Flavonoids and Cardiovascular Disease

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Abstract

Diets high in flavonoids have long been associated with nutritional recommendations, a healthy lifestyle, and the prevention of chronic diseases. However, identification of specific beneficial effects from specific flavonoids and flavonoid-rich foods has been a challenging area, probably due to a nonessential or conditional role for flavonoids in human nutrition. Nonetheless, recent efforts in the area of high flavonoid–containing foods and cardiovascular disease have begun providing the first demonstrations of specific effects and mechanisms of action in well-controlled studies. The early studies have shown that flavonoids have several anti-atherosclerotic activities including anti-inflammatory, antioxidant, anti-proliferative, antiplatelet, and provessel function activities. Cholesterol-lowering and antihypertensive effects of flavonoids have been studied and appear minimal in humans. The studies also demonstrate several possible mechanisms and pleiotropic effects of flavonoids that may be active in reduction in the risk of cardiovascular disease. Several subclasses of flavonoids may contribute toward the apparent beneficial effects and include flavones, flavonols, flavanones, catechins isoflavones, proanthocyanidins, and anthocyanidins. Further studies are necessary for confirmation of the beneficial effects, identification of dose-response relationships, and identification the most bioactive flavonoids.

Keywords: Anti-inflammation, antioxidant, atherosclerosis, bioactivity, cardiovascular disease, cell proliferation, flavonoids, platelets, vessel function.

Introduction

In recent years, flavonoids have been recognized as compounds with potent biological activities that may be active in the prevention of chronic diseases including cardiovascular disease. Significant dietary intakes of flavonoids occur with fruit and vegetable intakes, especially apples, onions, and certain beverages including tea and cocoa. Their antioxidant activity is well established, and epidemiologic studies have suggested associations between flavonoid intake and a lower risk of cardiovascular disease. Flavonoids may prevent oxidative damage and the oxidation of low-density lipoproteins (LDL), but importantly, several additional possible activities have been studied in cellular, experimental animal, and human experiments. Flavonoids may have anti-inflammatory, cholesterol-lowering, antihypertensive and antiplatelet activities. These compounds also may inhibit smooth muscle cell proliferation and migration and improve vessel function. Recent findings in each of these major areas of biological activity are reviewed herein, and further research needs are identified. The studies described herein include those evaluating the effects of flavonoids from all major subclasses (Fig. 1) with the exception of isoflavones, which have been the subject of several recent reviews (Knight & Eden, 1996; Anthony et al., 1998; St Clair, 1998; Tham et al., 1998; Setchell & Cassidy, 1999; Lissin & Cooke, 2000), and the compounds that may be classified as proanthocyanidins, which are described in Chapter 1 of this issue. Thus, the effects of the following major subclasses of flavonoids are described herein: flavones, flavonols, flavanones, catechins, and anthocyanidins.

Anti-inflammatory activities

A major hypothesis focuses on inflammation as a fundamental cause in the pathogenesis of coronary heart disease. Flavonoids may have anti-inflammatory activities that have been demonstrated in cellular systems and in animal models. Flavonoids may inhibit the production of pro-inflammatory cytokines, including interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interleukin-12 (IL-12), and may inhibit the expression of adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), on endothelial cells. Flavonoids may also inhibit the expression of matrix metalloproteinases (MMPs), which are important in the degradation of extracellular matrix proteins and contribute to the progression of atherosclerosis.

Keywords: Anti-inflammatory, antioxidant, atherosclerosis, bioactivity, cardiovascular disease, cell proliferation, flavonoids, platelets, vessel function.
The inflammatory response encompasses a wide range of activities including an increase in oxidative stress, an increase in capillary permeability, accumulation of white blood cells, release of cytokines (interleukins, TNF), induction of various enzyme activities (oxygenases, nitric oxide synthetase, peroxidases), and induction of arachidonic acid metabolism and cellular adhesion molecules, such as intercellular adhesion molecule (ICAM) and vascular cell adhesion molecule (VCAM). These activities may be diminished by flavonoids, which have a wide range of anti-inflammatory activities. The anti-inflammatory activities of flavonoids have been demonstrated in numerous cellular and animal models; however, human studies generally are lacking in this area. The various anti-inflammatory activities of flavonoids and some limitations of research in this area are described herein.

