# MINIREVIEW

# Flavonoids Attenuate Cardiovascular Disease, Inhibit Phosphodiesterase, and Modulate Lipid Homeostasis in Adipose Tissue and Liver

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**Plant flavonoids are widely distributed polyphenolic compounds of the human diet. They consist of six major classes based on specific structural differences: flavonols, flavones, flavanones, catechins, anthocyanidins, and isoflavones. All of the major classes of flavonoids are comprised of three six-membered rings: an aromatic A-ring fused to a heterocyclic C-ring that is attached through a single carbon-carbon bond to an aromatic B-ring. Population studies have shown that flavonoid intake is inversely correlated with mortality from cardiovascular disease, and numerous flavonoids of dietary significance have been shown to beneficially impact parameters associated with atherosclerosis, including lipoprotein oxidation, blood platelet aggregation, and vascular reactivity. Therapeutic effects of flavonoids on platelet aggregability and blood pressure have been attributed to competitive inhibition of cyclic nucleotide phosphodiesterase (PDE), an elevation in cAMP level, and subsequent activation of protein kinase A (cAMP-dependent protein kinase). In addition, flavonoids may induce neutral lipid hydrolysis from lipid stores through PDE inhibition in adipose tissue and liver. Indeed, the three-dimensional structure of many flavonoids is sterically and electrostatically compatible with the catalytic site of cAMP PDE3 and PDE4. Flavonoids have also been reported to suppress pathways of lipid biosynthesis and of very low-density lipoprotein production in cultured hepatocytes. Continued studies of the biochemical mechanisms underlying the biological effects of plant flavonoids may uncover new strategies for the treatment of cardiovascular disease, as well as associated conditions such as obesity, hepatic steatosis, and Type 2 diabetes.** Exp Biol Med 231:1287–1299, 2006

**Key words:** flavonoid; atherosclerosis; phosphodiesterase; lipid hydrolysis; adipose tissue; liver

## Introduction to Flavonoids: Structural Considerations

The flavonoids are a diverse group of polyphenolic compounds widely distributed in the plant kingdom. There are more than 6400 known flavonoid compounds (1). Flavonoids contribute to the flavor and pigmentation of the fruits and vegetables in the human diet (2). They also have important roles in plant growth, reproduction, and pathogen and predator resistance (1). Flavonoids are present in plants either as aglycones or as glycoside conjugates. Attached sugar moieties include D-glucose, L-rhamnose, glucorhamnose, galactose, lignin, and arabinose (3). The contents of flavonoids in common foods may be obtained from United States Department of Agriculture databases available on the World Wide Web (4, 5).

Flavonoids may be divided into 8 different classes (flavonols, flavones, flavanones, catechins, anthocyanidins, isoflavones, dihydroflavonols, and chalcones) based on differences in molecular backbone structure (Fig. 1, Ref. 6). Specific examples of the six major classes of flavonoids are shown as ball and stick models in Figure 2 (7–10). The flavonol quercetin and the flavone apigenin are found in many fruits and vegetables, including onions, apples, broccoli, and berries. Naringenin is a citrus flavanone. Catechin and other catechins are abundant in green tea. Cyanidin and other anthocyanidins are largely responsible

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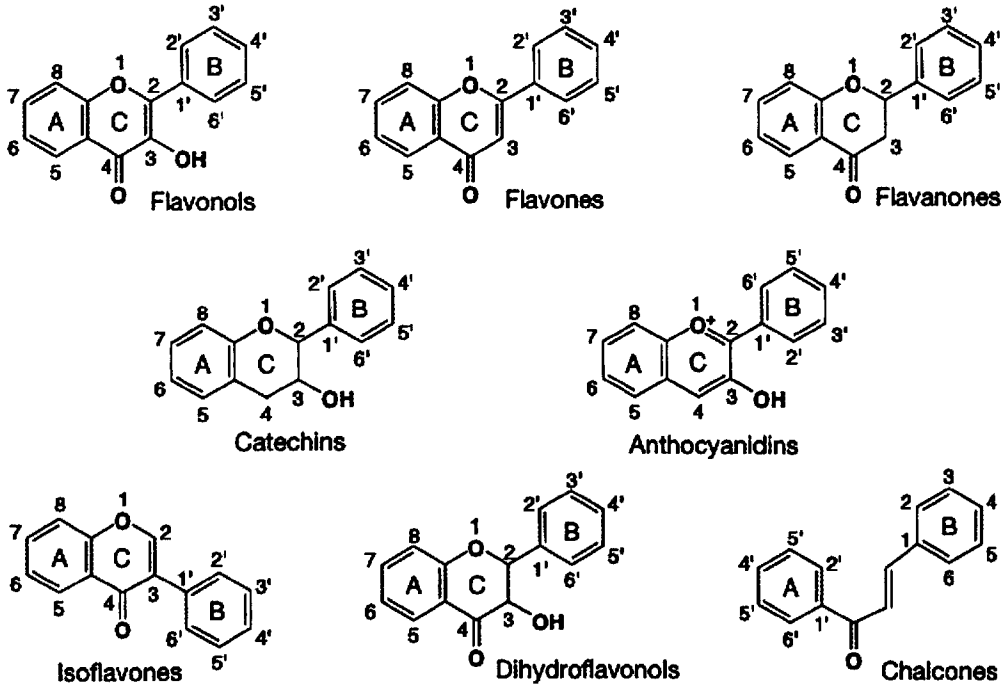


**Figure 1.** Backbone structures of the different classes of flavonoids. Backbone structural differences occur primarily at the C-ring. Flavonols, dihydroflavonols, catechins, and anthocyanidins are hydroxylated at the 3-position of the C-ring. The carbon 2–3 bond of the C-ring is saturated in the flavanones, catechins, and dihydroflavonols. For all of the flavonoids shown, the B-ring is attached to the C-ring at the 2-position of the C-ring, except the isoflavones for which the B-ring is attached to the C-ring at the 3-position of the C-ring. The catechins and anthocyanidins are lacking a carbonyl group at the 4-position of the C-ring. Chalcones have a five-membered open C-ring structure. All three rings of the anthocyanidins are aromatic, and electron delocalization is responsible for their characteristically bright colors.

