Flavonols in the Prevention of Diabetes-induced Vascular Dysfunction

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Abstract: As flavonols are present in fruits and vegetables, they are consumed in considerable amounts in the diet. There is growing evidence that the well-recognized antioxidant, anti-inflammatory, and vasorelaxant actions of flavonols may, at least in part, result from modulation of biochemical signaling pathways and kinases. It is well established that diabetes is associated with increased cardiovascular morbidity and mortality. Despite clinical management of blood glucose levels, diabetes often results in cardiovascular disease. There is good evidence that endothelial dysfunction contributes significantly to the progression of diabetic cardiovascular diseases. This review describes the biological actions of flavonols that may ameliorate adverse cardiovascular events in diabetes. We discuss evidence that flavonols may be developed as novel pharmacological agents to prevent diabetes-induced vascular dysfunction.

Key Words: endothelial dysfunction, diabetes, flavonols

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INTRODUCTION

Flavonoids are a class of polyphenolic compounds consisting of a benzene ring condensed with a 6-member ring with a phenyl ring attached either to the C2 or the C3 carbon position. Flavonoid classes are subdivided based on structural characteristics. One of the most abundant classes, flavonols, is widely found in plants. A hydroxyl group at the C3 carbon position distinguishes flavonols (Fig. 1). Flavonols can be found in virtually all vegetables and fruits, and high consumption of vegetables and fruits is associated with reduced risk for heart disease[1] and stroke.[2] Furthermore, a number of large-scale epidemiological studies reported that a greater flavonoid intake is associated with reduced incidence of cardiovascular diseases.[3–6] Thus, it is now recognized that flavonoids, in particularly flavonols, significantly contribute to the cardioprotective effects and may potentially be developed as pharmaceutical therapy for cardiovascular diseases.

Diabetes-induced vascular dysfunction is a leading cause of morbidity and mortality.[7] Patients with long-standing type I or type II diabetes frequently develop complications affecting both the macrovasculature and microvasculature. Current treatments aim to tightly regulate glucose levels; however, large-scale prospective studies for both type I and type II diabetes have shown that tight glycemic control slows, but does not prevent, the progression of cardiovascular complications.[8,9] The loss of the modulatory role of the vascular endothelium is one of the early hallmarks of diabetic vascular complications and is a risk factor for cardiovascular disease.[10–12] As a result, the endothelium offers a target for alternative therapies to reduce the mortality and morbidity associated with diabetic cardiovascular complications.

This review describes the biological actions of flavonols that we believe underlie the potential for this class of compounds to be developed as novel pharmacological agents to alleviate diabetes-induced vascular dysfunction.

FLAVONOLS

The simplest structure of a flavonol has a 3-hydroxyflavone backbone. The biological action of flavonols can be altered by chemical modification. For example, the number of hydroxyl groups on the flavonol backbone influences its antioxidant potency and vasorelaxant properties. The structure–activity relationships of flavonols have been extensively reviewed.[13–15] Based on previous studies, the flavonols with the greatest vascular activity are quercetin, which is most abundant in fruits and vegetables, and 3′,4′-dihydroxyflavonol (DiOHF), a synthetic compound. A summary of the biological actions of flavonols is shown in Figure 2.

Vasorelaxant Properties

Fruit and vegetable consumption is associated with a decrease in blood pressure, leading to suggestions that flavonols have blood pressure–lowering activity.[16] The blood pressure–lowering effects of quercetin were first reported in spontaneously hypertensive rats (SHRs)[17] and subsequently in other animal models of hypertension.[18] In SHRs, treatment with 10 mg/kg of quercetin for 5 weeks significantly reduced systolic, diastolic, and mean arterial pressure but similar treatment had no effect in Wistar-Kyoto (WKY) control rats.[17] Similarly, acute intravenous injection of quercetin metabolites significantly reduced mean arterial pressure in SHRs.[19] Numerous studies have shown that acute exposure to

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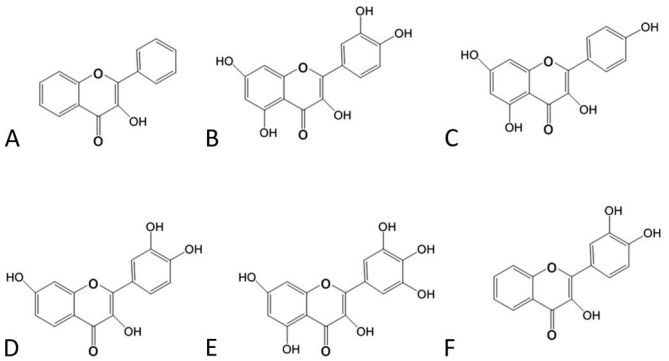

FIGURE 1. Structure of different types of flavonol: (A) flavonol, (B) quercetin, (C) kaempferol, (D) fisetin, (E) myricetin, and (F) 3′,4′-DiOHF.

flavonols can cause vascular relaxation in rat isolated blood vessels[20–24] and vasodilation in humans.[25] Flavonols mainly act in an endothelium-independent manner and their potency varies in different vascular beds. For example, quercetin and its methylated metabolites are more potent in coronary arteries[22] and resistance vessels compared with noncoronary conductance vessels.[20] Some reports suggest that the vasodilator effect of flavonols is also partially endothelium dependent and related to the release of nitric oxide (NO).[26–28] Indeed, in endothelial cells, flavonols cause phosphorylation of endothelial nitric oxide synthase (eNOS) on Ser^{1177} and dephosphorylation at Thr^{495}, leading to its activation and NO synthesis.[29] Similarly, quercetin ingestion increased plasma S-nitrosothiols, plasma nitrite, and urinary nitrate concentrations in healthy men. Indirectly, this is an indication of increased endothelial NO synthesis.[28] Thus, there is evidence of both endothelium-independent and endothelium-dependent components to flavonol-induced vascular relaxation.

