Nutritional Sciences 6(1), February, 2003, pp. 31-41

Cholesterol-lowering Properties of Citrus Flavonoids and Polyphenolic Compounds and Their Relevance to Antioxidative Activity*

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INTRODUCTION

The positive role of dietary polyphenolics such as citrus flavonoids in human health is the object of growing scientific interest. Over the past several years, dietary polyphenolics are reported to have anti-allergic, anti-inflammatory, anti-hypertensive and diuretic effects, as well as anti-cancer and hypolipidemic properties. Epidemiological evidence suggests that consumption of fruits and vegetables may reduce the risk of some forms of cancer and cardiovascular disease, and it is hypothesized that this may be due to their polyphenolic content. As a consequence, there is considerable interest in investigating the anti-atherogenic nature of these compounds. Polyphenolic compounds are naturally- occurring molecules abundant in fruits, vegetables, nuts, seeds, teas, and wine. Variations in the structure of polyphenolic compounds give rise to the major classes of flavonoids, dihydroflavonols, flavones, flavanones, catechins, anthocyanidins, isoflavones, dihydroflavonols, and chalones.

We have studied the cholesterol-lowering actions of various polyphenolic compounds such as naringin, naringenin, hesperidin, hesperetin, cinnamate, rutin, gallate, quercetin, and tannic acid. The roles of these compounds have recently received considerable attention with particular interest in their use as cholesterol-lowering and anti-atherogenic agents. This review focuses both on the authors' recent *in vivo* experiments designed to investigate the cholesterol-lowering potential of some bioflavonoids and polyphenolic compounds, and on other researchers' findings from similar *in vitro* and *in vivo* experiments.

STRUCTURES

Phenolic compounds are ubiquitous in the plant kingdom and the term refers to substances that possess an aromatic ring bearing one or more hydroxyl substituents. From this structure, larger molecules such as anthocyanins, coumarins, phenylpropamides, flavonoids, tannins, and lignin are formed. The terms polyphenols and phenolic are all-encompassing, ranging from simple phenolic acid to polymerized compounds like tannins. Taking the plant kingdom as a whole, polyphenols or phenolic compounds account for well over 4000 individual compounds. Phenolic compounds perform a variety of functions in plants including defence against herbivores and pathogens, light absorption, attraction of pollinators, reductions in the growth of competitive plants, and promotion of symbiotic relationships with nitrogen-fixing bacteria. In addition to wine and tea, polyphenols are found in many commonly-eaten fruits and vegetables, such as grapes, apples, grapefruits, onion, eggplant, and kale, as well as herbs, spices, cereals, legumes, and nuts.[1),2)]

There are a number of biosynthetic pathways used in the formation of phenolic compounds. Formation of phenolic acids occurs via reductive amination of phenylpyruvic acid to phenylalanine, which can undergo deamination and cytochrome P450-mediated hydroxylation to give either tyrosine or cinnamic acid and its hydroxylated analogues in plants (Fig 1).[3)] These hydroxylated phenylpropanoid analogues are the so-called polyphenolics. Hydroxylation of the cinnamic acid precursor usually first occurs in the para position followed by sequential hydroxylation at each of the meta positions. Species variation governs the degree of hydroxylation and methylation of these compounds. Polyphenols have generally been classified into three major groups: (1) simple phenols and phenolic acids, (2) flavonoids, and (3) hydroxycinnamic acid derivatives.[1)] In the flavonoids, the typical 5,7-hydroxyl pattern of the A ring and the 4'-, 3',4'- and 3',4',5'-pattern of the B ring are derived from different pathways. The major structural features of the chalcone, flavonol and flavone groups are shown in Fig 1.[3)] The isoflavonoids are formed by cyclization of the chalcones such that the B ring is located at the 3 position,

* This work was supported by Korea Research Foundation Grant (KRF-2001-005-D00020).
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as also indicated in Fig 1. Other major groups of flavonoids include the catechins (often occurring as esters with gallic acid in tea) and the anthocyanidines, which are highly-coloured pigments.