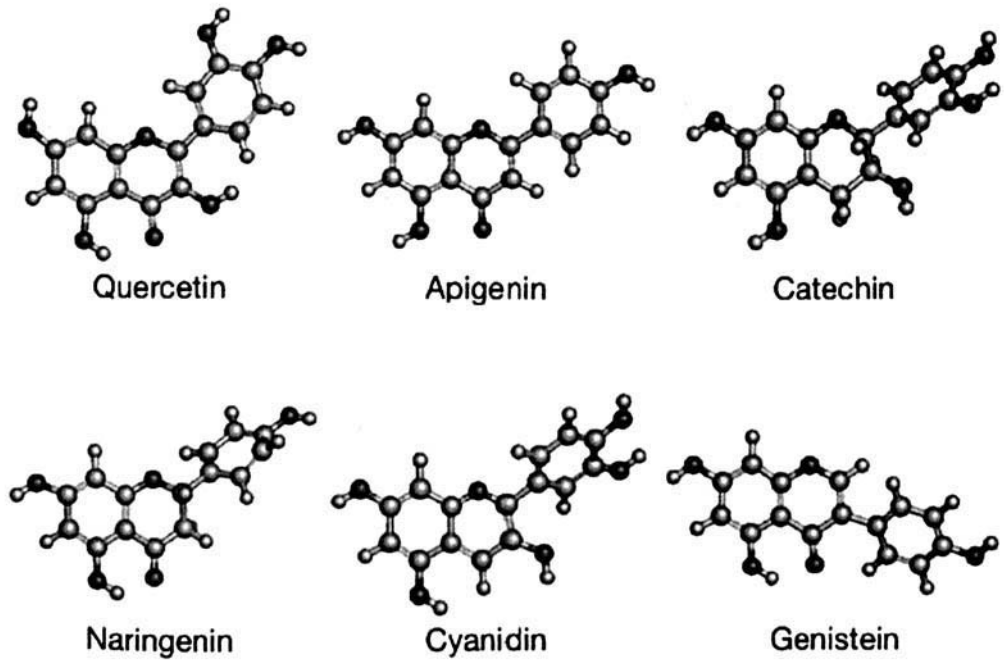


**Figure 2.** Ball and stick models of examples of the six major classes of flavonoids. Shown are the flavonol quercetin (3′,4′,5,7-tetrahydroxyflavan-3-ol, $C_{15}H_{10}O_7$, MW 302.2), the flavone apigenin (4′,5,7-trihydroxyflavone, $C_{15}H_{10}O_5$, MW 270.2), catechin (5,7,3′,4′-tetrahydroxyflavan-3-ol, $C_{15}H_{14}O_6$, MW 290.3), the flavanone naringenin (4′,5′,7-trihydroxyflavanone, $C_{15}H_{12}O_5$, MW 272.3), the anthocyanidin cyanidin (3,5,7,3′,4′-pentahydroxyflavylium, $C_{15}H_{11}O_6$, MW 287.2), and the isoflavone genistein (4′,5,7-trihydroxyisoflavone, $C_{15}H_{10}O_5$, MW 270.2). The three-dimensional structural coordinates for the genistein, quercetin, and naringenin models were obtained from the Protein Data Bank (7) files PDB 1qkm (8), PDB 1e8w (9), and PDB 1cgk (10), respectively, and represent actual x-ray crystallographic solutions. The three-dimensional structural coordinates for apigenin, catechin, and cyanidin represent theoretical models that were obtained from the ChemIDplus Web site (http://chem.sis.nlm.nih.gov/chemidplus). Graphical representations were rendered using PyMOL (http://pymol.sourceforge.net).

for the deep colors of berries, grapes, and red wine. Genistein is an isoflavone found predominantly in legumes. The major classes of flavonoids consist of two fused six-membered rings (an aromatic A-ring and a heterocyclic C-ring) connected through a carbon-carbon bridge to an aromatic B-ring (11). The fused A-ring and C-ring are generally planar, and each graphic in Figure 2 positions the A-C fused ring in the plane of the page. Rotation is possible at the linkage between the C-ring and the B-ring, and the B-ring is often not in the same geometric plane as the A-ring and the C-ring.

The biological effects of small molecules such as flavonoids are contingent upon interactions with proteins. Protein-flavonoid complex formation depends both on 3-dimensional structure and on electrostatic interactions. Figure 3 (12, 13) shows the electrostatic potential mapped to the van der Waals surface of each example flavonoid from Figure 2. Highly electronegative surface areas are shaded (black), and highly electropositive surface areas are unshaded (white). For each flavonoid, areas of highest electronegativity occur at the 5-hydroxyl and 7-hydroxyl positions of the A-ring, at the various hydroxylations of the B-ring, and at the 3-hydroxyl position of the C-ring that is specific to flavonols, catechins, and anthocyanidins. These positions of hydroxylation are potential sites for hydrogen bonding with protein residues, for interaction with metal cations, and for electron transfer. Many flavonoids are inhibitors of several isoforms of phosphodiesterase (PDE), and the positions of hydroxylation are important for differential PDE inhibition. For example, hydroxylation at the C-4′ position is important for PDE3 inhibition; hydroxylation at the C-5 position is important for inhibition of PDE1, PDE2, PDE4, and PDE5; and C-7 hydroxylation is important for inhibition of PDE1, PDE3, and PDE4 (14, 15).

## Flavonoids and Atherosclerosis: Lipoprotein Oxidation, Platelet Activation and Aggregation, Vascular Reactivity, and Plasma Lipid and Glucose Homeostasis

The development of cardiovascular disease is a function of numerous environmental factors (including diet), and epidemiological findings indicate that a diet rich in fruits and vegetables has a protective effect (16). At least part of the favorable effect of fruits and vegetables may be attributed to their flavonoid content (17). In the Zutphen Elderly Study, in the Seven Countries Study, and in a cohort study in Finland, flavonoid intake was inversely correlated with mortality from coronary heart disease (18–20). In another study of the Finnish population, a higher quercetin intake was reported to lower mortality from coronary heart disease; the incidence of cerebrovascular disease was reported to be lower with higher kaempferol, naringenin, and hesperetin intakes; and there was a trend toward a