The precise mechanism by which flavonols exert endothelium-independent relaxation remains unclear. Flavonols may disrupt calcium utilization in the vascular smooth muscle cell (VSMC) by several mechanisms. First, flavonols could inhibit calcium entry at voltage-gated or receptor-operated calcium channels. Alternatively, intracellular calcium release from the sarcoplasmic reticulum could be affected.[13,24,30] Ko et al[30] measured influx of radioactive calcium into aortic smooth muscle after contraction with noradrenaline or depolarization with high potassium. Under such conditions, flavonols inhibited the influx of calcium in a concentration-dependent manner by both voltage-gated and receptor-operated calcium channels.[30] Similarly, DiOHF could inhibit the contractile response caused by extracellular influx of calcium in rat isolated aorta, possibly by the voltage-gated calcium channels.[13,24] Consistent with the aorta, DiOHF also reduces vascular contraction through calcium desensitization in the rat mesenteric artery.[31]

There is also evidence that flavonols may inhibit the RhoA/Rho kinase signaling pathway when activated by protein kinase C or other stimuli[23,32,33] or through the opening of potassium channels on the VSMC causing hyperpolarization. Cogolludo et al[34] reported that quercetin can cause vascular relaxation by directly activating the large conductance calcium-activated potassium channels (BK_{Ca}) by a H_2O_2-dependent mechanism in the rat coronary arteries because these effects could be abolished by the BK_{Ca} channel blocker, iberiotoxin and catalase. However, other studies suggest that the contribution of potassium channels is minor[24] or nonexistent.[33]

Antioxidant Activity

In addition to their vascular activity, flavonols may modulate tissue antioxidant status. This may occur by direct scavenging of free radicals and enhanced expression and/or activity of antioxidant enzymes or by inhibition of prooxidant enzymes. Direct free radical scavenging by flavonols

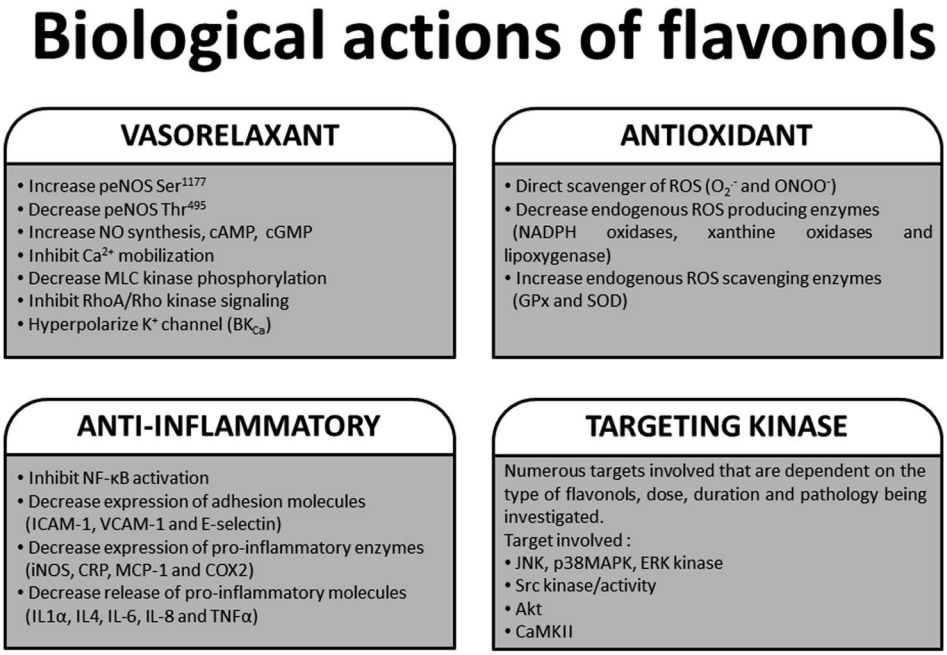

FIGURE 2. Summary of the biological actions of flavonols. cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; CRP, C-reactive protein; COX2, cyclooxygenase 2; E-selectin, endothelial cell selectin; MCP1, monocyte chemoattractant protein 1; MLC, myosin light chain; peNOS, phosphorylated endothelial NO synthase; VCAM-1, vascular cell adhesion molecule 1.

has been extensively reported.[24,35,36] Studies demonstrated that flavonols reduce superoxide levels in a concentration-dependent manner in both cell-free systems and in biological tissues.[24,35] Furthermore, acute exposure to flavonols significantly enhanced endothelium-dependent relaxation in the presence of oxidant stress. This suggests rapid scavenging of superoxide anions and preservation of NO.[24,35,36] In addition, acute exposure to a flavonol is also protective in other oxidative stress–related disorders, such as ischemia and reperfusion.[35,37–39] Apart from scavenging superoxide, flavonols can scavenge peroxynitrite.[40] It has been argued that the hydrogen-donating activity of flavonols is unlikely to account for all its antioxidant effect.[41]

Several studies have demonstrated that flavonols can also activate signal transduction pathways that regulate gene transcription. Activation of the antioxidant defense system occurred by increased expression of antioxidant enzymes, including superoxide dismutase (SOD), cytochrome c oxidase, and glutathione peroxidase.[42–45] The cellular pathway(s) regulated remain controversial but are likely to be dependent on the specific flavonols being investigated. For example, DiOHF had no significant effect on the gene expression of SOD and glutathione peroxidase.[45] In contrast, quercetin increases the level of glutathione through the activation of the protective NF-E2–related factor 2–dependent signaling pathway.[43]

