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Potential effects of flavonoids on the etiology of vascular disease

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Data from animal and epidemiological studies suggest that dietary flavonoids protect against the development of vascular disease. Despite the focus of attention on the ability of flavonoids to act as antioxidants and to alter endothelial cell eicosanoid production, a vast number of other mechanisms exist through which flavonoids could function to inhibit the development of vascular disease in humans. Reviewed here are six other factors that can influence the development of vascular disease in humans and the potential impact of flavonoids on each: adhesion receptor expression, bacterial replication, carbohydrate-induced AGE (advanced glycation end product) formation, estrogenic effects, proteolytic enzymes, and viral replication. Reviewed data suggest that if the total plasma flavonoid load exceeds a few micromoles per liter in vivo, flavonoids will protect humans against vascular damage that results from the aforementioned causes. (J. Nutr. Biochem. 9:560–566, 1998) © *Elsevier Science Inc. 1998*

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Introduction

Decades of studies into the bioactivities of plant flavonoids were largely ignored until Hertog et al.[1] reported an inverse relationship between flavonoid consumption and both the incidence of myocardial infarction and mortality from coronary heart disease in a broad spectrum of the human population. Subsequent to this report, a number of investigators used animal models to examine the effects of dietary flavonoids and flavonoid-containing plant extracts on experimentally induced vascular disease. In many of these studies, significant protection against vascular damage was demonstrated.[2–4] Because disease of the vascular system is a primary cause of dehabilitation and death in industrialized nations, a thorough understanding of how flavonoids affect animals at the systemic and cellular levels is necessary.

Flavonoids may inhibit disease of the vascular system that results from lipid oxidation and pathogenic vascular eicosanoid production. These mechanisms have received recent attention[2,5,6] and both can be observed in vitro with low micromolar concentrations of flavonoids. Despite the focus of attention on these mechanisms, a vast number of other mechanisms that have received comparatively little attention exist through which flavonoids could act to inhibit the formation of vascular disease. Reviewed here are the impact of viruses, bacteria, estrogens, proteases, adhesion molecules, and advanced glycation end products (AGE) on homeostasis of the vascular system, and mechanisms through which flavonoids can inhibit or mimic their effects (*Figure 1*).

Adhesion receptor expression

The physical interaction of leukocytes with endothelial cells is mediated by adhesion receptor–counter receptor interaction in a multiple-step process that includes primary adhesion, leukocyte activation, and activation-dependent integrin binding.[7] Primary adhesion of leukocytes to the endothelium (rolling) is mediated by the interaction between leukocyte or vascular selectins (e.g., L-selectin) and their ligand oligosaccharide carbohydrates (e.g., sialyl Lewis^x).[7] Leukocyte rolling induces an "activated" state as a result of the prolonged interaction with endothelial cell (EC) paracrine products. The activated state promotes the release of inflammatory mediators (e.g., cytokines) by leukocytes and the rapid upregulation of their cell surface integrins.[8] Activation-dependent (stable) leukocyte binding by the endothelium is dependent on the interaction of leukocyte integrins (e.g., β1 integrin) with endothelial cell counter-receptors (e.g., ICAM-1). As many of the cytokines

