towards a proinflammatory, procoagulatory and prothrombotic state [44]. Any damage to the endothelium results in a decrease in the bioavailability of endothelium-derived nitric oxide (NO). This predisposes the vessel wall to leukocyte and platelet adhesion, vasoconstriction and smooth muscle cell proliferation [45]. A significant association has been observed between endothelial dysfunction and increased risk of CVD [46]. Indeed, endothelial dysfunction is implicated in numerous cardiovascular pathologies including pre-hypertension, hypertension, atherosclerosis and stroke [47, 48]. Quercetin can potentially improve endothelial health through direct vasorelaxant activity as well as through the prevention of oxidant-induced endothelial dysfunction.

Flow-Mediated Dilatation In humans, ultrasonography is a common method used to assess vascular endothelial function as NO flow-mediated dilatation (FMD) of the brachial artery [49]. FMD in the peripheral circulation is primarily mediated by endothelium-derived NO in response to increased flow and shear stress and results in smooth muscle relaxation and arterial dilation [50]. The effect of quercetin on endothelial function has not been widely studied. Two studies have shown that quercetin has neither an acute (1095 mg quercetin aglycone) [37] nor chronic (quercetin-3-O-glucoside, 160 mg/day for 4 weeks) [36•] effect on FMD. Contrastingly, studies using whole foods (apples) or whole food extracts (onion extract) rich in quercetin have shown significant improvements in FMD (acute, 1.1 %) [51] and postprandial FMD (30 days, 1.6 %) [40•]. The above studies vary greatly in design and, as a result, are hard to compare. Further investigations should be undertaken to determine whether there is a difference between acute and chronic consumption of quercetin on FMD and whether different effects are observed in healthy subjects compared to subjects with risk factors for CVD such as hypertension or obesity.

Nitric Oxide NO is a potent vasodilator that is synthesised in the endothelium by endothelial NO synthase (eNOS) enzymes. It plays a key role in maintaining vascular integrity through its antithrombotic, antiproliferative and antiatherogenic properties [52]. Quercetin has been shown to increase eNOS activity, possibly through phosphorylation of adenosine monophosphate-activated protein kinase (AMPK), resulting in an increase in NO production both acute and long term [53]. Additionally, up-regulation of AMPK can increase NO bioavailability through inhibition of NADPH oxidase activity, reducing superoxide-activated NO depletion [54]. Quercetin increases intracellular Ca^{2+} levels in aortic endothelial cells and stimulates eNOS phosphorylation in a dose- and time-dependent manner, leading to an increase in NO production and relaxation of the vessel [55]. In humans, acute quercetin (200 mg) has been shown to significantly increase plasma *S*-nitrosothiols (metabolites of NO) [56]. Unfortunately, neither BP nor FMD was measured in this study. Similarly, quercetin-rich apple has been shown to significantly increase plasma nitric oxide status (assessed by measuring *S*-nitrosothiols + other nitrosylated species (RXNO)) [51]; however, it would be erroneous to attribute this to quercetin alone. The results of these studies provide some evidence of one possible mechanism behind the cardioprotective benefits of quercetin. Both Larson et al. [37] and Perez et al. [38•] reported no changes in plasma nitrite or NOx, despite seeing a decrease in BP and a large increase in brachial artery diameter, respectively. In both studies, nitrite was measured using the Griess reaction which is not suitable for measurement of submicromolar levels of nitrate and nitrite due to lack of sensitivity [57]. Correspondingly, Dower et al. found no changes in NO (measured by chemiluminescence) following 4 weeks ingestion of 160 mg/day quercetin-3-O-glucoside or after acute on chronic ingestion (NO measured 2 h after the last treatment) [36•]. It seems that while animal studies suggest that quercetin can improve endothelial function by increasing NO bioavailability, these results are not well replicated in human studies. It is important to note, however, that many animal studies use supra-physiological doses of pure quercetin; thus, care must be taken when interpreting these results. Interestingly, it has been shown that unlike quercetin aglycone, conjugated quercetin metabolites lack a direct vasorelaxant effect and are unable to modify endothelial function or NO bioactivity [23].