Flavonols can also inhibit the expression/activity of pro-oxidant enzymes. One critical pro-oxidant enzyme in VSMC is NADPH oxidase.[46] A number of studies have shown that flavonols inhibit the activity, and the expression, of NADPH oxidase, thereby decreasing the production of reactive oxygen species (ROS) in the vasculature.[47–50] In addition, quercetin can inhibit eNOS-derived superoxide production.[50] Other potential pro-oxidant enzymes contributing to ROS production include xanthine oxidase, lipoxygenase, COX-1, COX-2, cytochrome P450, and the enzymes of the mitochondrial respiratory chain.[51–54] Lipoxygenases and COX are oxidizing molecules that can increase ROS production in some tissues. Flavonols can reduce oxidative stress by inhibiting COX-2 and lipoxygenase.[51,52] Furthermore, the xanthine oxidase pathway is an important source of oxidative injury. Under ischemic conditions, flavonols decrease oxidative injury by inhibiting production of oxygen free radicals that are generated as a result of the conversion of xanthine dehydrogenase to xanthine oxidase.[53,54]

Anti-inflammatory Activity

In an inflammatory response, leukocyte adhesion to vascular endothelial cells is important for the recruitment and infiltration of leukocytes to the site of injury. These processes are mediated by a wide variety of adhesion molecules, including intercellular adhesion molecule 1 (ICAM-1) and CD54. In endothelial cells, ICAM-1 expression is induced by proinflammatory cytokines.[55] In a human endothelial cell line ECV304, quercetin treatment downregulated activator protein 1 (AP-1) and ICAM-1 messenger RNA (mRNA) levels, but had no effect on nuclear factor-κB (NF-κB) activation.[56] Quercetin and kaempferol also attenuated ICAM-1, vascular cell adhesion molecule 1, and endothelial cell selectin mRNA expression. They also inhibited AP-1 and NF-κB activity and degradation of the inhibitory protein of NF-κB (IκB).[57–59] These studies suggest the anti-inflammatory effects of flavonols can be partly attributed to inhibition of the transcription factor NF-κB and subsequent decreased expression of pro-inflammatory enzymes, such as inducible NO synthase (iNOS), C-reactive protein, and cyclooxygenase 2.[60–63] In activated macrophages, together with an inhibitory effect on expression of iNOS and NO, isorhamnetin inhibited NF-κB activation.[60] In addition, quercetin has the ability to stimulate anti-inflammatory cytokine interleukin (IL)-10 expression at low concentrations (<50 μM).[63]

Apart from modulating the expression or activity of pro-inflammatory enzymes, flavonols can inhibit secretion of pro-inflammatory mediators. Flavonols inhibited release of pro-inflammatory molecules, including IL-6, IL-8, and tumor necrosis factor α (TNF-α) from human mast cells.[64] In addition, the synthetic flavonol DiOHF reduced monocyte chemoattractant protein 1 (MCP-1) expression in rat VSMC. This effect was observed in unstimulated cells and with platelet-derived growth factor or IL-1β stimulation.[65] Moreover, in diet-induced obesity, dietary quercetin reduced circulating levels of the inflammatory markers interferon-γ, IL-1α, and IL-4.[66]

Targeting Kinases

There is growing evidence that flavonols could modulate the activity of different mitogen-activated protein kinases (MAPK). For example, a quercetin-induced reduction in ICAM-1 expression in endothelial cells is associated with the c-Jun NH_2-terminal kinases (JNK) pathway.[56] Specifically, quercetin treatment reduced c-jun mRNA expression and caused a concentration-dependent decrease in both protein kinase C-activated and TNF-α–mediated JNK activation.[56] Similarly, in A549 epithelial cells, kaempferol inhibited TNF-α–induced ICAM expression primarily by reduced c-jun mRNA expression and selective attenuation of JNK activity. Kaempferol had no effect on the p38 MAP kinase (p38MAPK) or extracellular signal-regulated kinase (ERK) pathways.[57] However, in cardiomyocytes, DiOHF reduced activation of p38MAPK after ischemia/reperfusion injury, without affecting the phosphorylation of ERK and protein kinase B (Akt).[67] Similarly in rats, quercetin reduced activation of p38MAPK in injured spinal cord tissue.[68] In addition to a reduction in p38MAPK activity, quercetin also decreased phosphorylation of ERK and Akt in the myocardium of rats with autoimmune myocarditis.[69] In another rodent study, quercetin prevented lead-induced hepatotoxicity, an effect that was mediated by activation of Akt and inhibition of lead-induced increases in JNK phosphorylation.[70] Kaempferol treatment also attenuated phosphorylation of ERK, p38MAPK, and JNK in human osteosarcoma[71] and epidermal cells.[72] Furthermore, although kaempferol had no effect on phosphorylation of the upstream regulators of MAP kinase, the Src kinase family, it did reduce Src kinase activity in a dorsal skin lysate.[72] Interestingly, this study showed that kaempferol directly competes with adenosine triphosphate binding to Src. This complements evidence from docking data, indicating kaempferol docks into the Src adenosine

triphosphate–binding site.[72] In a mouse model of lipopolysaccharide-induced acute lung injury, kaempferol treatment prevented lung inflammation and preserved function, an effect that was associated with reduced phosphorylation of ERK, p38MAPK, and JNK.[73]

In a sheep model of myocardial ischemia/reperfusion, DiOHF attenuated p38MAPK and JNK phosphorylation with no effects on ERK and Akt phosphorylation.[74] These findings[74] are consistent with observations in cardiomyocytes under oxidative and chemical stress, where DiOHF inhibited the JNK and p38MAPK pathways. Furthermore, DiOHF prevented stress-induced activation of the direct upstream regulators MKK4/7 (MAPK kinase 4/7) and MKK3/6.[74] Interestingly, this effect was selective for DiOHF s, under the same conditions, flavonol did not attenuate the JNK and p38MAPK pathways.[74] These findings suggest that modulation of p38MAPK and JNK signaling requires the 3′- and 4′-hydroxyl groups of the B ring to form a catechol group (Fig. 1). Using small molecule affinity purification, Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) was identified as a DiOHF target. It is important to note that under identical conditions, DiOHF inhibited CaMKII with a potency (IC^{50} 0.25 μM) superior to that of the well-established CaMKII inhibitor, KN-93 (IC_{50} 3.3 μM).[74] Thus, there is good evidence that modulation of p38MAPK and JNK signaling by DiOHF is mediated by inhibition of CaMKII.