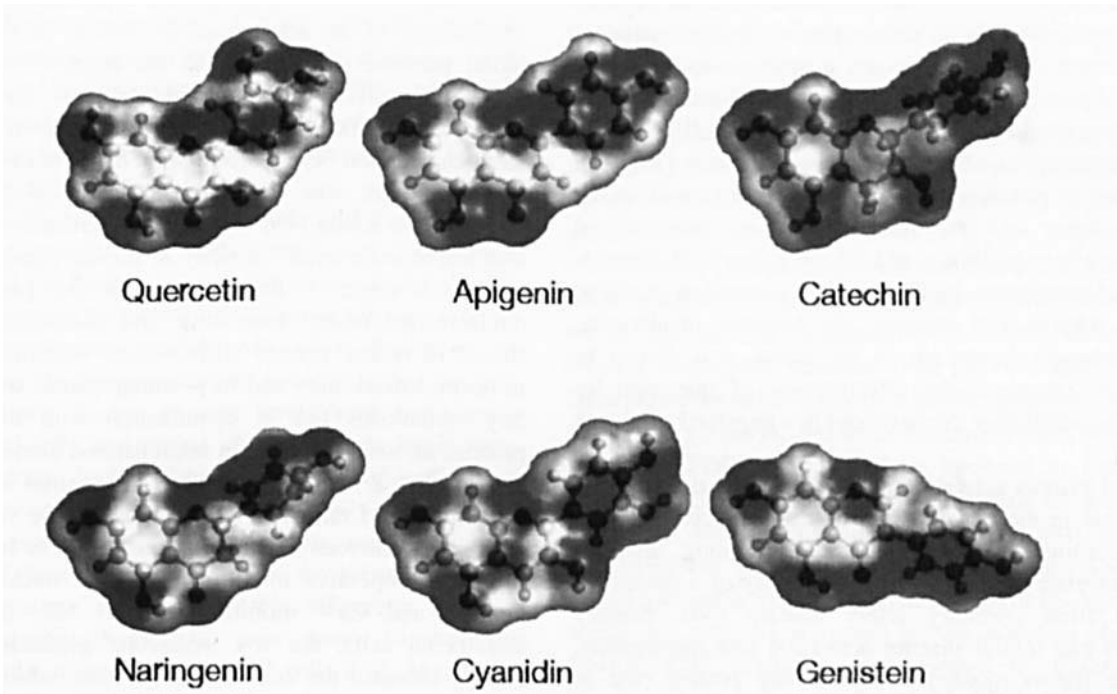


**Figure 3.** The electrostatic potential mapped to the van der Waals surface of examples of the six major classes of flavonoids. Partial atomic charges for each example flavonoid from Figure 2 were calculated using the General Atomic and Molecular Electronic Structure System (GAMESS, Ref. 12). The following GAMESS parameters were set: GBASIS=N21, NGAUSS=3, SCFTYP=ROHF. The electrostatic potential was calculated with the Adaptive Poisson-Boltzmann Solver (APBS, Ref. 13) and mapped to the van der Waals surface (1.4-Å solvent probe radius) of each example flavonoid. Highly electronegative surface areas are shaded (black), and highly electropositive surface areas are unshaded (white). The grayscale gradient from black to white was set to span between –5 kT/e and 5 kT/e. Graphical representations were rendered using PyMOL.

reduction in the incidence of Type 2 diabetes at higher quercetin and myricetin intakes (21). Furthermore, in cynomolgus monkeys fed an atherogenic diet, isoflavones fed in combination with isolated soy protein were shown to attenuate atherosclerotic lesion development (22). Numerous other studies illustrate antioxidant, antithrombotic, and anti-inflammatory properties of flavonoids that likely play a role in the lower cardiovascular mortality observed with higher flavonoid intake (6, 23–25).

An elevated plasma LDL concentration is a primary risk factor for the development of atherosclerosis and coronary artery disease (26). Very low-density lipoproteins (VLDLs) are produced in the liver primarily for the transport of newly synthesized triglyceride to peripheral tissue. In the capillary beds, plasma VLDL is converted to cholesteryl ester–enriched triglyceride-poor LDL. Reactive oxygen species generated through lipid peroxidation can oxidatively modify the amino acid residues of LDL, and LDL oxidation in the arterial intima can initiate the atherosclerotic process (27). Flavonoids may suppress LDL oxidation and inflammatory progression in the artery wall. Soybean isoflavones administered to human subjects and to hamsters were shown to attenuate $Cu^{2+}$-catalyzed oxidation of isolated LDL (28). Flavonoids such as morin, fisetin, quercetin, and gossypetin (1–2 $\mu M$) were shown to inhibit the oxidative modification of LDL by macrophages, at least partly by conserving the α-tocopherol content of the LDL particles (29). In cholesterol-fed rabbits, dietary isoflavones reduced the number of oxidized LDL-positive macrophage foam cells in atherosclerotic lesions found in the aortic arch (30). The flavonol quercetin was shown to scavenge lipid alkoxyl and peroxyl radicals and to repair neutral tryptophan and tyrosine radicals, as well as superoxide radical anions and α-tocopheroxyl radicals (31). The mechanism is postulated to involve the flavonoid as an electron donor and the formation of a semioxidized flavonoid radical. Inhibition of LDL oxidation by flavonoids is strictly dependent on binding of the flavonoid molecule to the LDL particle. For example, the presence of albumin, which transports flavonoids in the blood, was shown to significantly attenuate the effectiveness of quercetin by binding and stabilizing the flavonoid in a negatively charged state (32).