The most commonly occurring flavonoid, quercetin, and its conjugate, rutin, has demonstrated significant anti-inflammatory activity in several systems, including the rat paw system and neutrophils. In these systems, rutin suppresses the inflammatory reaction, most obviously indicated by a reduction in edema and a reduction in the chemotaxis of neutrophils (Selloum et al., 2003). Intraperitoneal administration of rutin and quercetin reduce both acute and chronic inflammation in a rat model (Guardia et al., 2001). Lipopolysaccharide (LPS)-induced inflammatory reactions are associated with an elevated level of NO release, which may lead to an increase in oxidative stress. Quercetin, rutin, and wogonin inhibit this release through suppression of inducible NO synthase (iNOS) expression (Shen et al., 2002). They also suppress cyclooxygenase-2 (COX-2) gene expression and prostaglandin E2 formation (Chen et al., 2001). Thus, these flavonoids have potent anti-inflammatory activities that influence several inflammatory responses.

Specific types of oxidative stress are associated with inflammatory responses and may be reduced by flavonoids. Inflammation produces hypochlorous acid, a strong oxidant, via myeloperoxidase activity. The common dietary flavonols quercetin and rutin become chlorinated in the presence of HOCl and may prevent the oxidative damage associated with exposure to HOCl (Binsack et al., 2001). An overproduction of nitric oxide has been associated with inflammatory responses. The flavones apigenin and luteolin are potent inhibitors of nitric oxide formation by activated macrophages (Scuro et al., 2004), a major cellular component involved in atherosclerosis. These specific examples illustrate the types of antioxidant activity that various flavonoids may have in the prevention of oxidative damage associated with inflammatory responses.

Specific anti-inflammatory activities of individual flavonoids include the following. Nobiletin has shown a number of anti-inflammatory activities in cell systems. These include suppression of cyclooxygenases, prostaglandin E2, and several proinflammatory cytokines including interleukin (IL)-1α, IL-1β, tumor necrosis factor (TNF)-α, and IL-6 (Lin et al., 2003). In addition, nobelitin suppresses the production of promatrix metalloproteinase (Ishiwa et al., 2000; Sato et al., 2002), a factor associated with vascular plaque stability. Luteolin is identified as the active anti-inflammatory factor in *Perilla frutescens* (L.) Briton var. acuta kydoformaviridis makino. In a mouse model, it inhibits TNF-α activity, arachidonic acid–induced and 12-O-Tetradecanoylphorbol-13-acetate (TPA)-induced ear edema, and oxazolone-induced allergic edema (Ueda et al., 2002). The expression of TNF-α and
intercellular adhesion molecule-1 (ICAM-1) in lipopolysaccharide-treated mice is reduced by luteolin treatment. The treatment also reduces lung and liver infiltration by leukocytes (Kotanidou et al., 2002). Similar results also were found in an earlier study (Xagorari et al., 2001). Luteolin effectively inhibited asthma-like responses in several studies (Park et al., 1999; Kimata et al., 2000a, 2000b), and pathways for this inhibitory activity have been identified (Xagorari et al., 2001, 2002). The activities of several inflammatory factors are diminished and include phospholipase A2, platelet cyclooxygenase, platelet thromboxane A2, 5′-lipoxygenase, and leukotriene B4. The thrombocyte synthesis index, the prostaglandin (PG) E2/thromboxane A2 ratio, is increased by tea catechins. Myricetin has anti-inflammatory activity in the rat paw model of inflammation (Hiermnn et al., 1998). Its activity may involve inhibition of COX-1, COX-2, and 5′-lipoxygenase (5-LOX).

Blackberry extracts contain anthocyanins, primarily cyanidin-3-O-glucoside, which may have anti-inflammatory activity. In a lung acute inflammation model (carrageenan-induced acute inflammation), the blackberry extracts reduce fluid accumulation, neutrophil accumulation, polymorphonuclear leukocyte accumulation, and lipid peroxidation. Nitrite/nitrate (products of NO production) and prostaglandin E2 levels also are reduced by the extract (Rossi et al., 2003). Purified anthocyanins, malvidin and cyanidin, and galloyl derivatives of catechins have shown COX inhibitory activities (Seeram et al., 2003). Thus, anthocyanins in blackberry extracts are active agents and have anti-inflammatory activities. The blackberry extract may affect several pathways, and its multifaceted actions may be the basis for its potent inhibitory activity. Importantly, the anthocyanin effect may be unique. Anthocyanins induce TNF-α production in a cellular system, RAW264.7 macrophages, which are activated by LPS/interferon-γ. This is in contrast to several other flavonoids that inhibit TNF-α production. Thus, anti-inflammatory effects of anthocyanins may use mechanisms that differ from those of other flavonoids.

Mediterranean diets have a high content of flavonoids, in particular, resveratrol. Resveratrol inhibited expression of the vascular cell adhesion molecule-1 (VCAM-1) in response to bacterial LPS (Carluccio et al., 2003). The activities of resveratrol and other flavonoids in the Mediterranean diet suggest that this diet may have an anti-inflammatory effect.