In summary, the actions of flavonols on MAP kinase signaling and disease are diverse. This may be attributed to structural differences, duration, and doses used and the nature of the disease under investigation. Regardless of the underlying mechanisms, flavonol treatment often results in improved pathology.

FLAVONOLS AS NOVEL ANTI-DIABETIC AGENTS

Type I diabetes is associated with autoimmune destruction of insulin-producing β cells in the pancreas, leading to impaired insulin secretion and hyperglycemia. In contrast, insulin resistance is defined as reduced blood glucose clearance in response to a given amount of insulin. Insulin resistance is associated with progressive development of hyperglycemia and type II diabetes. Flavonols may be used to target pancreatic β-cell survival and function and thus also glucose homeostasis to prevent diabetes. The anti-diabetic properties and underlying mechanisms of flavonols in animal studies are summarized in Table 1.

Oxidative stress is recognized as an important contributing factor in the pathogenesis of diabetes, with death of pancreatic β-cells being one consequence.[102] Thus, oxidative stress is a potential target for antioxidant flavonols. Kaempferol caused a concentration-dependent improvement in cell viability, decreased caspase-3 activity, and prevented apoptosis in β-cells and human islets exposed to high glucose or palmitate.[75,76] Furthermore, kaempferol improved the survival and function of β-cells and human islets cultured in the presence of high glucose, leading to enhanced insulin secretion.[75,76] The proposed mechanism for these effects is by upregulation of pancreatic and duodenal homeobox 1 (PDX-1) expression. This leads to increased protein kinase A–dependent cyclic adenosine monophosphate levels, phosphorylation of cyclic adenosine monophosphate–responsive element binding protein, and upregulation of anti-apoptotic (*Akt* and *Bcl-2*) gene expression.[75,76] small interfering RNA (siRNA) knockdown of PDX-1 in β-cells abolished all these effects.[76] Interestingly, upregulation of PDX-1 was selective to kaempferol because other flavonols, including quercetin, had no effect on PDX-1 expression.[76] In contrast, a recent study showed that quercetin suppressed fructose-induced upregulation of PDX-1 expression in INS-1 β-cells and in the pancreas of fructose-fed rats.[77]

In other studies, quercetin potentiated glucose-induced insulin secretion in pancreatic β-cells.[78,79] In addition, quercetin protected β-cell function, viability, and oxidative damage induced by hydrogen peroxide[78] and inflammatory cytokines.[79] This was associated with reduced expression of iNOS, inhibition of NF-κB nuclear translocation, and suppressed cytochrome C release from mitochondria.[79] The beneficial effects of quercetin are proposed to be mediated by the extracellular regulatory kinase kinase[78] and/or the mitochondrial and NF-κB signaling pathways.[79]

Type II diabetes is associated with reduced muscle glucose disposal. Adenine monophosphate activated protein kinase (AMPK) may be a key modulator of whole-body glucose homeostasis. Thus, activators of AMPK, such as metformin, provide one approach to treatment of type II diabetes.[103] Indeed, in skeletal muscle C2C12 cells, quercetin and its metabolites stimulated AMPK phosphorylation, resulting in enhanced muscle cell glucose uptake.[80] This suggests that the action of quercetin is similar to that of metformin. In another study in C2C12 cells, quercetin attenuated the effects of TNF-α and enhanced basal and insulin-stimulated glucose uptake in a concentration-dependent manner.[81] This is associated with the activation of Akt and AMPK pathways, leading to the suppression of NF-κB signaling and iNOS expression.[81] Consistent with the studies in C2C12 cells, quercetin treatment also suppressed TNF-α and iNOS expression and increased glucose transporter 4 (GLUT4) expression and glucose uptake in insulin-resistant L6 myotubes.[82] Similarly, treatment with quercetin (30 mg/kg) for 10 weeks in *ob/ob* type II diabetic mice upregulated skeletal muscle GLUT4 expression, resulting in a reduced fasting blood glucose levels and improved whole-body insulin sensitivity.[82]

The anti-diabetic effect of quercetin in vivo was studied in a streptozotocin (STZ)-induced type I animal model of diabetes. Quercetin treatment (15 mg/kg intraperitoneal) was initiated 3 days before STZ injection and continued for 4 weeks. Treatment reduced blood glucose levels and increased serum insulin concentrations. This was associated with pancreatic reduction in markers of oxidative stress, increased antioxidant enzyme activity, and improved β-cell survival.[83] Similarly, in other studies, quercetin reduced blood glucose levels[84,85] and improved glucose tolerance in rodents with STZ-induced diabetes.[84] Quercetin treatment of diabetic animals also increased hepatic hexokinase and glucokinase activity and the number of pancreatic islets.[84] Furthermore, quercetin reduced lipid peroxidation and apoptosis in the livers of diabetic mice.[85] Analysis of hepatic and pancreatic tissue also revealed that quercetin had the greatest effect on

TABLE 1. Animal Studies of Flavonols for the Treatment and Prevention of Diabetes