Blood platelet activation and aggregation also have an integral role in the development of cardiovascular disease (33). Variations in platelet sensitivity among different species correlate directly with susceptibility of a species to mortality from coronary artery disease (34). Dietary flavonoids can inhibit platelet activation and aggregation. In female rhesus monkeys, isolated soy protein (fed in combination with 350 mg of isoflavones per kilogram of diet for 6 months) relative to isoflavone-depleted soy protein was reported to lower the aggregation of isolated platelets stimulated with thrombin *in vitro* (35). Another study of soybean isoflavones showed that thrombin-stimulated serotonin release was suppressed in platelets isolated from soy protein–fed rats (578 mg of isoflavones per kilogram of diet) compared with serotonin release in platelets isolated from casein-fed rats (36). Platelet responses can involve activation of phospholipase C and phospholipase $A_2$, as well as hydrolytic release of arachidonic acid and its subsequent conversion through cyclooxygenase to thromboxane $A_2$, which potentiates platelet activation (37). Both genistein and daidzein (0.4–110 $\mu M$) have been shown to exert a dose-dependent inhibition of thromboxane receptor binding in human platelets *in vitro* (38). Apigenin, genistein, and luteolin (1–100 $\mu M$) were also shown to bind and antagonize the thromboxane receptor (39). In rabbit platelets, fisetin, kaempferol, and quercetin (13–22 $\mu M$) were shown to suppress thromboxane formation and were suggested to antagonize the thromboxane receptor (40). *In vivo*, the intravenous injection of myricetin (3.6 μg per kilogram of body weight) was shown to inhibit cat platelet aggregation, and as little as 60 n$M$ was shown to disperse platelet aggregates *in vitro* (41). Platelet antiaggregatory effects have generally not been observed with flavonoid glycosides. It has been suggested that flavonoid aglycones, but not flavonoid glycones, suppress platelet activation through inhibition of platelet PDE activity (42). Cyclic nucleotide PDE inhibitors, including quercetin, have been shown to raise platelet cAMP levels, which subsequently blocks cytoplasmic $Ca^{2+}$ mobilization and platelet activation (43). The effect of quercetin on $Ca^{2+}$ mobilization may also be related to inhibition of phospholipase C (44).

Atherosclerosis is accompanied by a narrowing and loss of elasticity of the artery wall, as well as an elevation in blood pressure. In addition to the antioxidant and antithrombotic effects demonstrated for plant flavonoids of dietary significance, flavonoids have been shown to improve vascular function (45). Consumption of soybean isoflavones with soy milk was shown to lower blood pressure in hypertensive adults (46). Intravenous genistein administration improved arterial elasticity in female rhesus monkeys (47). Red clover isoflavones rich in the genistein and daidzein precursors biochanin and formononetin were shown to reduce arterial stiffness and vascular resistance in normotensive men and in postmenopausal women (48). Soy isoflavones fed in combination with isolated soy protein, as well as genistein administered alone, improved flow-mediated vasodilation in postmenopausal women (49, 50). In isolated rat aortic strips and rings, the vasorelaxant effects of numerous flavonoids were shown to be mediated through competitive inhibition of cyclic nucleotide PDE activity and $Ca^{2+}$ mobilization (51, 52). In vascular endothelial cells, the soy isoflavone genistein (10 n$M$) acutely elevated the cellular cAMP level, which activated protein kinase A (PKA), cAMP response element binding protein (CREB), and CREB-mediated gene expression (53). One of the targets of PKA, endothelial nitric oxide synthase, elevates nitric oxide production, which has produced a vasodilatory effect in humans (54). Vasodilation through PDE inhibition has also been suggested for other flavonoids

(52, 55–57). The PKA-mediated vasodilatory effect of genistein was shown to be unrelated to tyrosine kinase inhibition or interaction with the estrogen receptor (58).

Despite the beneficial effects shown for plant flavonoids on parameters associated with the progression of atherosclerosis, an ability of flavonoid compounds to lower plasma lipid levels remains a contentious issue (59). Dietary supplementation with isoflavone tablets has failed to lower plasma lipid levels in humans (60, 61). Nevertheless, other studies have shown that isoflavones fed in combination with isolated soy protein lower plasma cholesterol more effectively than soy protein devoid of isoflavones (62, 63). Furthermore, there is some evidence of an independent reduction of plasma lipid levels by ingested flavonoids. Total flavonoid and quercetin intakes were inversely correlated with plasma total and LDL cholesterol concentrations in Japanese women (64). Isoflavones (1–2 g per kilogram of diet) added to a casein protein diet lowered plasma triglycerides (65) and plasma total cholesterol and triglycerides (66) in healthy male Sprague-Dawley rats. Dietary hesperidin (7 g per kilogram of diet) lowered plasma total cholesterol in ovariectomized mice (67). Dietary isoflavones lowered plasma total and LDL cholesterol concentrations in male spontaneously hypertensive rats, lowered plasma triglyceride concentrations in the lean phenotype, and tended to raise plasma triglycerides in the obese phenotype. However, the isoflavone diet also suppressed plasma testosterone levels and growth of both lean and obese animals (68, 69). In addition, supplementation of a high-fat diet with genistein (2 g per kilogram of diet) suppressed growth and lowered plasma total cholesterol and free fatty acid concentrations in mice (70). Additional studies are needed to delineate the effects of flavonoids on plasma lipid homeostasis that are independent of the apparent effects on growth.

Few studies have measured an effect of flavonoids on plasma glucose levels. Soy isoflavones fed in combination with isolated soy protein were shown to accelerate glucose clearance from the blood during a glucose tolerance test in fasted obese female Zucker rats (71). However, in spontaneously hypertensive male obese rats fed an isoflavone mixture, plasma glucose levels were not significantly altered (69), and fasting plasma glucose levels were elevated in male Zucker diabetic fatty rats fed isoflavones with soy protein (72). Flavonoids physically interact with glucose transporters (GLUTs) *in vitro* and are potent inhibitors of glucose transport. Fisetin, myricetin, quercetin, apigenin, genistein, cyanidin, daidzein, hesperetin, naringenin, and catechin (8–50 $\mu M$) were shown to attenuate glucose uptake in myelocytic U937 cells (73). Myricetin, quercetin, and catechin at similar concentrations were shown to inhibit glucose uptake in isolated rat adipocytes (74). Genistein has directly inhibited GLUT1, GLUT2, and GLUT4 in several cell types (75–77). In addition, perfusion of ovariectomized rat liver with genistein was shown to markedly lower the surface expression of high-affinity insulin receptors (78). Incubation of cultured rat adipocytes with genistein (100 μg/ml) was shown to completely inhibit insulin-mediated glucose oxidation, as well as the antilipolytic effect of insulin, downstream from and unrelated to insulin receptor tyrosine kinase activity (79). In the fowl tapeworm *Raillietina echinobothrida*, genistein reduced the activity of glucose-6-phosphate dehydrogenase and elevated the activities of pyruvate carboxylase and phosphoenolpyruvate carboxykinase (80). Collectively, results of these studies suggest that flavonoids have the potential to compromise tissue insulin action and glucose utilization and, perhaps, to induce gluconeogenesis.