Nitric Oxide: Endothelin-1 Endothelial dysfunction is associated with a diminished bioavailability of the vasodilator NO resulting in less opposition to vasoconstrictors such as endothelin-1 (ET-1) [50]. Quercetin has been shown to

significantly down-regulate ET-1 gene expression [58], prevent eNOS from endothelin-1-induced uncoupling [54] and prevent endothelial dysfunction induced by incubation with ET-1 [23] in isolated rat aortas. These studies support the hypothesis that quercetin improves vascular function by altering the balance between NO and ET-1. Loke et al. showed that urinary ET-1 was significantly lower in people given quercetin (200 mg) compared to those receiving the placebo [56]. This was accompanied by an increase in plasma nitrite. In contrast, no changes in plasma ET-1 were observed in people following 4 weeks ingestion of 160 mg/day quercetin-3-O-glucoside [36•] or after acute administration of 1095 mg quercetin aglycone [37]. As results are inconsistent, further clinical intervention studies are needed to determine whether a reduction in circulating levels of ET-1 is one of the mechanisms behind the cardioprotective effects of quercetin.

Direct Action on Vascular Smooth Muscle

Up until now, we have been looking at the effects of quercetin on the endothelium as a way of improving blood vessel reactivity and decreasing BP. There is evidence that quercetin and its methylated metabolite isorhamnetin can act directly on the smooth muscle layer, inducing vasorelaxation independent of the endothelium [59–61]. The results of a recent study by Hou et al. demonstrate that quercetin possesses vasospasmolytic effects and suggest that depression of Ca^{2+} influx through L-type voltage-gated Ca^{2+} channels and augmentation of voltage-gated K^{+} channel activity in the muscle cells may underlie coronary relaxation [62]. It is important to note that the above-mentioned studies use quercetin aglycone. It has not been confirmed whether this is a representative of in vivo conditions as pure quercetin is rapidly metabolised upon ingestion; however, evidence shows that metabolites are cleaved by β-glucuronidase in the target tissues, releasing the active aglycone [24].

Angiotensin-Converting Enzyme

Angiotensin-converting enzyme (ACE) is a crucial element in the renin-angiotensin-aldosterone system (RAAS) which regulates BP and fluid loss. The role of ACE is to convert angiotensin I into angiotensin II: the peptide responsible for increasing BP [31]. Consequently, chronic over-activation of RAAS is associated with hypertension [63]. Inhibition of ACE is a method used for down-regulating RAAS. There is evidence that plant extracts, rich in flavonoids such as quercetin, can be effective ACE inhibitors. Evidence is derived from in vitro models and animal studies [64, 65], and several quercetin metabolites have been shown to be moderate inhibitors of ACE [66–68]. Quercetin has been shown to blunt the increase in BP observed after administration of angiotensin I in rats, findings which were further supported by a 31 % decrease in plasma ACE activity [64]. As well as decreasing BP by blocking angiotensin II production, resulting in an increase in urinary volume and sodium output, quercetin has also been shown to inhibit ACE activity through the down-regulation of renal angiotensin II receptors [65]. In a study by Larson et al., quercetin lowered blood pressure in hypertensive men; however, they observed no accompanying changes in plasma ACE activity [37]. A study by Knab et al. found an increase in urinary output of approximately 30 mL/day after 12 weeks of quercetin supplementation in humans [26]. Increased urine output has been proposed as a mechanism behind the decrease in mean arterial pressure (MAP) that has been reported in animal models after quercetin supplementation [65]. As evidence of ACE inhibitory effects of quercetin from in vitro and animal studies has been presented, more studies are required to investigate this mechanism in humans.

Atherosclerosis

Atherosclerosis, a deposition of lipids in the subendothelial layer of injured blood vessels, is believed to be the primary cause of many fatal CVDs [69]. Plaque disruption is initiated by damage to endothelial cells by inflammatory responses or reactive oxygen species (ROS) and oxidation of low-density lipoprotein (LDL). Cardiovascular risk factors, such as hypertension and diabetes, accelerate this process [70]. Primary cell cultures derived from the damaged endothelium have reduced expression of eNOS [71] and greater production of oxygen-derived free radicals (ROS) and have been shown to take up more LDL and oxidised LDL (oxLDL) [72]. Animal studies have shown that long-term quercetin consumption leads to a significant reduction in atherosclerotic lesion formation [73, 74]. Histological and gene expression analyses of aortas treated with quercetin show that quercetin affects lesional smooth muscle cell proliferation and inflammatory factors associated with atherogenesis [73]. Quercetin-3-glucuronide (Q3G) can accumulate in human atherosclerotic lesions and macrophage-derived foam cells, but not in healthy aorta cells [75]. Inflammatory stimulation of macrophages may initiate lactate secretion, leading to acidification of the surrounds which enhances the activity of β-glucuronidase [76]. This means that macrophages may serve as a potential pool of Q3G which they deconjugate into the aglycone at the site of pathogenic lesions [77]. The effect of quercetin on atherosclerotic lesions has not yet been measured in humans, as the required long-term interventions are not feasible. However, several studies have investigated the effects of quercetin on oxidative stress and inflammation, which have been implicated in the development of atherosclerosis [78]. Overall, quercetin has been shown to have little effect on markers of inflammation such as C-reactive protein (CRP), cytokines (such as IL-6, IL-1 and TNF-α) and adhesion molecules (such as ICAM-1, VCAM-1 and selectins) (refer to Table 1). Interestingly, quercetin