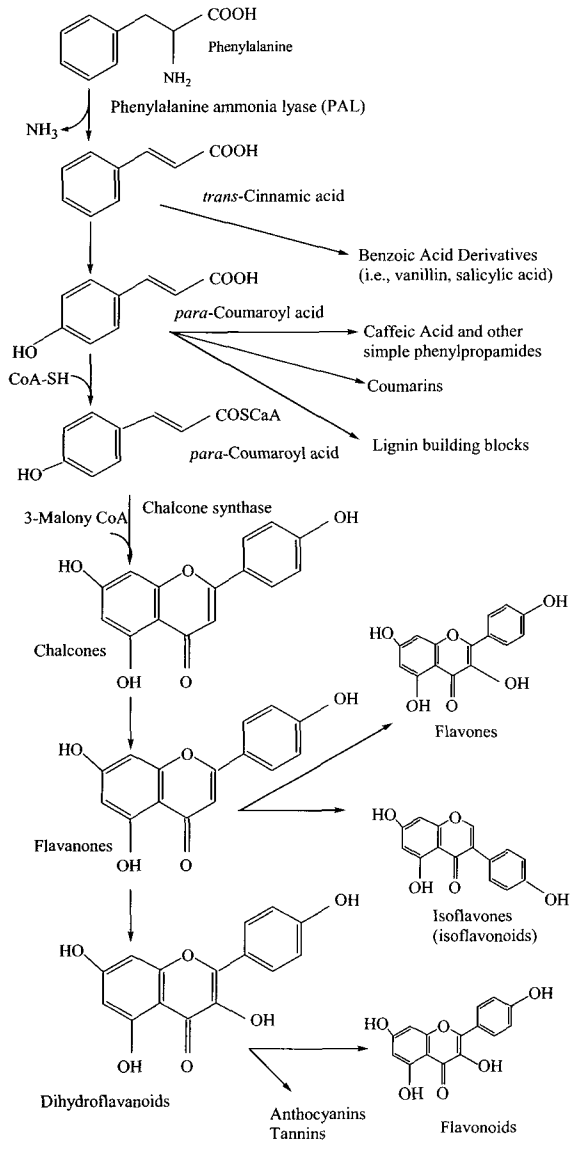

Fig 1. Production of plant phenolic molecules via phenylalanine[3]

DISTRIBUTION AND DIETARY SOURCES OF PHENOLIC ACIDS

The phenolic acids are known to be involved in the formation of lignin, which is a polymer present in the cell walls of plants. These phenolic compounds are present in abundance wherever vascular bundles or structural features of plants are found. They play a role in the structural stability of plant material by virtue of the fact that their chemical structure enables them to form a variety of esters and ester cross-linkages. Thus, the seeds and skins of fruits, and the stems and leaves of vegetables, are rich sources of phenolic acids. Red wine and tea are known to provide concentrated sources of these compounds.[4),5)] Presently, there are very limited amounts of food consumption data available for the polyphenols.

There are, however, many individual studies in which levels of phenolic acids in selected samples of specific foods and beverages have been quantified. Red wine has been found to contain phenolic acids in concentrations of approximately 200 mg/l.[6)] Table 1 presents a range of fruits,[7)-13)] vegetables,[14),15)] and grains[15),16)] that are good sources of phenolic acids.

Table 1. Some dietary sources of phenolic acids

Source	Compounds
Red wine and grapes	Cinnamic acid derivatives, flavonoids
Tea	Gallic and caffeic acid, complex polyphenols
Apricots	Caffeic acid (as chlorogenic acid)
Cherries	Caffeic and gallic acids
Apples and peaches	Caffeic, coumaric and ferulic acids
Raspberry	Ellagic, gallic and cinnamic acids
Apple cider	Caffeic acid
Citrus fruit	Cinnamic acids
Olives, olive oil	Dihydroxyphenylethanol (tyrosol), oleuropeine
Alfalfa, cabbage, spinach	Cinnamic acids, mainly caffeic acid
Wheat	Cinnamic acid derivatives, minor amounts of coumaric and caffeic acids
Potato	Caffeic, gallic and protocatechuic acids

BIOAVAILABILITY AND METABOLISM AFTER INGESTION

An understanding of the absorption and bioavailability of phenolic compounds is critical before evaluation of their biological activity or potential nutritional value can be made. In general, the absorption, distribution, metabolism and excretion of dietary phenolic compounds are little studied in humans.[17)] Because such compounds have enormous variability and occur as complex mixtures in plant materials, it is difficult to study their bioavailability and their physiological effects. Polyphenols rarely occur in a free form.

Earlier studies in the United States estimated that the daily intake of flavonoids was about 1 g/day when expressed as glycosides, or 650 mg/day when expressed as aglycones.[18)] Hollman and colleagues,[19)] however, have questioned these values as too high, and others have estimated that the average intake of all flavonoids from

dietary sources is between 23 and 170 mg/day.[20),21)] In a Dutch study, daily intake of all flavonoids was estimated at 23 mg/day with quercetin accounting for 16 mg/day;[20),21)] this is in keeping with the observation that, of the flavonoids, quercetin is generally found in the highest concentration in food. Its concentration in grapes is reported as 1.4 mg/kg, whereas green tea contains >10,000 mg/kg of quercetin glycosides and kaempferol.[22)] In addition, Hollman and colleagues[19)] reviewed six studies and concluded that the average daily flavonol intake as 4 to 68 mg/day. Interestingly, on a mg/day basis, flavonoid intake exceeds the average daily requirement of vitamin E and beta-carotene.