## Flavonoid Modulation of Lipid Homeostasis in Adipose Tissue: Role of Cyclic Nucleotide PDE Inhibition

The incidence of obesity has reached epidemic proportions in the United States, and it represents a primary risk factor for the development of cardiovascular disease and Type 2 diabetes (81, 82). The hydrolysis and diminution of cellular neutral lipids (cholesteryl ester and triglyceride) in adipocytes are governed by a complex interplay between several components (83, 84). A schematic representation of the primary components regulating adipose tissue lipid hydrolysis, including the effect of flavonoids, is shown in Figure 4 (85–88). Flavonoids can induce lipolysis in adipose tissue, likely through competitive inhibition of PDE and antagonism of cAMP degradation. For example, subcutaneous injection and dietary administration of genistein were shown to lower adipose tissue weight in ovariectomized mice (89). In adipocytes isolated from both ovariectomized female and healthy male rats, incubation with genistein (20–300 $\mu M$) was shown to elevate epinephrine-induced lipolysis and to reduce insulin-stimulated incorporation of glucose into lipid (90, 91). In 3T3-L1 preadipocytes, genistein (50–100 $\mu M$) inhibited differentiation, triglyceride accumulation, and peroxisome-proliferative-activated receptor (PPAR) gamma expression (92). In fully differentiated 3T3-L1 adipocytes, genistein (100 $\mu M$) elevated basal and epinephrine-induced lipolysis. Luteolin, apigenin, quercetin, diosmetin, genistein, and other flavonoids have been shown to inhibit PDE3 and to induce lipolysis in isolated rat adipocytes (93). Augmentation of lipolysis by genistein in adipocytes was shown to depend on PKA (94), and grape seed cyanidins were shown to elevate cellular cAMP levels and to induce lipolysis through activation of PKA in 3T3-L1 adipocytes (95). An argument for the induction of lipolysis through PDE inhibition is supported by findings from investigations using nonflavonoid PDE inhibitors. For example, intravenous injection of the PDE3 selective inhibitor milrinone in rats blocked insulin-mediated suppression of lipolysis in both adipose tissue and liver (96). Thus, in addition to flavonoid-mediated inhibition of platelet activation and aggregation and improvement of vascular

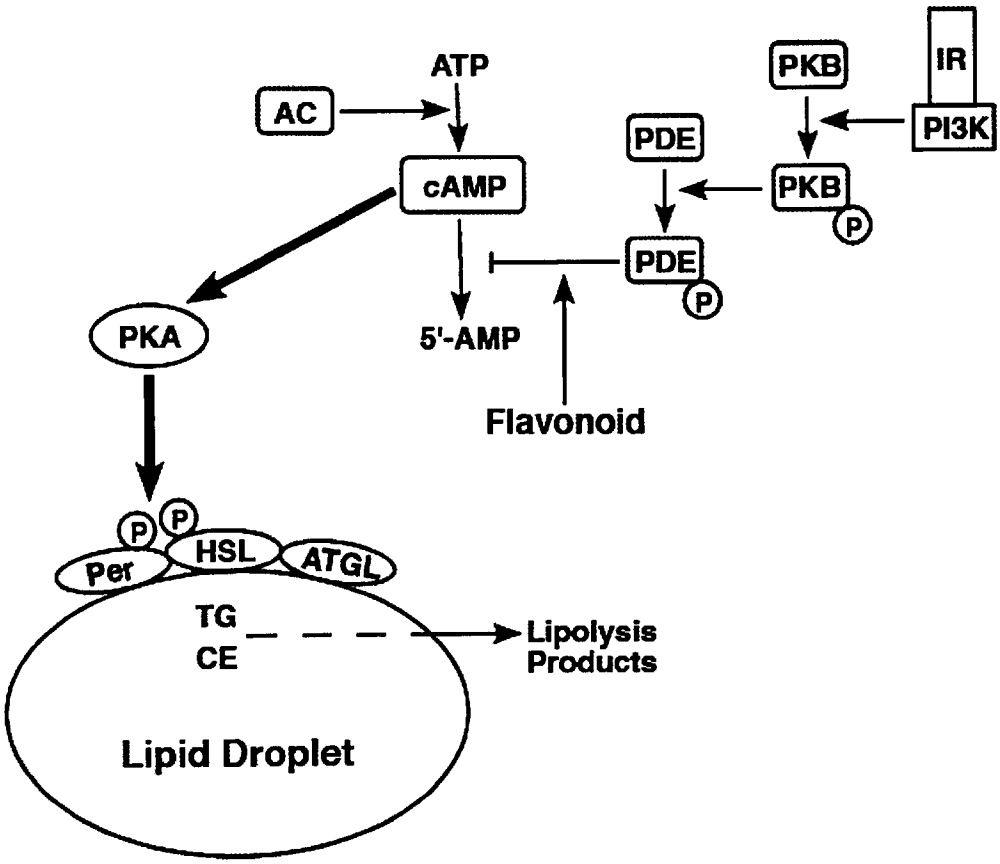


**Figure 4.** Schematic representation of a model for stimulation of neutral lipid hydrolysis by plant flavonoids through PDE Inhibition in adipose tissue. Adenylyl cyclase catalyzes cAMP synthesis from ATP. Cyclic AMP activates PKA, which phosphorylates and activates HSL and perilipin. Phosphorylated perilipin facilitates HSL translocation to the lipid droplet for lipid mobilization (85, 86), which is initiated by a recently discovered adipose triglyceride lipase (87). PDE3 and PDE4 catalyze cAMP degradation to 5'-AMP. Insulin signaling through its receptor activates phosphoinositide 3-kinase, which phosphorylates and activates protein kinase B, which in turn phosphorylates and activates PDE. Thus, insulin has an antilipolytic effect via stimulation of cAMP degradation and termination of signaling through the cAMP pathway (88). AC, adenylyl cyclase; ATGL, adipose triglyceride lipase; CE, cholesteryl ester; IR, insulin receptor; Per, perilipin; PI3K, phosphoinositide 3-kinase; PKB, protein kinase B; TG, triglyceride.

compliance, the lipolytic effect of flavonoids very likely involves PDE inhibition.