Antibacterial activities have been demonstrated for quercetin, quercitrin, and morin. Moreover, synergy is demonstrated between these flavonoids and rutin in their antibacterial activity (Arima et al., 2002). Flavonoids have antibacterial activities against several antibiotic-resistant bacterial strains. Myricetin, datiscetin, kaempferol, quercetin, flavone, and luteolin inhibit methicillin-resistant Staphylococcus aureus. Myricetin may have a broad spectrum of activity as it inhibits several additional antibiotic-resistant organisms (Xu & Lee, 2001). Thus, flavonoids may be active in the reduction of bacteria infections and the associated inflammatory response.

In summary, several flavonoids have demonstrated potent anti-inflammatory activities and may reduce inflammation that results from a variety of causes including antibiotic-resistant bacteria. These flavonoids inhibit several key steps in inflammation and have been used as anti-inflammatory agents in some instances. Anti-inflammatory activity may be a common property of flavonoids as well as being found in compounds from several flavonoid subclasses. Thus, total flavonoid exposure may be more important than exposures to specific flavonoid compounds for the prevention of inflammation. It is possible that the anti-inflammatory activity of flavonoids influences the inflammation associated with atherosclerosis and is a promising area of investigation. Several types of information are essential for progress in this area of investigation. In particular, human studies and dose-response studies are lacking. It is unknown whether typical flavonoid intakes influence the low levels of inflammation that now have been associated with an increased risk of coronary heart disease (Libby & Ridker, 2004; Ridker et al., 2004; Willerson & Ridker, 2004). Also, the influence of human flavonoid metabolism on anti-inflammatory activities remains unknown. Fundamental studies in these areas may provide an understanding of the role of flavonoids in the prevention of coronary heart disease. Thus, the influence of flavonoids on inflammation in humans should receive additional attention and remain an active area of investigation.

Vascular smooth muscle cells

The proliferation of vascular smooth muscle cells is a consistent feature in the development of atherosclerotic plaque. Apigenin is a potent inhibitor of growth factor–induced rat aortic vascular smooth muscle cell growth (Kim et al., 2002). A major flavonoid constituent of tea, epigallocatechin gallate (EGCG), is a potent inhibitor of rat aortic smooth muscle cells. This activity is mediated by Ras/JNK and the downregulation of c-jun (Hwang et al., 2002). Red wine polyphenols inhibit the migration of vascular smooth muscle cells. The inhibition occurs through two pathways, the PI3K activity and p38 (MAPK) pathways (Iijima et al., 2002). Thus, flavonoids may be a significant inhibitor of vascular smooth muscle growth and migration. Further studies are necessary for confirmation of the effect and evaluation in additional models.
Antioxidant activity

Flavonoids are potent antioxidants that may affect initial steps in the development of atherosclerosis through the prevention of LDL oxidation, blockage of LDL uptake by macrophages, and prevention of foam cell formation. In support of these possible activities, flavonoids prevent atherosclerosis in an animal model (Hayek et al., 1997). Apolipoprotein E–deficient mice develop atherosclerotic lesions that are readily measured by 10 weeks of age. These lesions are prevented by the consumption of red wine and catechin- and quercetin-supplemented water for 6 weeks. Susceptibility to oxidation also was reduced in the mice receiving red wine or the flavonoid-supplemented water. In addition, various flavonoids display several antioxidant activities that are relevant to the prevention of atherosclerosis. For example, the isoflavone genistein has demonstrated antioxidant activities in cell-free and cell-mediated systems. These include the prevention of LDL oxidation and protection of vascular cells against oxidized LDL particles (Kapiotis et al., 1997). Nobiletin has extensive antioxidant activities. It suppresses the formation of oxidants by three systems including the xanthine oxidase system, TPA-induced oxidative stress, and NO (nitric oxide) generation by the RAW264.7 cell line (Murakami et al., 2000a, 2000b). These are just a few examples of activities that are found in a wide range of flavonoids.

The antioxidant activity of flavonoids may occur through several mechanisms including scavenging of reactive oxygen/nitrogen species, chelation of metals, inhibition of propagation reactions in lipid peroxidation, and sparing of LDL-associated antioxidants (Fuhrman & Aviram, 2001; Mira et al., 2002). Their antioxidant activity also may occur through the inhibition of cellular oxygenases and the enhancement of cellular antioxidants. Detailed studies have identified structural requirements for the antioxidant activity of flavonoids. These involve primarily the 4-oxo group of the C-ring, hydroxyl groups at positions 5 and 3', 4' and 5', and the double bond between positions 2 and 3 of flavonoids (see Fig. 2 for position numbers). Recent studies have evaluated several derivatives of flavonoids and found additional structural features that influence antioxidant activity, such as an effect of alkylation at position 7 on antioxidant activity (Kessler et al., 2003). These studies also identified possible pro-oxidant activities of flavonoids, which appeared to be dependent on the presence of a free hydroxyl group at position 3. Thus, the major structural requirements and mechanisms are known for many of the antioxidant activities of flavonoids.