Type of Flavonols	Animal or Cell Model	Glycemia Function and Key Findings	Proposed Mechanism of Action	References
Kaempferol	β-Cells	↑ insulin secretion	↑ PDX expression	75,76
	Human islets	↓ caspase-3 activity	↑ PKA phosphorylation	
		↑ cell viability	↑ cAMP levels	
			↑ CREB phosphorylation	
			↑ anti-apoptotic expression	
Quercetin	INS	↓ β-cell proliferation	↓ pancreatic PDX expression	77
	Fructose	↓ insulin hypersecretion	↓ pancreatic PKA phosphorylation	
		↓ serum insulin and leptin levels	↓ pancreatic FoxO1 expression	
		↓ insulin gene expression		
Quercetin	Pancreatic β-cells	↑ glucose-induced insulin secretion	↓ NF-κB activation	78,79
		↑ cell viability	↓ iNOS expression	
		↑ β-cell function	↑ ERK1/2 phosphorylation	
			↓ cytochrome c release	
Quercetin and its metabolites	C2C12 skeletal muscle cells	↑ basal and insulin-stimulated glucose uptake	↑ Akt phosphorylation	80,81
			↑ AMPK phosphorylation	
			↓ iNOS expression	
			↓ NF-κB activation	
Quercetin	L6 myotubes	↑ glucose uptake	↑ GLUT4 expression	82
			↓ iNOS expression	
			↓ TNF-α expression	
Quercetin	*ob/ob* type II diabetic mice	↓ fasting blood glucose levels	↑ skeletal muscle GLUT4 expression	82
		↑ whole-body insulin sensitivity	↓ NF-κB activation	
			↓ JNK phosphorylation	
Quercetin	STZ-induced diabetic rat	↓ serum blood glucose levels	↑ pancreatic antioxidant enzymes activities	83,84
		↑ serum insulin concentrations	↓ pancreatic oxidative stress markers	
		↑ β-cell survival	↑ hepatic glucokinase activity	
		↓ blood glucose levels	↑ pancreatic islets numbers	
		↑ glucose tolerance		
		↓ plasma cholesterol and triglycerides		
Quercetin	STZ-induced diabetic mice	↓ blood glucose levels	↓ hepatic and pancreatic Cdkn1a expression	85
		↑ plasma insulin levels	↓ hepatic lipid peroxidation	
			↓ hepatic apoptosis	
Fisetin	STZ-induced diabetic rat	↓ blood glucose levels	↑ hepatic glycogen content	86,87
		↑ plasma insulin levels	↑ hepatic glycogen synthase activity	
		↓ HbA1C	↑ hepatic antioxidant enzymes	
			↑ hepatic glutathione levels	
			↓ hepatic oxidative stress markers	
			↓ serum aminotransferases and alkaline phosphatase activities	
Fisetin	STZ-induced diabetic rat	↓ blood glucose levels	↓ plasma nitrite and IL-1β levels	88
		↑ plasma insulin levels	↓ pancreatic NF-kB p65 levels	
		↓ HbA1C	↑ pancreatic antioxidant enzymes	
		↑ β-cell survival	↓ pancreatic oxidative stress markers	

TABLE 1. (*Continued*) Animal Studies of Flavonols for the Treatment and Prevention of Diabetes

Type of Flavonols	Animal or Cell Model	Glycemia Function and Key Findings	Proposed Mechanism of Action	References
Quercetin	db/db mice	↓ blood glucose levels	↓ hepatic thiobarbituric acid reactive substances levels	89
		↓ insulin resistance	↑ hepatic SOD, GPx, and catalase activities	
		↑ plasma adiponectin levels		
		↔ insulin levels		
		↓ plasma cholesterol		
		↑ HDL levels		
Quercetin	Obese Zucker rats	↓ free fatty acid levels	↓ plasma nitrite levels	90
		↓ plasma insulin levels	↓ adipose tissue TNF-α production	
		↓ plasma cholesterol levels	↓ adipose tissue iNOS expression	
		↓ triglyceride levels	↑ adipose tissue eNOS expression	
		↑ plasma adiponectin levels		
Pentamethylquercetin	Neonatally STZ-induced diabetic rat	↓ postprandial glucose	Prevented the onset of overt diabetes	91
		↓ triglyceride levels		
		↓ glucose intolerance		
		↑insulin sensitivity		
Quercetin	HFD-induced insulin resistance	↓ fat mass	↑ skeletal muscle PGC1α expression	92
		↓ body weight		
		↑ whole-body insulin sensitivity		
Quercetin	HFD, high sucrose-induced insulin resistance	↔ adipose tissue mass	↔ skeletal muscle PGC1α expression	93
		↔ body weight	↔ skeletal muscle PPARγ expression	
		↓ glucose levels		
		↓ insulin levels		
		↑ whole-body insulin sensitivity		
Quercetin and kaempferol	Mature 3T3-L1 adipocytes	↑ insulin-stimulated glucose uptake	Partial agonist on PPARγ receptors	94
Pentamethylquercetin	Mature 3T3-L1 adipocytes	↑ adiponectin expression	↑ PPARγ gene and protein expression	95
		↓ TNF-α expression and secretion		
		↓ IL-6 expression and secretion		
Quercetin	STZ-nicotinamide-induced type II diabetic rat	↓ total cholesterol	Inhibitor of 11β-HSD1	96
		↓ glucose levels		
		↑ HDL levels		
		↓ LDL levels		
Quercetin	STZ-induced diabetic mice and rats, Akita mice	↔ blood glucose levels		97–101
DiOHF		↔ HbA1C		
Fisetin				

↑, increase; ↓, decrease; ↔, no effect; PKA, protein kinase A; cAMP, cyclic adenosine monophosphate; CREB, cAMP-responsive element binding protein; ERK1/2, extracellular signal-regulated kinase 1/2; GLUT4, glucose transporter 4; GPx, glutathione peroxidize.

suppression of STZ-induced expression of cyclin-dependent kinase inhibitor p21 (WAF1/Cip1) (Cdkn1a).[85] This suggests that quercetin reversal of diabetes-induced, ROS-related damage in the liver and pancreas may be partly because of suppression of Cdkn1a expression.

Another flavonol, fisetin, also possesses anti-diabetic activity. Oral administration of fisetin (10 mg/kg, 30 days) decreased blood glucose and glycosylated hemoglobin A1C levels and increased plasma insulin in STZ-induced diabetic rats.[86–88] Two mechanisms for the anti-diabetic action of fisetin have been proposed. First, that fisetin increases glycogen content and glycogen synthase activity while inhibiting glycogen phosphorylase in the liver.[86] Second, that fisetin protects hepatic and pancreatic function by increasing activity of antioxidant enzymes and decreasing oxidative stress.[91,92] Similarly, fisetin reduced circulating IL-1β, nitrite, and NF-kB levels in

the diabetic pancreas, leading to improved β-cell survival,[88] possibly by antioxidant, anti-inflammatory pathways.