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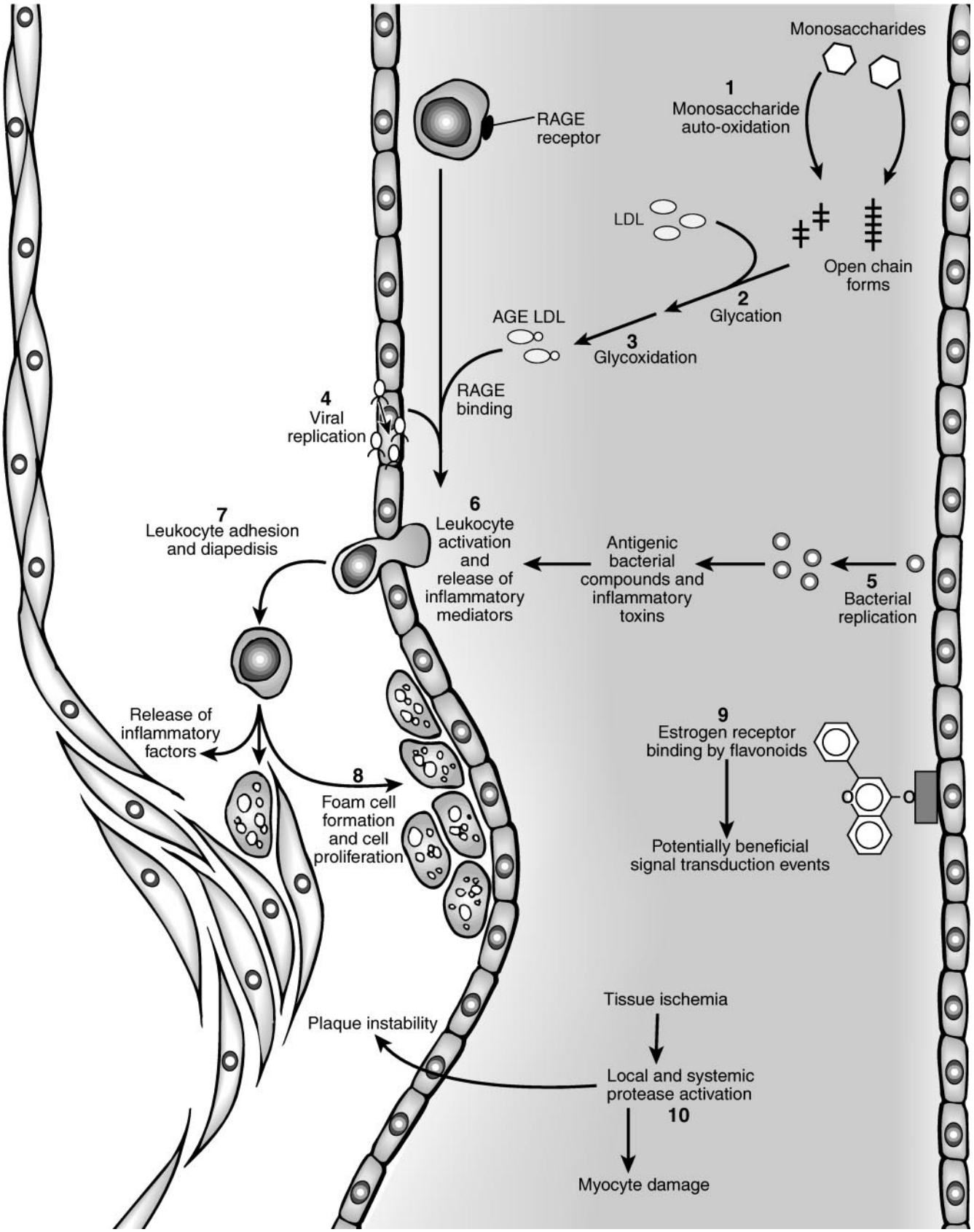

Figure 1 An illustration of events that flavonoids could block or mimic to inhibit the formation of vascular disease in humans: (1) monosaccharide autoxidation, (2) glycation, (3) glycoxidation, (4) viral replication, (5) bacterial replication, (6) leukocyte activation and release of inflammatory mediators, (7) leukocyte adhesion and diapedesis, (8) foam cell formation and cell proliferation, (9) estrogen receptor binding, and (10) local and systemic protease activation.

that trigger strong adhesion also act as chemotactic factors, leukocyte diapedesis usually occurs following stable leukocyte binding. Although leukocyte–endothelial cell interaction is essential to maintaining vascular homeostasis, it is also integral to disease.[8,9]

Because of the importance of leukocyte diapedesis in disease formation (e.g., vascular disease formation), disruption of the cascade of events that allows leukocyte adhesion and diapedesis is being studied as a method of disease prevention. The flavonoid apigenin was identified as an effective inhibitor of cytokine-mediated E-selectin expression.[10] This effect was relatively structure specific in that most flavonoids (e.g., genistein) are not inhibitors. Despite the lack of effect of most flavonoids on EC expression of E-selectin, EC expression of the VCAM-1 integrin was inhibited by most flavone flavonoids (e.g., luteolin) and flavonol flavonoids (e.g., fisetin) at IC_{50} concentrations as low as 12.5 μmol/L.[10–12] Effects may be mediated through a NF-κB mediated mechanism.[10,12] Although the general effect of flavonoids may be to decrease adhesion molecule expression, one isoflavone examined, genistein, stimulated ICAM-1 expression.[13] In vivo, flavonoids may not only inhibit the induction of adhesion molecule expression induced by inflammatory factors like cytokines, but also may inhibit leukocyte activation through their ability to alter the production of cell-derived inflammatory factors.[14]