Although flavonoids clearly have antioxidant activity from a purely chemical standpoint, a major question is do they act as antioxidants or pro-oxidants in biological systems and, if so, under what conditions? A description of recent in vitro and in vivo studies of antioxidant activities is given below for specific flavonoids and flavonoid-rich foods.

The antioxidant activity of luteolin-7-glucoside has been investigated in an in vivo system, which involved the use carbon tetrachloride, a well-known oxidizing agent, and an evaluation of liver damage. Luteolin suppresses several indicators of liver and oxidative damage including serum levels of glutamic pyruvic transaminase and glutamic oxaloacetic transaminase. Liver malondialdehyde and 8-hydroxydeoxyguanosine levels are lower in the luteolin-treated as compared with controls. Glutathione is elevated in the luteolin-treated group compared with the control group (Qiusheng et al., 2004). Each of these results indicates that luteolin is a potent antioxidant in vivo.

Quercetin and its derivatives can prevent oxidative damage in a variety of systems, including those with apparent relevance to atherosclerosis. The flavonols myricetin and quercetin prevent lipid peroxidation and may be active in the regenerations of α-tocopherol (Gordon & Roedig-Penman, 1998; Morel et al., 1998). Synergistic

Figure 2. Basic structure and position numbers of flavonoids.
activities of rutin, a derivative of quercetin, with the anti-
oxidants ascorbic acid and γ-terpinene were found in the
inhibition of LDL oxidation (Milde et al., 2004). Radi-
olytic oxidation of apolipoprotein B in LDL particles
can be repaired by quercetin, but not rutin. Quercetin
bonds effectively with LDL particles and through intra-
molecular electron transfer repairs phenoxyl radicals
(Filipe et al., 2002a, 2000b). Oxidative damage from
UV radiation and methyl violagen can be prevented by
rutin (Palmer et al., 2002). Neutrophils are significant
sources of reactive oxygen species that may be overex-
pressed in immune responses and cause tissue damage.
This response can be inhibited by two flavonoids, trihy-
droxyethylrutin and disodium flavodate (Wenisch &
Biffignandi, 2001). Rutin was an effective inhibitor of
free-radical production by monocytes and neutrophils
from rheumatoid arthritis patients (Ostrakhovitch &
Afanas’ev, 2001). Thus, quercetin or its derivatives can
inhibit oxidation in several of the major components that
are involved in atherosclerosis. In addition, antioxidant
status may be improved by the intake of quercetin or
its derivatives. The intake of rutin enhances the antioxi-
dant status of mouse liver tissue and Mn-superoxide dis-
mutase activity (Gao et al., 2002). Higher amounts
decrease catalase activity and reduce the concentration
of antioxidant minerals including iron, zinc, and copper.
These later responses of antioxidant enzymes and miner-
als may reflect a decreased need for their activity as a
result of improved antioxidant status.

Antioxidant concentrations and oxidative damage
indicators have been evaluated in a human study of rutin
supplementation. The consumption of 500 mg of rutin
per day increased plasma levels of quercetin, kaempferol,
andisorhamnetin. It did not change the levels of oxidized
lymphocyte DNA, urinary malondialdehyde, oxidized
DNA, and F2-isoprostanes. Thus, rutin remains unpro-
ven as an in vivo antioxidant. Nonetheless, an important
result was that no adverse changes were found in blood
chemistries of the subjects (Boyle et al., 2000).

The antioxidant activity of common anthocyanidins
and their aglycones has been evaluated in several lipid
environments, including LDL particles, and bulk and
eмуsified methyl linolate. The anthocyanidins are
strong antioxidants and effectively prevent oxidative
damage in these environments (Kakhkonen & Heinonen,
2003). Structure-activity relationships for the antioxidant
activity have been identified for many of the commonly
consumed anthocyanidins and anthocyanins (Seeram &
Nair, 2002). Galloylation is an important determinant
of antioxidant activity in anthocyanidins (Plumb et al.,
1998).