In type II diabetic animal models, chronic quercetin treatment decreased plasma glucose and triglyceride levels and reduced dyslipidemia and insulin resistance.[89,90] Similarly, pentamethylquercetin treatment for 10 weeks dose-dependently reduced postprandial glucose and triglyceride levels, preventing onset of overt diabetes by attenuating glucose intolerance and enhancing insulin sensitivity.[91] Four weeks of treatment with quercetin in fructose-fed rats decreased body weight, fasting serum glucose, insulin and leptin levels, and reduced islet size and mass.[77] Furthermore, quercetin downregulated fructose-induced upregulation of Akt and forkhead box protein O1 (FoxO1) phosphorylation and suppressed the expression of the PDX-1 and insulin genes (Ins1 and Ins2) in islets.[77] Thus, quercetin may prevent compensatory β-cell hyperplasia by decreasing pancreatic Akt/FoxO1 activation and FoxO1 nuclear translocation.[77]

In high-fat diet–induced insulin resistance, a low (50 μg/d), but not high (600 μg/d) dose of quercetin for 8 weeks attenuated increases in fat mass, body weight, and also improved whole-body insulin sensitivity.[92] Similarly, a low, but not high, dose of quercetin increased the expression of peroxisome proliferator-agonist receptor gamma (PPARγ) coactivator 1 alpha (PGC1α) in skeletal muscle.[92] These findings suggest that the effects of quercetin, at least in a high-fat diet–induced insulin resistance model, are not dose dependent. In contrast, a recent study demonstrated that quercetin (30 mg/kg) for 6 weeks reduced basal and insulin-induced glucose uptake but had no effect on skeletal muscle PGC1α expression.[93] The discrepancies between studies could be due either to the duration or the dose of quercetin used.

Identified Molecular Targets of Flavonols to Reduce Hyperglycemia

A current approach to the treatment of type II diabetes is the use of agonists of peroxisome proliferator-agonist receptor gamma, PPARγ. These drugs are commonly referred to as the "glitazones". Kaempferol and quercetin were weak, partial agonists in PPARγ reporter assays.[94] Competitive ligand-binding assays confirmed that kaempferol and quercetin could bind to the same pocket as rosiglitazone. Kaempferol and quercetin improved insulin-stimulated glucose uptake in mature 3T3-L1 adipocytes,[94] suggesting that they may reduce hyperglycemia by acting on PPARγ receptors. In support of this concept, pentamethylquercetin increased adiponectin and PPARγ mRNA and protein expression in 3T3-L1 adipocytes.[95] Pentamethylquercetin-induced upregulation of adiponectin expression is dependent on the PPARγ pathway because blockade of PPARγ by GW9662 eliminated that effect.[95]

11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) is recognized as a novel therapeutic target for the treatment of type II diabetes.[104] Investigation of the structure–activity relationship of flavonoid analogs showed that treatment with either quercetin, flavones, or chrysin in the type II diabetes, STZ-nicotinamide rat model significantly reduced total cholesterol, glucose, triglyceride, and low-density lipoprotein while increasing high-density lipoprotein levels. In contrast, treatment with 3-hydroxyflavone, 6-hydroxyflavone, or 7-hydroxyflavone was not effective. Molecular modeling studies using the most effective flavonoid, quercetin, showed that it docked into the crystal structure of 11β-HSD1, supporting inhibition of 11β-HSD1 as a potential mechanism of its diabetic activity.[96]

Effect of Flavonols in Human Type I and Type II Diabetes

Despite experimental evidence supporting the potential use of flavonols as anti-diabetic agents, there are no human studies on flavonol intake as a therapeutic approach to treat type I diabetes. Furthermore, not all experimental studies in type I and II diabetes showed that flavonols reduced blood glucose levels.[97–101] In addition, there are 2 conflicting epidemiological studies. In a Finish population, a trend ($P = 0.07$) toward reduced type II diabetes risk was associated with higher quercetin and myricetin intake.[105] In a subsequent larger, multi-national, multi-center epidemiological study of European populations, higher dietary flavonol intake was also associated with a lower incidence of type II diabetes.[106–108] In contrast, other studies failed to establish any relationship between the intake of flavonols and flavones with the risk of type II diabetes or insulin resistance in women.[109,110] However, it is interesting to note that in the latter study, the intake of apples and tea, both good sources of flavonols, was modestly and inversely associated with the risk of type II diabetes and insulin resistance.[110] The only published controlled clinical trial involving diabetic patients of whom we are aware demonstrated that quercetin treatment for 10 weeks significantly decreased systolic blood pressure in diabetic patients.[111] Unfortunately, there was no measure of plasma glucose or insulin sensitivity and the number of subjects was low (n = 62), leaving much scope for further investigation.

FLAVONOLS AS NOVEL PHARMACOLOGICAL AGENTS TO PREVENT DIABETES-INDUCED VASCULAR DYSFUNCTION

Endothelial Dysfunction in Diabetes

The vascular endothelium is a single layer of cells lining the interior surface of blood vessels. It serves as a barrier between blood and tissues, and importantly, it actively contributes to the regulation of vascular tone. Endothelial dysfunction is broadly defined as impaired capacity of a blood vessel to relax in response to an endothelium-dependent vasodilator, such as acetylcholine (ACh) or bradykinin, or to flow-mediated vasodilatation.[11,112] Endothelial dysfunction is recognized as an early and independent predictor of poor prognosis in cardiovascular disease, including diabetes-induced macrovascular and microvascular dysfunction.[11,112] Besides the diminished production and activity of NO, prostacylin, and endothelium-dependent hyperpolarization (EDH), there are also changes in the expression of adhesion molecules, proinflammatory molecules, and overproduction of vasoconstrictors, including endothelin-1, ROS, and prostaglandins. For instance, in

diabetic arteries, reduced endothelium-dependent relaxation is often accompanied by increased expression of proinflammatory molecules and ROS production.[113–117] The mechanisms underlying vascular dysfunction in diabetes have been extensively reviewed,[11,112,118–120] so we will now focus on the potential for flavonols to be used as novel treatments for diabetic vascular dysfunction.