## Flavonoid Modulation of Lipid Homeostasis in the Liver

The liver is the central organ of whole-body cholesterol homeostasis, and it is the primary organ involved with the distribution of VLDL triglyceride to peripheral tissue. Hepatic overproduction of apolipoprotein B (apoB) and VLDL is associated with obesity, hepatic steatosis, and Type 2 diabetes, and it forms the basis for the development of combined hyperlipidemia, metabolic syndrome, and coronary heart disease (97). Hepatic secretion of apoB is largely dependent on the availability of lipids (cholesterol, cholesteryl ester, triglyceride, and phospholipid) for complexation with apoB and VLDL assembly (98). Although a hypolipidemic effect of dietary flavonoids has not been demonstrated, several *in vitro* studies indicate that flavonoids can reduce the availability of lipid substrates for hepatic VLDL production. Isoflavones were shown to inhibit the activity of the catalytic domain of 3-hydroxy-3-methylglutaryl (HMG) CoA reductase, the rate-limiting enzyme for cholesterol synthesis (99). In HepG2 cells, isoflavones elevated HMG-CoA reductase mRNA and protein, as well as the mature form of sterol regulatory element binding protein-2, a transcriptional inducer of the HMG-CoA reductase gene (100). Similar effects have been shown for the statin drugs, which are competitive inhibitors of HMG-CoA reductase. Other flavonoids such as taxifolin, hesperetin, and naringenin were shown to suppress lipid synthesis and VLDL production in HepG2 cells (101–103). The mechanism responsible for attenuation of lipogenesis by flavonoids in cultured liver cells has not been elucidated, and similar effects *in vivo* remain to be clearly demonstrated.

In addition to biosynthetic pathways, the mass of neutral lipid in hepatocyte cytosolic lipid bodies is regulated by lipid hydrolysis. Dietary studies have shown that soy isoflavones and citrus bioflavonoids can reduce liver cholesteryl ester and triglyceride mass and the extent of hepatic steatosis in rats and mice (36, 70, 104–106). The soy isoflavone genistein has been shown to inhibit several PDE

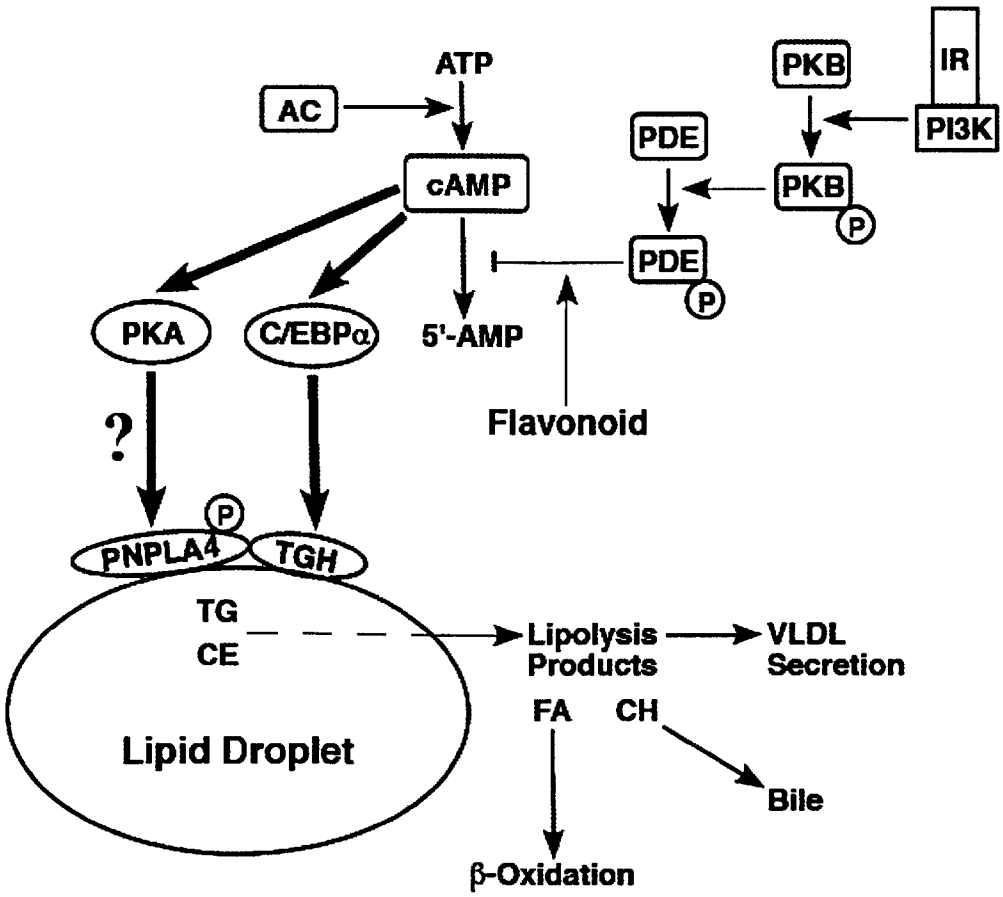


**Figure 5.** Schematic representation of a theoretical model for stimulation of neutral lipid hydrolysis by plant flavonoids through PDE Inhibition in the liver. See text for discussion. AC, adenylyl cyclase; CE, cholesteryl ester; CH, cholesterol; FA, fatty acid; IR, insulin receptor; Per, perilipin; PI3K, phosphoinositide 3-kinase; PKB, protein kinase B; TG, triglyceride.

isoforms and to enhance cellular cAMP accumulation in numerous cell types (14, 107–110). Furthermore, daidzein, hesperetin, prunetin, apigenin, diosmetin, and myricetin were shown to inhibit several PDE isoforms isolated from guinea pig lung and heart (15). In rat hepatocytes, genistein was shown to augment the glucagon-mediated elevation in cAMP (111). Collectively, the results of these studies suggest that flavonoids could induce neutral lipid hydrolysis in the liver through a mechanism involving PDE inhibition and cAMP-mediated PKA activation, similar to that observed in adipose tissue. This concept is supported by studies showing cAMP-mediated triglyceride mobilization in rat hepatocytes (112) and cAMP-mediated activation of triglyceride lipase and cholesterol esterase activities through PKA in mouse myocardial cells (113).