The anthocyanins demonstrate antioxidant activity
in several cell and animal systems as well as in humans.
Anthocyanins inhibit NO formation in LPS/interferon-
γ-activated RAW264.7 macrophages (Wang & Mazza,
2002), and the mechanism of antioxidant activity has
been described (Tsuda et al., 2000b). Anthocyanins
(elderberry extracts) protect endothelial cells against
several oxidative stressors, including hydrogen peroxide,
AAPH (2,2′-azobis(2-aminopropane), and Fe/ascorbic
acid (Youndim et al., 2000). The anthocyanidin cyanidin 3-
O-beta-D-glucoside was an effective antioxidant in the
hepatic ischemia-reperfusion rat model. It suppressed
the formation of thiobarbituric acid–reactive substances
and spared reduced glutathione (Tsuda et al., 2000a).
Acylated anthocyanins reduce oxidative stress in the rat
model of paraquat-induced oxidative damage (Igarashi
et al., 2000). Notably, anthocyanin-rich diets did not
influence cholesterol or fatty acid patterns in the livers
of rats. The rats fed black currant and elderberries did
appear to spare vitamin E. Similar results are found for
cyanidin-3-O-glucoside (Frank et al., 2002). Postprandial
oxidative stress is reduced in human subjects by the con-
sumption of a proanthocyanidin-rich grape seed extract
(Kakhkonen & Heinonen, 2003; Natella et al., 2002).
Consumption of blueberries increased Oxygen Radical
Absorption Capacity (ORAC) activity in the blood of
human subjects (Mazza et al., 2002). These studies sug-
stect that anthocyanins act as antioxidants in biological
systems and may be active in human subjects. Their
relative importance in humans remains unknown and
requires substantial additional experimentation.

The most commonly consumed flavonoids, catechins,
are in tea. These compounds have a wide range of anti-
oxidant activities. Electron paramagnetic resonance
experiments demonstrated the quenching of singlet oxy-
gen, superoxide anion, and hydroxyl radicals by black
and green tea extracts (Thiagarajan et al., 2001). Hydro-
gen peroxide- and primaquine-induced lipid peroxidation
is prevented by prior incubation with tea polyphenols.
The tea polyphenols inhibit hydroxyl radical fluxes gen-
erated by an iron-ascorbic acid system, suggesting that
iron chelation may be a mode of polyphenol action.
Tea flavonoids inhibit macrophage- and human umbilici-
un vein endothelial cell–induced LDL oxidation
(Yoshida et al., 1999). Theaflavin digallate was the most
effective. The mechanism of action may be the chelation
of iron and decreased formation of superoxide anion.
Green tea catechins also were effective in the prevention
of cell-mediated LDL oxidation (Yang & Koo, 2000b).
Black and green tea polyphenols protect in vitro red
blood cells against oxidative stressors (Grinberg et al.,
1997).

Regular tea intake did not alter in vivo lipid peroxi-
dation in intervention studies. Urinary F2-isoprostanes
were measured following 7 days (1000 ml/day of each
black and green tea) and 4 weeks of black tea
(1250 ml/day). The studies were performed with hyper-
tensive and hypercholesterolemic subjects, respectively.
No differences were found in urinary F2-isoprostane
concentrations between the control and tea treatments.
In another clinical trial, black tea and onions (a good source
The moderate consumption of red wine, a beverage high in flavonoids, has been associated with a low risk of cardiovascular disease. A portion of this beneficial effect has been attributed to the alcohol in wine, which may alter blood lipoprotein levels including an increase in high-density lipoprotein (HDL) concentrations (Vogel, 2002). However, the flavonoid content of red wine also has been indicated as another major factor in the prevention of cardiovascular disease. Dealkoholized red wine inhibits the expression of epinephrine-induced platelet antigens in vitro but did not affect the activation-dependent platelet antigen expression in either unstimulated platelets or after ex vivo activation with epinephrine (Rein et al., 2000). Many of the flavonoids in wine also are contained in purple grape juice. Thus, interest in the influence of purple grape juice on the risk of cardiovascular disease has grown in recent years. Purple grape juice and its flavonoids have antiplatelet and antioxidant activities. Several studies have shown an inhibition of platelet activity in the Folts dog model of acute platelet thrombus formation by purple grape juice and quercetin, a constituent of grape juice (Demrow et al., 1995; Folts et al., 1982). Purple grape juice inhibits platelet activity in dogs, monkeys, and humans (Folts, 2002). Further studies have identified the inhibition of platelet aggregation as occurring through an enhancement of platelet-derived nitric oxide release, a central regulator of platelet activity, and a reduction in superoxide production in vitro (Freedman et al., 2001). Consumption of purple grape juice (7 ml kg$^{-1}$ day$^{-1}$) for 14 days resulted in the ex vivo reduction of platelet aggregation, an increase in NO release, and a decrease in superoxide formation (Freedman et al., 2001). A feeding study investigated the effect of flavonoid-rich foods on hemostasis. The study fed onions, a source of quercetin, and parsley, a source of apigenin, for treatment periods of 7 days. No effect was found on ex vivo platelet aggregation, thromboxane B$_2$, and factor VII (Janssen et al., 1998). A flavonoid-rich beverage, tea, did not alter platelet activity in cardiovascular disease patients (Duffy et al., 2001b). Platelet activity was examined within 2 h following the consumption of tea (450 ml) and after a long-term exposure (900 ml/day for 4 weeks). A response may have been mitigated as a result of aspirin use and antiplatelet agent usage by many of the patients. Green tea catechins may inhibit platelet aggregation by the inhibition of an increase in cytoplasmic calcium (Kang et al., 2001).