Vascular Actions of Flavonols in Diabetes

In earlier sections of this review, the ability of flavonols to protect pancreatic β-cell function and to improve glucose homeostasis was discussed. However, tight glycemic control does not always prevent progression of diabetic vascular complications.[7] Flavonols exert actions, other than those on glucose or insulin levels, that may affect vascular function. The roles of flavonols in preventing diabetic-induced vascular dysfunction are summarized in Table 2.

The acute effect of quercetin on vascular reactivity in diabetic thoracic aorta was studied by Ajay et al.[121] They reported increased ACh-evoked relaxation and decreased phenylephrine (PE)-induced contraction in diabetic aortae. Oral treatment with quercetin (10 mg/kg) for 6 weeks restored impaired ACh-induced relaxation and PE-induced contraction in STZ-induced diabetic rats.[122] In addition, quercetin treatment reduced plasma markers of oxidative stress, malonaldehyde and 4-hydroxyalkenal content, and increasing SOD activity and total antioxidant capacity in diabetic rats.[122] Similarly, in 2 different rat diabetic models, STZ- and fructose-induced insulin resistance, 6 weeks of quercetin (50 mg/kg) treatment also attenuated thoracic aorta contractile responses to PE and KCl.[100] In addition, quercetin inhibited diabetes-associated adventitial leukocyte infiltration and increased collagen deposition.[100] These effects were accompanied by a reduction in serum TNF-α and CRP and inhibition of aortic NF-κB.[100] In cell culture studies, quercetin (100 μM) attenuated high glucose (25 mM)–induced MCP-1 gene expression and protein synthesis and ROS production in rat aortic endothelial cells.[123] Quercetin treatment also decreased cytosolic p65 protein expression and prevented high glucose-induced p65 and c-jun nuclear localization. Furthermore, quercetin attenuated high glucose–induced activation of NF-κB and AP-1 activity in rat aortic endothelial cells.[123] It was proposed that quercetin attenuates MCP-1 expression, probably by regulating both NF-κB and AP-1.[123] In another study, metabolites of quercetin, sulfate, and glucuronide prevented high glucose–induced apoptosis of human umbilical vein endothelial cells.[124] Specifically, the quercetin metabolites reduced ROS production and attenuated high glucose–induced increases in JNK activity and protein expression.[124] Quercetin metabolites also reduced caspase-3 activity and apoptosis in human umbilical vein endothelial cells exposed to high glucose.[124]

Beside quercetin, exposure to the synthetic flavonol DiOHF ex vivo caused a concentration-dependent reduction in superoxides in aortae from normal and diabetic rats.[99] In the presence of DiOHF (100 μM), ACh-induced relaxation was improved in the diabetic aorta.[99] Interestingly, the presence of SOD did not improve ACh-induced relaxation in the diabetic aorta,[99] suggesting, at least with acute exposure, that DiOHF more effectively preserves endothelial function. This might relate to the high lipid solubility of flavonols favoring absorption into cells. In the same study, the in vivo effects of DiOHF were investigated in STZ-induced diabetic rats. Short-term DiOHF (5 mg/kg) treatment for 7 days reduced aortic superoxide levels in the diabetic rats.[99] This was associated with improved endothelium-dependent relaxation.[99]

The beneficial effects of flavonols in diabetic conduit arteries seem to involve antioxidant and anti-inflammatory actions and preservation of NO bioavailability. Less is known about the effect of flavonols in diabetic resistance arteries, where EDH is an important contributor to endothelium-dependent vasodilator function.[113] Actions of DiOHF were investigated in mesenteric arteries from type I and type II diabetic rats. The acute presence of DiOHF (10 μM) ex vivo reduced superoxide levels and improved endothelium-dependent relaxation in the mesenteric arteries of STZ-treated and obese Zucker rats.[125] Short-term DiOHF (1 mg/kg) treatment for 7 days also reduced vascular superoxide levels. This was associated with a reduction in NAPDH oxidase (Nox2) protein levels in the diabetic mesenteric arteries. Furthermore, DiOHF treatment improved ACh-mediated relaxation and attenuated enhanced contractile responses to endothelin-1 in the diabetic mesenteric arteries.[97] Overall improvement of endothelial function in DiOHF-treated diabetic rats is mediated by reversal of eNOS uncoupling, leading to enhanced NOS activity and improved NO-mediated relaxation.[97] DiOHF treatment had no significant effect on EDH responses in diabetic mesenteric arteries.[97]

A further benefit of flavonols might be as antithrombotic agents. Previous studies demonstrated flavonols inhibited platelet aggregation and reduced thrombus formation.[126,127] A recent study investigated the in vivo effects of quercetin and DiOHF in STZ-induced type I diabetic mice.[98] In response to protease-activated receptor-4 platelet stimulation, treatment with quercetin (6 mg/kg) or DiOHF (6 mg/kg) for 7 days reduced diabetes-induced platelet aggregation.[98] Treatment with quercetin or DiOHF also inhibited dense, but not alpha, granule exocytosis in diabetic and control mice. In addition, flavonol-treated mice displayed improved carotid artery blood flow compared with vehicle-treated diabetic mice.[98]

In summary, the vasoprotective effects of flavonols in diabetes seem to be largely related to their antioxidant properties because in most cases flavonol treatment reduces oxidative stress (Table 2). This is likely achieved either by direct scavenging or by inhibition of ROS production as discussed earlier. The consequence of reduced oxidative stress is to increase NO bioavailability, thereby promoting vasorelaxation, anti-platelet aggregation, and reduced inflammation in the diabetic vasculature.