(500 mg twice daily for 4 weeks) significantly reduced plasmacytoid dendritic cells in healthy males [79] which have been shown to contribute to the initiation of atherosclerosis by amassing in the lipid plaque [80].

Antioxidant

Quercetin has been shown to be an excellent antioxidant in vitro and has been described as a potent free radical scavenger in numerous papers [81]. These properties are primarily attributed to the presence of a catechol or gallate group in the B-ring [82]. These claims are supported by studies which found that quercetin increased total plasma antioxidant capacity 6.24 times more than trolox, a reference antioxidant [83]. Thus, the role of quercetin in preventing CVD has largely been associated with its free radical scavenging antioxidant properties. However, the interpretation of plasma antioxidant capacity in human blood is difficult, and there is little evidence to support the antioxidant activity of quercetin in vivo. A recent review by Forman et al. has presented a new concept by which antioxidants exert their beneficial effects. Rather than acting as free radical scavengers in vivo, as was previously thought, antioxidant compounds generate signals that induce the transcription of protective enzymes [84•]. The physiological concentration of polyphenols in the plasma is insufficient to scavenge a significant portion of free radicals, as is seen in many in vitro studies. Rather, polyphenols will be oxidised to electrophilic hydroquinones and quinones during their reaction with free radicals. This step appears to be crucial to its ability to activate nuclear factor erythroid 2-related factor 2 (Nrf2), which maintains protective oxidoreductases and their nucleophilic substrates. Quercetin and many other plant polyphenols have been recommended as antioxidants for many years, despite little evidence that they decrease oxidative stress in humans [35, 56, 85–87, 88•, 89, 90].

Oxidative Stress

Oxidative stress and inflammation are commonly thought to contribute to the initiation and progression of atherosclerosis [78]. Oxidative stress is defined as an increase in the production of ROS over the ability to degrade them. Many functions of the endothelium are known to be affected by ROS, including endothelial cell apoptosis [91] and adhesion of inflammatory cells [92], initiating and augmenting the progression of CVD, namely atherosclerosis. As discussed above, quercetin may decrease oxidative stress by stimulating protective defences and repair systems [84•]. F_2-isoprostanes are considered to be the "gold standard" in vivo biomarker of oxidative stress [93]. Another commonly used marker of oxidative stress is malondialdehyde (MDA); however, the methods of detection are less reliable and MDA itself is not a specific marker of oxidative stress [94]. The ability of quercetin to reduce oxidative stress levels has been demonstrated in some animal studies [95]. To date, only a few studies have shown this in humans; Boots et al. found a decrease in MDA and markers of inflammation in the blood of patients with the inflammatory disease, sarcoidosis [96], and Perez et al. found a decrease in urinary F_2-isoprostanes 5 h after supplementation of quercetin aglycone (200 and 400 mg). Other studies in humans have failed to find decreases in oxidative stress after quercetin consumption [35, 56, 90, 97, 98]. Some of these results may be explained by the fact that the studies were done in healthy volunteers or volunteers without elevated levels of oxidative stress; the above results suggest that quercetin may reduce markers of oxidative stress only when levels are high. To test this hypothesis, Shanely et al. gave 500 mg or 1000 mg/day doses of quercetin for 12 weeks to a large population of subjects ranging widely in age, BMI and disease state [85]. They did not find an improvement in antioxidant capacity or a decrease in oxidative stress in any group. Overall, indications that quercetin may reduce or prevent CVD risk by reducing levels of oxidative stress are much stronger in animal studies than human studies. This may be because higher doses of quercetin per kilogram body weight are generally used in animal studies; the resulting differences in plasma quercetin concentrations may be responsible for the contrasting effects of quercetin on oxidative stress. Another explanation may lie within the methods used to quantify oxidative stress.