Thus, the results are mixed for a relationship between flavonoid-rich food consumption and an inhibition of platelet activity: however, the relationship may be complex. A comparison of results of platelet studies with foods and purified flavonoids suggests synergy between flavonoids in the inhibition of platelet activity (Violi et al., 2002). Metabolism of flavonoids also may influence their antiplatelet activities, as some studies
suggest that quercetin is not active in vivo. Further studies are necessary for an understanding of the antiplatelet activities of flavonoid-rich foods. In particular, identification of the agents in purple grape juice and a better understanding of flavonoid metabolism are important areas of investigation. Various in vitro studies have shown antiplatelet activity for various purified flavonoids and have been described previously (Folts, 2002).

**Vessel function**

Blood vessel function has been characterized by flow-mediated vasomotor responses and arterial stiffness. These characteristics typically are measured with vascular ultrasonography and have been related to the risk of cardiovascular disease. Vasomotor responses are reduced and arterial stiffness increases when there is vascular endothelial dysfunction as commonly occurs with atherosclerosis and cardiovascular disease (Tiritilli, 1998). Several studies have shown that vasomotor responses and arterial stiffness can be improved in coronary artery disease patients with drug usage (statins) and dietary manipulation, especially antioxidant intakes (Keaney & Vita, 1995). One group of antioxidant intakes that improves vessel function has been flavonoid-rich foods. The flavonoid-rich beverage, tea, increases the vasomotor response of cardiovascular disease patients (Burns et al., 2000). The induction of NOS is the primary mechanism for vasodilation (McHugh & Cheek, 1998). Flavonoids may maintain stability of the NOS system, which improves NO production and reduces superoxide production (Freedman et al., 2001). This activity may occur through the antioxidant activity of the flavonoids and regulation of signal pathways. The mechanisms of myricetin involves an induction of calcium influx, which subsequently induced the activation of phospholipase A2 and the cyclooxygenase pathway with the release of thromboxane A2 (Jimenez et al., 1999). Another mechanism that may be active is inhibition of the NFκB/inhibitor-κB (IκB) system, which is a key regulator of numerous genes that respond to oxidative stress. For instance, its activation is associated with monocyte adhesion to vascular cells (Tsai et al., 1999; Kim et al., 2003). This system is regulated by cytokines, such as TNF-α and, importantly, redox status. Thus, it can coordinate several proatherogenic events including changes in vessel function. These regulatory mechanisms may individually or together influence vessel function.

Additional evidence for a flavonoid effect on vessel function comes from several animal models. In rabbit vessels, quercetin induced vasorelaxation of conductance and resistance vessels, which were unaffected by a moderate red wine exposure (Rendig et al., 2001). In reperfusion injury experiments, quercetin decreased superoxide anion exposures and increased nitric oxide, a cytoprotective agent. Each of these events is tracked in a rabbit hindlimb ischemia model (Huk et al., 1998). Flavonols and flavonoids induced vasorelaxation in isolated rat thoracic aorta (Chan et al., 2000). The treatment of rats with red wine polyphenols lowered systolic blood pressure, increased endothelium-dependent relaxation, and increased inducible NO synthase and cyclooxygenase (Diebolt et al., 2001).

The relaxation of vascular smooth muscle cells in rat aorta ring preparations by quercetin and rutin prompted...
the use of these agents in a clinical setting (Fusi et al., 2003). Two studies indicate that venous insufficiency may be improved through treatment with rutin and its derivatives (Cataldi et al., 2001; Petruzzellis et al., 2002). An extract of Ruscus aculeatus and hesperidin methylchalcone inhibits the activation of endothelial cells by hypoxia and may account for the efficacy of these substances in the treatment of venous insufficiency (Bouaziz et al., 1999).

The promotion of vessel function by flavonoids is supported by both animal model and human studies. Mechanisms of action have been identified, and their activity may have clinical relevance in the regulation of vessel function, but these aspects of flavonoid activity require further validation. Further studies are needed for the identification of subjects whose vessel function will benefit from an enhancement in flavonoid intake. The amount, type, and form of flavonoid intake also require further definition.