CONCLUSIONS

Flavonols have a broad range of biological actions, including vasorelaxant, antioxidant, and anti-inflammatory effects, which are linked to inhibition of a range of kinases. These actions may account for the strong positive correlation between a high dietary intake of flavonols and reduced risk of

TABLE 2. Flavonols in the Prevention of Diabetes-induced Vascular Dysfunction

Type of Flavonols	Animal or Cell Model	Route of Administration, Dose, and Treatment Duration	Vascular Bed or Cells	Key Findings	Proposed Mechanism of Action	References
Quercetin	STZ-induced diabetic rats	Ex vivo incubation, 10 μM, 20 min	Thoracic aorta	↑ ACh ↓ PE ↔ SNP	↑ NO bioavailability	121
Quercetin	STZ-induced diabetic rats	Oral, 10 mg/kg, 6 wk	Thoracic aorta	↑ ACh-induced relaxation ↓ PE-induced contraction ↓ plasma MDA and 4-HNE ↑ SOD and total antioxidant activity	↑ NO bioavailability ↓ ROS production	122
Quercetin	STZ-induced diabetic rats and fructose-induced insulin resistance	Oral, 50 mg/kg, 6 wk	Thoracic aorta	↓ PE-induced contraction ↓ KCl-induced contraction ↓ leukocyte infiltration ↓ collagen deposition ↓ serum TNF-α and CRP ↓ NF-κB activation	↓ vascular inflammation	100
Quercetin	High glucose (25 mM)	*In vitro* incubation, 100 μM	RAECs	↓ MCP1 and p65 expression ↓ p65 and c-jun nuclear translocation ↓ NF-κB and AP1 activation ↓ ROS production	↓ ROS production ↓inflammation	123
Quercetin sulfate/ glucuronide	High glucose (33 mM)	In vitro incubation, 100 nM, 300 nM, 1 μM	HUVECs	↓ ROS production ↓ JNK activity and expression ↓ caspase-3 activity	↓ ROS production ↓ apoptosis	124
DiOHF	STZ-induced diabetic rats	Ex vivo incubation, 100 μM, 20 min Ip, 5 mg/kg, 1 wk	Thoracic aorta	↑ ACh-induced relaxation ↓ superoxide production ↔ SNP-induced relaxation	↓ ROS production	99
DiOHF	STZ-induced diabetic rats and Obese Zucker rats	Ex vivo incubation, 10 μM, 20 min	Mesenteric arteries	↑ ACh-induced relaxation ↓ superoxide production	↓ ROS production	125
DiOHF	STZ-induced diabetic rats	Sc, 1 mg/kg, 1 wk	Mesenteric arteries	↑ ACh-induced relaxation ↓ ET-1–induced contraction ↔ SNP-induced relaxation ↓ superoxide and ROS production ↓Nox2 expression and eNOS uncoupling	↑ NO bioavailability ↓ ROS production	97

TABLE 2. (*Continued*) Flavonols in the Prevention of Diabetes-induced Vascular Dysfunction

Type of Flavonols	Animal or Cell Model	Route of Administration, Dose, and Treatment Duration	Vascular Bed or Cells	Key Findings	Proposed Mechanism of Action	References
				↑ NO activity ↔ EDH-type relaxation		
Quercetin and DiOHF	STZ-induced diabetic mice	Ip, 6 mg/kg, 1 wk	Carotid artery and platelets	↑ carotid artery blood flow ↓ platelet aggregation	↓ thrombus formation	98

↑, increase; ↓, decrease; ↔, no effect; AP1, adapter protein 1; ET-1, adapter protein 1; CRP, C-reactive protein; HUVECs, human umbilical vein endothelial cells; 4-HNE, 4-hydroxyalkenal; ip, intraperitoneal; MCP1, monocyte chemoattractant protein 1; MDA, malonaldehyde; RAECs, rat aortic endothelial cells; sc, subcutaneous; SNP, sodium nitroprusside.

cardiovascular diseases. The precise mechanism(s) underlying the effects of flavonols on diabetes and associated vascular dysfunction is an area of particular interest. There is evidence that flavonols may improve pancreatic β-cell survival and function and increase glucose uptake and insulin sensitivity in skeletal muscle, liver, and adipocytes, leading to an overall improvement in glycemic status. However, epidemiological findings are limited in type I diabetics and inconsistent for type II diabetes. Regardless of these inconsistent findings, phase II clinical trial to evaluate the effects of quercetin on glucose levels and vascular function in type II diabetes are currently under development (www.clinicaltrials.gov). In addition to hypoglycemic action, flavonols may preserve endothelial function, predominantly through augmenting NO activity and thereby reducing diabetic vascular complications. Experimental studies indicate that flavonols are vasoprotective and have potential, in combination with standard therapy, as adjunctive treatments for diabetic vascular disease.

Flavonols have the advantage that as small, lipid-soluble molecules they can readily enter cells and exert their biological actions. Equally, this property makes it challenging for flavonols to be translated into clinical studies because their low aqueous solubility makes formulation for human use difficult. Furthermore, because of multiple biological actions, it is hard to achieve specificity and there is potential for unwanted side effects. For example, to achieve pharmacologically active concentrations (micromolar) of flavonols to exert their antioxidant activity in vivo, the possibility of hypotension arises given the vasorelaxant effect that may also occur at this concentration. Fortunately, water-soluble flavonol analogs retaining the biological activities of natural compounds have been synthesized.[24,36,39,67] Furthermore, a water-soluble flavonol with anti-oxidant but not vasorelaxant activity has been synthesized, thus limiting potential side effects attributed to natural flavonols. A greater understanding of the cellular actions of these water-soluble flavonols will reveal whether they can be developed as therapies for diabetes and its associated vascular complications.

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