Bacterial replication

Bacteria (e.g., *Streptococcus*) and their products (e.g., lipopolysaccharide) are hypothesized to contribute to infective endocarditis, congenital heart disease, rheumatic heart disease, and brain abscess through immune cell activation and platelet aggregation.[15–17] Bacteria that are threats to vascular health include *Helicobacter pylori*, *Streptococcus mutans*, *Chlamydia pneumoniae*, *Prevotella intermediaved*, *Porphyromonas gingivalis*, and *Actinobacillus actinomycetencomitans*.[17–21] Epidemiological data[22,23] support clinical and experimental data suggesting that the frequency and severity of bacterial infections correlate with the occurrence and severity of vascular disease.

Although growth of some soil bacteria such as *Rhizobium* may be stimulated by flavonoids,[24] flavonoids appear to inhibit the growth and replication of bacteria that are pathogenic to the vascular system of humans. Growth and replication of some species of the *Escherichia*, *Gingiva*, *Pseudomonas*, *Staphylococcus*, and *Canadida* genera can be inhibited by a variety of flavonoids (e.g., kaempferol) and flavonoid-containing plant extracts (e.g., *Gomphrena*).[25–28] Investigators observed IC_{50} values as low as 4 μmol/L. The bactericidal effects of flavonoids have been hypothesized to be mediated by flavonoid-induced damage to bacterial DNA.[29]

In addition to flavonoid-related reductions of bacterial growth, specific phytochemicals may inhibit bacteria-induced vascular damage through other mechanisms. For example, the cell wall constituents of live and heat-killed *Staphylococcus* (e.g., lipopolysaccharide) activate pathways in animals that induce vessel clot formation;[14] thus, flavonoids may protect humans against bacterially induced vascular damage through their ability to reduce endothelial cell endothelin synthesis.[30] Also, since many flavonoids inhibit leukocyte activation they may protect against damage resulting from bacterial DNA-induced leukocyte activation.[31]

Carbohydrate-induced AGE formation

The binding of protein, lipid, and DNA amine groups by carbohydrates can result in fixed structural alterations called *advanced glycation end products* (AGE), which have been quantitated structurally and by their characteristic fluorescence.[32,33] The molecular damage caused by AGE can contribute to the pathology of cataracts, renal disease, Alzheimer's disease, atherosclerosis, and heart disease.[34,35]

Evolutionary adaptation to carbohydrate-induced damage has resulted in a process for the removal of AGE. Scavenger receptors such as the receptor for advanced glycation end products (RAGE) are present on most cells of the vascular (e.g., endothelial cell) and immune (e.g., mononuclear phagocyte) systems and mediate the removal of AGE.[36–38] Unfortunately, as with many immune reactions, receptor-mediated removal of AGE can itself induce damage. In endothelial cells, for example, both oxidation and the expression of the adhesion molecule VCAM-1 increase as a result of RAGE binding.[35,37–39] Therefore, in diabetic patients, where carbohydrate-induced tissue damage is integral to the early onset of vascular disease seen in these patients, the benefits of suppressing some immune reactions may outweigh the risks.

Three steps in the pathway of AGE formation that can be targeted for inhibition are carbohydrate autoxidation, the covalent attachment of a carbohydrate to a free amine group (glycation), and glycate oxidation (glycoxidation). Carbohydrate autoxidation was suggested to be the most important step in the formation of AGE characteristic fluorescent adducts because autoxidation of carbohydrates produces reactive-oxygen species (e.g., H_2O_2) and carbohydrates that are far more likely to form glycates (e.g., glyoxal and arabinose) than their parent compounds.[40] Flavonol flavonoids (e.g., quercetin) and monophenol flavonol metabolites (e.g., 3,4-dihydroxyphenylacetic acid) inhibit glucose autoxidation.[40]

Glycation is the nonenzymatic condensation of a reducing sugar with an amine.[41] This reaction produces an Amadori or Heyns product called a glycate. In vitro, the rate of glycation is dependent on a sugar's concentration and its anomerization rate (e.g., glucose < mannose < galactose < xylose < fructose < arabinose < ribose < 2-deoxy-D-ribose < glyoxal).[35] In addition, reaction rate is inversely proportional to the number of carbon atoms in the reducing sugar, and phosphorylated sugars are more reactive than their unphosphorylated counterparts. Flavonol flavonoids with vicinal B-ring hydroxyl groups (e.g., quercetin) are efficient inhibitors of collagen glycation.[42] In contrast, flavonoids without vicinal hydroxyl groups (e.g., naringin) did not inhibit glycation. A similar relationship was observed with flavonoid metabolites (e.g., 3,4-dihydroxyphenylacetic acid).[43] Because vicinal hydroxyl group compounds can form quinones, the inhibition of glycation may have resulted from amine group binding by flavonoids.