**Epidemiology**

Several epidemiologic studies have associated flavonoid intake with a low risk of cardiovascular disease. One such study found a relative risk of nonfatal myocardial infarction of 0.77 among male smokers was high as compared with a low intake of flavonols and flavones (Hirvonen et al., 2001). The risk of aortic calcification is lower among subjects with a high tea intake (>4 cups/day) as compared to nondrinkers in the Rotterdam Study. The odds ratio is 0.31 (CI = 0.16 to 0.59) following adjustment for age, sex, body mass index, smoking, education, and the intakes of alcohol, coffee, vitamin antioxidants, and several components of diet (Geleijnse et al., 1999). The Physicians Health Study did not find an association between flavonol and flavone intake and the risk of nonfatal coronary heart disease. A nonsignificant association was found between flavonol and flavone intake and coronary mortality rates (Rimm et al., 1996) in a prospective study with 4 years of follow-up. A prospective study of postmenopausal women found an inverse relationship between catechin intake and coronary heart disease death. In 12 years of follow-up, 767 cases occurred among 34,492 participants. Individuals with the highest catechin intake have a risk ratio of 0.76 (Arts et al., 2001). A study of Finnish men and women found a lower risk of several chronic diseases among subjects with a high versus low intake of flavonoids. Subjects with the highest intakes of quercetin had a relative risk of 0.79 (CI = 0.63 to 0.99) for mortality from ischemic heart disease (Knapp et al., 2002). A similar level of relative risk for cerebrovascular disease was found for the highest intakes of kaempferol, naringenin, and hesperidin (Gebhardt, 2003). These results generally indicate an association between flavonoid intake and a lower risk of cardiovascular disease. However, several studies do not show an association, and flavonoid intake may be confounded by the intake of other dietary and other lifestyle factors (Peters et al., 2001). In addition, the level of intake varies significantly between countries and may be the influencing factor in identification of associations. The major food sources of flavonoids are tea, apples, red wine, soy products, and onions. In a meta-analysis of a more specific exposure, tea intake, high intakes had a significant but relatively small protective effect (RR = 0.89) (Peters et al., 2001). Significant heterogeneity existed between studies, and some of these are not considered in the analysis. Thus, overall the epidemiologic evidence suggests a slight reduction in risk of cardiovascular disease, but the issue remains debatable.

**Blood cholesterol**

Several studies have investigated the effect of flavonoids on cholesterol synthesis and levels in cellular models, experimental animals, and humans. The cellular models, hepatocytes and HepG2 cells, have shown both stimulation and inhibition of cholesterol synthesis by flavonoids, depending on the specific flavonoid and dosage (Gebhardt, 2001). Quercetin, luteolin, and taxifolin inhibit cholesterol synthesis, and kaempferol and myricetin stimulate cholesterol synthesis. The effects may occur through indirect mechanisms that modulate the complex regulatory network of HMG-CoA reductase activity. Luteolin may induce biliary secretion of cholesterol and thereby reduce cholesterol levels. Dealkoholized red wine extracts reduce the production of ApoB100 and increase LDL receptor expression by HepG2 cells (Pal et al., 2003). A hypocholesterolemic effect has been shown in animal models for tea, naringenin, naringenin 7-O-cetyl ether (Lee et al., 2003; Miura et al., 2001; Yang & Koo, 2000a). Hesperidin supplementation lowered plasma cholesterol levels in rats fed a high-cholesterol diet (1%). It is accompanied by a reduction in HMG-CoA reductase activity (Park et al., 2001). Hesperidin is an inhibitor of lipases and may reduce plasma triglycerides in rats. A reduction in plasma triglycerides is found with a 10% dietary supplement (Kawaguchi et al., 1997). Another study did not find an effect of hesperidin on triglycerides (Park et al., 2001). In most animal studies, flavonoids decreased total cholesterol concentrations and increased the relative concentrations of high-density lipoprotein concentrations and the HDL:LDL ratio (Muramatsu et al., 1986; Matsumoto et al., 1998; Vinson & Dabbagh, 1998). On the other hand, the results of human observational studies have been mixed. Black tea intake has been associated with low cholesterol levels in six observational studies (Little et al., 1966; Green & Jucha, 1986; Tuomilehto et al., 1987; Prineas et al., 1980; Sovoll et al., 1989; Stensvold
Flavonoids and cardiovascular disease

and may have substantial health effects. Flavonoids disease. Several areas of investigation appear promising activities that may act in the prevention of cardiovascular activities of these compounds, especially in the area of substantial progress in the identification of the biological research have included recent advances in flavonoid research have included flavonoids occurring in vivo with biological activity in vivo can be extensive, and the actual flavonoid or metabolite effects, such as an improvement in vessel function. Another important area that affects interpretation of such studies is the need for a better understanding of flavonoid absorption and metabolism. Although some progress has been made in this area, flavonoid metabolism can be extensive, and the actual flavonoid or metabolite with biological activity in vivo generally is not known. Identification of the flavonoids occurring in vivo and their biological activities is an important next step in understanding the role of flavonoids in the prevention of cardiovascular disease. Another limitation of many current human studies is that generally flavonoid-rich foods have been evaluated, and these often contain a variety of flavonoids and phytochemicals. Thus, identification of a specific flavonoid that precipitates the effects has been difficult.