Lipoproteins

It has been established that the conversion of LDL to oxidised LDL is an early event in atherosclerosis [78]. High levels of oxidised LDL are found in patients with the metabolic syndrome and are associated with an increase in the risk of myocardial infarction [99]. Reactive species generated by myeloperoxidase (MPO) are thought to modify LDL in vivo [100]. In a recent in vitro study, quercetin and some of its major metabolites were found to protect LDL from MPO-mediated modification in vitro [101]. One potential mechanism behind this may be increasing the activity of paraoxonase 1 (PON1), an esterase related to the anti-atherogenic properties of high-density lipoprotein (HDL) [102] that has been shown to inhibit the oxidation of LDL [103]. Quercetin has been shown to up-regulate PON1 activity and free radical scavenging and protect against LDL oxidation and lipid peroxidation [104]. In a study by Egert et al., 150 mg of quercetin dehydrate per day for 6 weeks reduced plasma oxidised LDL concentrations in overweight subjects with a high CVD risk phenotype [34]. In agreement with these results, Chopra et al. showed that 30 mg of quercetin per day for 2 weeks significantly inhibited LDL oxidation [105]. In contrast to these results, several other human intervention studies found no changes in oxidised LDL after chronic quercetin

supplementation [85, 87, 106]. Some animal studies support improvements in lipid profile following quercetin supplementation [74, 107]. A potential mechanism for this may be in the reduction of proprotein convertase subtilisin/kexin 9 (PCSK9) which promotes LDL receptor degradation. Q3G has been shown to increase LDL receptor expression, stimulate LDL uptake and reduce PCSK9 secretion [108]. However, results of human studies measuring lipid profiles after quercetin supplementation are equivocal. Overall, most human studies, with both acute and chronic quercetin supplementation, have not reported any changes in the levels of plasma LDL or HDL cholesterol (refer to Table 1). As hyperlipidemia is an independent risk factor for atherosclerosis, it is imperative to investigate the potential for quercetin to decrease the levels of LDL and oxidised LDL.

Heme Oxygenase-1

As mentioned previously, quercetin can act as an antioxidant by signalling the induction of protective enzymes, one of these being heme oxygenase-1 (HMOX-1) [84•]. HMOX-1 is the inducible form of the enzyme that catalyses the degradation of heme, producing iron, carbon monoxide and biliverdin, which is then converted to bilirubin [109]. Several reports have highlighted the important physiological and beneficial roles of HMOX-1 in the vasculature, and this has recently been reviewed by Calay et al. [110•]. HMOX-1 exerts an anti-inflammatory and antioxidant action within the vasculature and may modulate endogenous cellular ROS generation [111]. Pharmacological inducers of HMOX-1, such as probucol, protect against vascular disease in three different animal models of atherosclerosis [112]. Moreover, this beneficial effect is associated with enhanced protection of arteries against endothelial dysfunction induced by oxidative stress. Increasing evidence suggests a central role for HMOX-1 in cardiovascular protection, and induction of vascular HMOX-1 could be an important therapeutic target [113]. Blood vessels exposed to the oxidant hypocholorous acid (HOCl), a physiologically relevant oxidant produced by myeloperoxidase, exhibit a defect in endothelium-dependent NO bioavailability as shown by impaired endothelium-dependent relaxation [114]. Pre-treatment of aortic rings from control mice with quercetin (5 and 10 μmol/L) dose-dependently attenuated endothelial dysfunction while maintaining eNOS activity [53]. This effect was not seen in the rings from HMOX-1 heterozygous knockout mice suggesting that HMOX-1 is essential for the protective effects of quercetin. This notion is supported by the increased expression of HMOX-1 protein in arteries isolated from mice fed a diet supplemented with quercetin which was also associated with a decrease in atherosclerotic lesions [95, 115]. Several other studies have shown that quercetin induces the production of HMOX-1 [116, 117]. Although HMOX-1 is not detected in healthy human arteries, high expression is seen in foam cells and atherosclerotic plaques [118]. The majority of studies indicate a protective role for HMOX-1 and its products in atherosclerosis; however, the exact mechanisms remain to be determined. The potential induction of HMOX-1 by dietary flavonoids, such as quercetin, is of substantial significance; clinical trials are now required to determine whether the benefits of quercetin in relation to CVD are associated with HMOX-1 in humans.