Glycoxidation is the oxidative rearrangement of a gly-

cate that can result in the formation of both AGE and reactive-oxygen species.[44] For example, glycoxidation of amines previously glycated by glyoxal forms the AGE N^{Σ}-(carboxy-methyl)lysine (CML) and the glycoxidation of amines previously glycated by arabinose forms the AGE pentosidine.[45] The same flavonoids that inhibited glucose autoxidation also inhibited glycoxidation.[42] Glucose autoxidation and glycoxidation are oxygen and metal ion-dependent reactions; therefore, both may have been inhibited through the same mechanism(s).

Quercetin and its monophenol metabolites, which inhibit carbohydrate autoxidation, glycation, and glycoxidation, were tested as inhibitors of collagen-linked pentosidine formation. Formation of the AGE pentosidine on collagen incubated with glucose in phosphate buffer was inhibited by these compounds. When combined, quercetin and its metabolites exhibited synergy, and in vitro data suggest that a flavonoid concentration of one micromole per liter of blood would be effective in vivo.[42] In addition, flavonoids may inhibit carbohydrate-induced protein damage in vivo as oral rutin inhibited the formation of AGE-characteristic fluorescent adducts in streptozocin-induced diabetic rats.[46]

Estrogenic effects

Gender differences in the incidence of vascular disease have been recognized since at least 1939.[47] In 1976, Kannel et al.[48] reported data from the Framingham Study showing that although disease of the vascular system was decreased in premenopausal women compared to same-age men, the decreased incidence disappeared following menopause. Further study reaffirmed this observation.[49]

In rabbits, rats, chicks, and pigeons fed atherogenic diets, the administration of estrogen prevented atherogenesis.[50] Ovariectimized cynomolgus monkeys without estrogen replacement therapy developed atherosclerosis twice as fast as ovariectamized cynomolgus monkeys given estrogen therapy.[51] In humans, data show that the relative risk of developing cardiovascular disease in human estrogen users is 0.56 compared with nonusers.[52]

Although a complete understanding of the vasoprotective effects of estrogen is lacking, various potential mechanisms are hypothesized. As reviewed by Nathan and Chaudhuri,[49] estrogens may inhibit the progression of atherosclerosis at multiple steps through mechanisms such as decreasing lipoprotein(a), preventing lipid oxidation, increasing the high-density lipoprotein concentration of blood, modulating endothelial cell expression of chemokines and adhesion molecules, inhibiting vascular smooth muscle cell proliferation and collagen synthesis, preventing platelet aggregation, and ensuring adequate tissue perfusion by promoting the dilation of blood vessels. Flavonoids and estrogen share a variety of properties, including the ability to inhibit lipid oxidation, platelet aggregation, and the expression of chemokines and adhesion molecules.[6,53,54] In addition, they promote vasodilatation and bind type II estrogen receptors. Competition binding studies have revealed estrogenic properties of isoflavone (e.g., genistein), chalcone (e.g., isoliquiritigenin), flavonol (e.g., quercetin) and flavone (e.g., apigenin) flavonoids, with estrogenic effects noted at concentrations as low as 10^{-9} M.[54,55]

Although investigators have acknowledged the role of estrogen receptor binding in the protective effects of flavonoids against melanoma,[55] and flavonoid-induced vasoprotective effects were hypothesized to result from flavonoid binding of type II estrogen receptors,[55] the extent to which the vasoprotective effects of flavonoids and estrogens are linked to the binding of type II estrogen receptors is not established.