The identification of flavonoid-specific effects in humans, the active compounds, and their affect on the risk of cardiovascular disease requires further progress in several areas. Following these developments, it should be possible to design clinical trials that can identify the human health effects of specific flavonoids.

References

Blood pressure
Several animal studies have shown a reduction in blood pressure with flavonoid supplementation. The long-term administration (15 weeks) of hesperidin and glucosyl hesperidin lowered the blood pressure and heart rate in spontaneously hypertensive rats (SHR) but not in normotensive rats (WKY) (Ohtsuki et al., 2002). N-nitro-L-arginine methyl ester (L-NAME)-induced hypertension and its complications are reduced in rats by quercetin supplementation. Quercetin supplementation prevented increases in left ventricular and kidney weight, proteinuria, renal lesions, and decreased antioxidant status. Quercetin and its metabolites have antihypertensive effects and provide end-organ protection (Duarte et al., 2002). These effects generally are not found in human populations. Several studies evaluating blood pressure have not found an effect, and it appears unlikely that typical flavonoid intakes significantly reduce blood pressure in humans (Hodgson et al., 1999; Washburn et al., 1999).

Conclusions
Recent advances in flavonoid research have included substantial progress in the identification of the biological activities of these compounds, especially in the area of activities that may act in the prevention of cardiovascular disease. Several areas of investigation appear promising and may have substantial health effects. Flavonoids may have several anti-atherosclerotic activities including anti-inflammatory, antioxidant, antiproliferative, antiplatelet, and provessel function activities. Cholesterol-lowering and antihypertensive effects appear minimal for the flavonoids. Many of these activities have been demonstrated in cellular systems and experimental animals. Human studies have been performed in the evaluation of a few of these activities. However, further human studies are essential for the establishment of most flavonoid effects and evaluation of their health impact. In particular, studies are essential for the evaluation of dosage effects, which generally are lacking in current studies. Design and analysis of such experiments should consider the possible pleiotropic effects of flavonoids and the possibility that their cumulative effect will affect the risk of cardiovascular disease more than individual effects, such as an improvement in vessel function. Another important area that affects interpretation of such studies is the need for a better understanding of flavonoid absorption and metabolism. Although some progress has been made in this area, flavonoid metabolism can be extensive, and the actual flavonoid or metabolite with biological activity in vivo generally is not known. Identification of the flavonoids occurring in vivo and their biological activities is an important next step in understanding the role of flavonoids in the prevention of cardiovascular disease. Another limitation of many current human studies is that generally flavonoid-rich foods have been evaluated, and these often contain a variety of flavonoids and phytochemicals. Thus, identification of a specific flavonoid that precipitates the effects has been difficult.

The identification of flavonoid-specific effects in humans, the active compounds, and their affect on the risk of cardiovascular disease requires further progress in several areas. Following these developments, it should be possible to design clinical trials that can identify the human health effects of specific flavonoids.


et al., 1992) and has not been associated with cholesterol levels in many (10) other studies. Five of six clinical trials did not show a change in cholesterol levels with treatments of black tea (Bingham et al., 1997; Ishikawa et al., 1997; van het Hof et al., 1997; McAnlis et al., 1998; Princen et al., 1998; Davies et al., 2003). Similar results are found with green tea, with mixed result for observational studies (Kono et al., 1992, 1996; Imai & Nakachi, 1995; Tsubono & Tsugane, 1997; Arai et al., 2000; Tokunaga et al., 2002) and clinical trials (van het Hof et al., 1997; Princen et al., 1998; Maron et al., 2003). A theaflavin-enriched green tea extract decreased cholesterol levels in a randomized controlled trial of Chinese subjects consuming a low-saturated fat diet (Maron et al., 2003). Both total cholesterol and LDL-cholesterol are reduced by the treatment. These studies suggest that certain flavonoids may have a hypocholesterolemic effect, but the effect is dependent on dosage and the complete diet. The higher dosages used in animal experiments and extracts have hypocholesterolemic effects, whereas typical human intakes generally did not have an effect. Thus, typical dietary intakes appeared ineffective in lowering cholesterol levels in human subjects.


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