Quercetin in Human Studies

In the past decade, a significant number of human intervention studies investigating the effect of quercetin on risk factors for CVD have been conducted. The goal of these studies was to determine the mechanisms of action by which quercetin may protect against CVD. Despite these studies, which have looked at the effects of both pure quercetin (described in Table 1) and quercetin-rich foods, the precise mechanisms have not been elucidated. There are a number of limitations of these studies, but perhaps the most important is the sample size. Half of the studies presented in Table 1 have a sample size $n<20$, therefore decreasing the statistical power to detect changes in the outcome of interest. There is a large range in the doses and form in which quercetin is given; a strength of these studies is that majority were assessed and showed significant increases in plasma quercetin levels. Over half of the studies discussed in Table 1 were done in a healthy population; however, studies looking at the antioxidant effects of quercetin need to be done on a cohort of people with increased levels of inflammation and oxidative stress [119]. A healthy and diverse diet generally supplies sufficient antioxidants to counteract ROS production in healthy individuals, and this may explain the lack of effect observed in studies with healthy volunteers [35, 56, 86]. Additionally, quercetin has only been shown to decrease BP in a hypertensive population. Table 1 highlights the variety of endpoints measured to demonstrate the biological effects of quercetin; however, many of these are not standardised or validated. Unfortunately, this makes it difficult to compare results and draw conclusions from studies using a different methodology.

Conclusions and Future Studies

Quercetin is a promising molecule in cardiovascular health and has been widely researched. Nonetheless, interesting results seen in animal models and cell culture studies have not been replicated in humans. There are several possible reasons for this: firstly, many of these studies use supra-physiological doses of quercetin, which are not reflective of quercetin content in the human diet. Secondly, many in vitro studies use quercetin aglycone,

which may not accurately represent conditions in vivo. Thirdly, pathways for quercetin metabolism differ between humans and rats/mice [120], and the resulting metabolites may function very differently. Another vital factor to take into consideration when planning future studies is the metabolism of quercetin by gut microflora [121]. The variability in bacterial species found in the gut between people and animals, and even between people, may account for a lot of the variability found in flavonoid research. Consequently, there is a real need for more human intervention studies, specifically (i) long-term intervention studies, (ii) dose–response studies, (iii) studies administering quercetin glucosides and (iv) studies that use co-ingestion of quercetin with other flavonoids and food constituents to improve its bioavailability and bioactivity. Due to reported high inter-subject variability, future flavonoid studies need to use a cross-over design [120]. Health status and age should play a significant role in the selection of volunteers as structural damage and arterial diseases can reduce the potential to improve vascular function by nutrients alone [34]. As discussed previously, many studies have reported that quercetin only lowers BP in hypertensive individuals; it remains to be determined whether quercetin benefits all forms of hypertension, irrespective of pathological cause. One study [34] suggests that future studies should incorporate 24-h ambulatory blood pressure measurements, rather than rely on one blood pressure measurement taken at resting state, in order to gain a better understanding of the effects of quercetin on blood pressure throughout the day and night. Food databases are potentially powerful tools in epidemiological studies; however, there is a large amount of variation in flavonoid content due to agricultural growth methods and differing analytical techniques. Spencer et al. have stressed the importance of establishing quality biomarkers of flavonoid intake as a means of overcoming food questionnaire and food database limitations [122]. Although many human studies administering pure quercetin yield a negative result, there is certainly evidence that quercetin contributes to the beneficial effects of a diet rich in fruits and vegetables.

Acknowledgments NP Bondonno acknowledges the support of an Australian Postgraduate Award. NC Ward acknowledges the support of a MRF/UWA Fellowship. JM Hodgson was supported by an NHMRC Senior Research Fellowship.

Compliance with Ethics Guidelines

Conflict of Interest Nicola P. Bondonno declares that she has no conflict of interest.

Catherine P. Bondonno declares that she has no conflict of interest.

Jonathan M. Hodgson has received research support through grants from the National Health and Medical Research Council of Australia, Fruit West, Horticulture Australia Limited and Nestlé.

Natalie C. Ward declares that she has no conflict of interest.

Kevin D. Croft has received research support through grants from the National Health and Medical Research Council of Australia, Fruit West and the Australian National Apple Breeding Program.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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