Proteolytic enzymes

Blood vessel plaques are characteristic advanced atherosclerotic lesions in humans. These lesions have lipid cores covered with a fibrous cap that is composed of smooth muscle cells, immune cells, and extracellular matrix. As a plaque occludes a vessel, ischemia results and proteases are activated. Local and systemic activation of proteases induces plaque instability and myocyte damage.[56–59] Multiple proteases can mediate damage that leads to aortic plaque instability and plaque fragmentation.[56,59–61] Two of these damage causing proteases, collagenase and elastase, also mediate the age-related loss of collagen and fibrous protein in veins, which contributes to the formation of aneurysm and varicose veins.[62–64]

In vitro, flavonoids (e.g., catechin) can decrease proteolytic attack on fibrous proteins and the accumulation of proteoglycans and hyaluronan. Anthocyanidin flavonoids and flavonoid-containing extracts from plants (e.g., *Ribes nigrum*) inhibit the activity of elastase, trypsin, and alpha-chymotrypsin.[65,66] IC_{50} values as low as 11 μmol/L were observed. In addition, anthocyanidins can inhibit protease-induced increases in the permeability of the blood-brain barrier in rats.[66] Various proteases not currently known to be involved in the regulation of vessel integrity (e.g., tissue-type plasminogen activator [t-PA], HIV-1 proteinase, and microbial leaf protein proteases) are also inhibited by some flavonoids.[67–69] Last, since leukocyte activation causes protease release, the inhibition of leukocyte activation by flavonoids could mediate reduced protease-induced tissue damage.

Viral replication

Myocarditis is the inflammatory infiltration of myocardium that results in myocyte degeneration and necrosis.[70,71] This pathology is associated primarily with viruses, but its etiology is poorly understood and its prevalence is believed to be largely represented by chronic but subclinical cardiomyopathy.[70,72] Both enteroviruses and cardiotropic viruses are hypothesized to contribute to myocarditis in humans.[71,72] Following invasion of myocytes, enteroviruses replicate and ultimately cause myocyte death by cell lysis.[71] Lysed myocytes exude myosin, which activates and attracts leukocytes through cytokine-mediated pathways. In contrast, cardiotropic viruses replicate in myocytes until T-cell-mediated autoimmunity causes myocyte death.[72]

In humans, mumps virus, influenza virus, herpes viruses, picornaviruses, and hepatitis C virus are hypothesized causes of viral myocarditis.[73–77] Antiviral activity has been noted with both flavonoids and flavonoid containing plant extracts. For example, the common dietary flavonoid quer-

cetin and the flavonoid polymer SP-303 (2100 Da) have anti-herpes virus and anti-influenza virus activity.[78–83] Observed IC_{50} values were as low as 2 μmol/L. The activity of both large and small molecular weight phytochemicals was also noted in relation to endothelial cell eicosanoid release,[84] suggesting that a structural feature common to many phytochemical monomers and polymers may mediate a variety of phytochemical effects.

Flavonoids can also protect cells against a multitude of other viruses, including Epstein-Barr virus, hepatitis virus, HIV virus, mengovirus virus, and polio virus.[78,85–87] In addition to inhibiting replication of viral particles, flavonoids can enhance the efficacy of antiviral agents (e.g., interferon) and inhibit the activity of tyrosine kinase $_p56^{lck}$, a molecule that is key to the formation of at least one type of viral-mediated myocarditis.[6,78,79,85,88]

Conclusions

Although the effects of flavonoids on oxidation and eicosanoid production are commonly discussed in relation to vascular disease, this review illustrates that flavonoids may act through a multitude of mechanisms to protect the vascular system from conditions that would otherwise cause damage. Information concerning flavonoid absorption and metabolism is essential to a better understanding of mechanisms through which orally consumed flavonoids affect human health. Although data from Paganga et al.[89] suggest that the steady-state concentrations of various flavonoids in human plasma are between 0.5 and 1.6 μmol/L, this data is available for only for a few of the 4000+ known flavonoids. Knowing the absorption, metabolism, and steady-state plasma concentrations of flavonoids and flavonoid metabolites will not only allow investigators to work within concentrations and time frames of exposure that are relevant in vivo but will also allow investigators to calculate the total flavonoid load of human plasma. In addition, information concerning cellular flavonoid transport and the sequestration of flavonoids or their metabolites by animal cells will increase the speed at which investigators can determine the physiologically relevant molecular mechanisms through which flavonoids and other phytochemicals affect animals. A thorough understanding of each of these will enable investigators to determine the contribution of each mechanism of vascular protection described here.

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