Elevated liver neutral fat content is part of the etiology of nonalcoholic steatohepatitis, hyperlipidemia, obesity, insulin resistance, cardiovascular disease, and Type 2 diabetes (114). Hepatic steatosis (fatty liver) was estimated to affect 33% of the participants in the Dallas Heart Study (115). Hepatocyte neutral lipid hydrolysis is regulated by a microsomal carboxylesterase (CES1 in human liver), also referred to as triacylglycerol hydrolase (TGH), which similar to hormone-sensitive lipase (HSL), possesses cholesterol ester hydrolase activity (116, 117). Although a cholesterol ester hydrolase that is activated through phosphorylation by PKA has been demonstrated in rat liver (118), CES1 (TGH) apparently is not activated by phosphorylation (119). Figure 5 shows a theoretical model for the induction of hepatic neutral lipid hydrolysis by plant flavonoids through inhibition of PDE, elevation of the cellular cAMP level, activation of PKA, and induction of TGH. Cyclic AMP-induced triglyceride hydrolysis may require PKA-mediated phosphorylation and activation of one or more proteins associated with the lipid droplet (120). In addition to perilipin, which is expressed in adipose tissue, several lipid body–associated proteins with a patatin-like domain have been identified that participate in lipid hydrolysis in mammals (121). One such protein that is highly expressed in human liver, patatin-like phospholipase domain containing protein-4 (PNPLA4), also referred to as GS2, contains a putative PKA phosphorylation site (122). A flavonoid-mediated elevation in cellular cAMP could theoretically activate this protein or another lipid body–associated protein to initiate neutral lipid hydrolysis. In addition, CCAAT/enhancer-binding protein α (C/EBPα) is a cAMP-responsive nuclear regulator (123) that has been shown to induce transcription from the TGH promoter

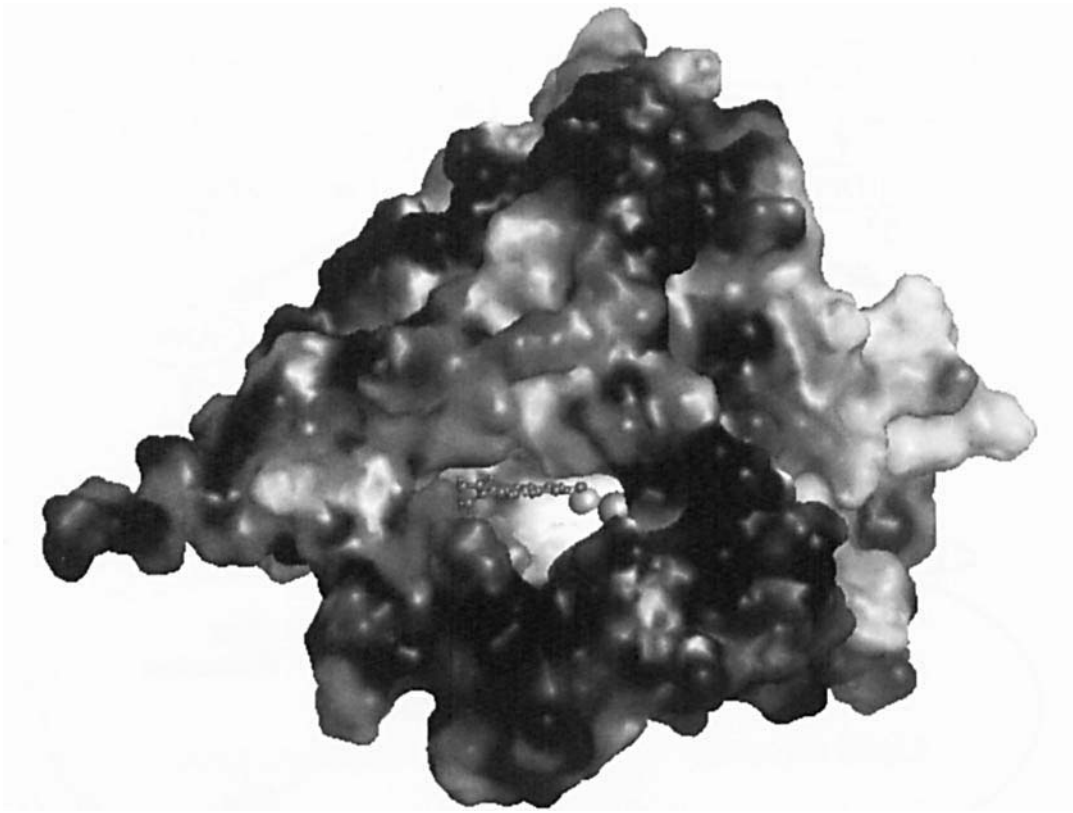

**Figure 6.** The electrostatic potential of human PDE4B2 mapped to the van der Waals surface of the x-ray crystallographic model. An x-ray crystallographic model of the catalytic domain (residues 153–487) of human PDE4B2, PDB 1ror (131), was obtained from the Protein Data Bank (7). Hydrogen atoms were added, and a reduced model was built with the program Reduce (132). The electrostatic potential was calculated with the APBS (13) and mapped to the van der Waals surface (1.4-Å probe radius) of the x-ray crystallographic model. Genistein was docked into the catalytic site using the PatchDock server (133). The electrostatic interaction of genistein with residues of the catalytic site of the enzyme was optimized using the SZYBKI Web server (http://demo.eyesopen.com/cgi-bin/szybki). Ligand-protein contacts (LPCs) were derived using LPC software (134). In this model, the normalized complementarity of genistein with the residues of the catalytic site is 80%. Highly electronegative surface areas are shaded (black), and highly electropositive surface areas are unshaded (white). The grayscale gradient from black to white was set to span between −10 kT/e and 10 kT/e. The net charge on the protein was −12.33 e. The catalytic site (cAMP binding pocket) is predominantly electropositive due to the presence of two zinc cations that would tend to attract a sterically compatible flavonoid molecule with electronegative surface charge. The graphical representation was rendered using PyMOL.

(124). An interesting adjunct to elevated liver TGH expression is the utilization of lipid hydrolysis products as part of a lipolysis-esterification pathway for the assembly and secretion of VLDL (125, 126). Studies showing an isoflavone-mediated elevation in plasma triglycerides in obese male spontaneously hypertensive rats (69), as well as a reduction in hepatic neutral lipids accompanied by an elevation in plasma triglycerides in hyperinsulinemic obese Zucker rats (71), are consistent with flavonoid-mediated PDE inhibition and induction of lipolysis as proposed in Figure 5. It has been suggested that flavonoids such as genistein and daidzein may act as PPAR ligands to stimulate lipid catabolism (71, 127, 128). However, the established PPAR ligands of biological significance are fatty acids and prostaglandins, and fatty acids, PPAR alpha, and PPAR gamma did not affect neutral lipid hydrolysis or TGH expression in liver and adipocytes (129). Flavonoid-mediated inhibition of PDE activity and subsequent induction of lipolysis may indirectly activate PPAR pathways (including fatty acid β-oxidation) through an elevation in cellular free fatty acid levels.

## Flavonoids Fit in the Catalytic Site of Some PDE Isoforms

Flavonoid-mediated PDE inhibition is dependent on the ability of the flavonoid to sterically fit in the cyclic nucleotide binding pocket (130), and findings from molecular docking investigations support the contention that many of the biological effects of plant flavonoids are attributable to competitive inhibition of specific cyclic nucleotide PDE isoforms. Indeed, flavonoids such as apigenin, genistein, daidzein, and quercetin fit very well in the catalytic site of x-ray crystallographic models of human PDE3B, PDE4B, and PDE4D.[1] Figure 6 (7, 13, 131–134) shows the electrostatic potential mapped to the van der Waals surface of the x-ray crystallographic solution model

[1] M.R.P., unpublished data, 2005.

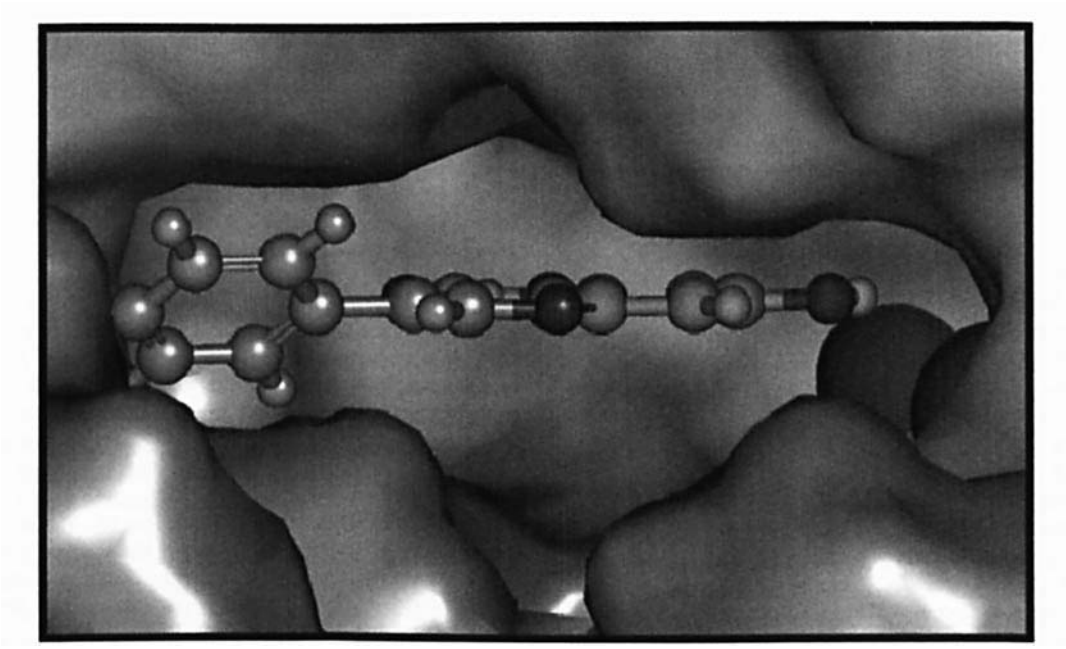

**Figure 7.** Close-up view of genistein in the substrate binding cavity of human PDE4B2. Genistein was docked in the catalytic site of human PDE4B2 as described in the legend to Figure 6. The electrostatic surface shading is not shown. The two zinc cations are shown on the right as spheres. The graphical representation was rendered using PyMOL.

of the catalytic domain of human PDE4B2, with the isoflavone genistein docked at the catalytic site. Highly electronegative surface areas of the protein are shaded (black), and highly electropositive surface areas are unshaded (white). Note the electropositive area, primarily due to the presence of two zinc cations that are essential for catalysis, on the internal surface of the catalytic site (lower center of the PDE4B2 protein). Thus, the PDE4B2 catalytic site would tend to attract a flavonoid molecule with electronegative surface charges both sterically and electrostatically. A close-up view (without electrostatic surface shading) of the internal surface of the PDE4B2 catalytic site with docked genistein is provided in Figure 7.

## Conclusions and Future Directions

Population investigations indicate that flavonoid consumption is inversely related to mortality from coronary artery disease. Aberrant lipid homeostasis and lipid accumulation in liver and adipose tissue are part of the root etiology of atherosclerosis, as well as associated conditions such as obesity, hepatic steatosis, and Type 2 diabetes. Through PDE inhibition, flavonoids can induce neutral lipid hydrolysis from lipid stores. Therefore, flavonoids may potentially be used as part of a treatment regimen for obesity, hepatic steatosis, and Type 2 diabetes. However, some flavonoids (dietary isoflavones) have been shown to elevate fasting plasma triglyceride and glucose levels in obese hyperinsulinemic Zucker rats, and the therapeutic effectiveness of dietary plant flavonoids may depend on suppression of lipogenesis and efficient metabolism and elimination of lipolytic products. Indeed, flavonoid-mediated PDE inhibition and elevation in cellular cAMP may theoretically have additional related metabolic implications that could be explored. Cyclic AMP inhibits the posttranslational processing of sterol regulatory element binding protein-1 to the mature transcriptionally active form (135), and cAMP inhibits transcription of the malic enzyme gene (136), suggesting that flavonoid-mediated elevations in cAMP may retard lipogenesis. Cyclic AMP also upregulates expression of carnitine palmitoyltransferase-1, which is rate limiting for mitochondrial fatty acid import and oxidation (137). A potential contraindication of elevated cellular cAMP is upregulation of phosphoenolpyruvate carboxykinase and gluconeogenesis (138). Future investigation of lipolytic and additional metabolic effects of plant flavonoids may lead to more effective strategies for the treatment of cardiovascular disease, as well as associated conditions such as obesity, hepatic steatosis, and Type 2 diabetes.

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