The absorption characteristics of polyphenols vary depending on the type of food, the chemical form of the polyphenols, and their interactions with other substances in the food, such as protein, ethanol, and fiber. Taking onions as an example, quercetin absorption was about 52% from quercetin glucosides, about 17% from quercetin rutinoside, and about 24% from quercetin aglycone;[22)] urinary excretion was about 0.5% of the amount absorbed. Flavonoids such as quercetin can be absorbed either as the free aglycone or as glycoside, as demonstrated by detection in blood and urine following consumption of non-supplemented diets.[23-25)] It has also been reported that polyphenols from wine may be better absorbed than the same substances from fruits and vegetables, because the ethanol in wine may enhance the breakdown of the polyphenols into smaller-sized products that are absorbed more readily.[26)]

Studies indicate that polyphenols can be absorbed in experimental animals and humans after ingestion. Formerly, it was expected that polyphenol glycosides would remain unchanged in most of the gut, since it was presumed that humans lack the appropriate β-glycosidases to metabolize the sugars. However, the glycosidases originating from bacteria that colonize the ileum and cecum can break down flavonoids. For instance, the flavonoid glycosides ingested by germ-free rats are recovered intact in the feces.[27)] Others have found that the administration of 0.5 g/day of catechin or tannic acid to rats over a 3-week period resulted in less than 5% of these substances being excreted unchanged in the feces.[28)] Moreover, glycones from onions have been shown to cross through the mucosal layer of the intestinal cells, suggesting that hydrolases may be present in humans to remove sugar components, forming aglycones.[29)] Further research is needed before it can be confirmed that deglycosylation of flavonoids can occur independently of gut microbial action. Between 10% and 20% of oral doses of quercetin were absorbed in some animal[30)] and human studies[31)], whereas only 0.5% of the quercetin was excreted unchanged after tea drinking in another study.[32)] Several studies have found that a wide range of phenolic compounds enter the circulation, and are found in the plasma or excreted in urine.[24),33),34)] In general, peak blood levels of flavonoids occur between 2 and 3 hours after consumption, and its half-life varied between 5 and 17 hours depending on the particular flavonoid or the food source[35),36)].

Dietary intake of citrus flavonoids is substantial, especially in countries with a high consumption of citrus juices. However, the bioavailablity of these compounds is still poorly understood. Recent studies in humans demonstrated that the free forms of hesperidin and naringin present in citrus juices can be absorbed into the blood system,[37)] most likely following their liberation from the glycosides by intestinal bacteria.[38)] Some flavonoids, especially those possessing methoxy groups such as hesperetin and polymethoxylated flavonoids, were reported to remain longer in the body due to their facilitated uptake by cells.[38)] Plasma levels of caffeic acid and the gallic acid metabolite 4-*O*-methylgallic acid are significantly increased within 1-4 hours following consumption of red wine.[39)] Caffeic acid is absorbed from the gut by rabbits,[40)] and Jacobsen et al.[34)] have observed the absorption, metabolism and excretion of caffeic and ferulic acids in humans. More recently, Bourne and Rice-Evans[41)] identified the urinary excretion of chlorogenic, caffeic, p-coumaric and ferulic acids, and rutin, in people consuming tomatoes and apricots, as well as the excretion of anthocyanidine following the consumption of raspberries. While many aspects of flavonoid and phenolic acid absorption and metabolism remain unknown, there is enough evidence to suggest that some of these compounds will be absorbed in sufficiently high concentrations to have physiological effects.

The liver seems to be the primary site of polyphenol metabolism, although other sites such as the kidney or intestinal mucosa may be involved. In the liver, these compounds can undergo methylation, hydroxylation, reduction of the carbonyl group in the pyrane ring, and conjugation reactions. The most common degradation pathway for flavonoids is through conjugation with glucuronides or sulfate.[42)] In addition, some flavonoid metabolites can be recycled via the enterohepatic biliary route. Improvements in HPLC and gas chromatography-mass spectrometry (GC-MS) methodologies for measuring phenolic compounds or their metabolites in plasma and urine will continue to provide useful information in this area.

POTENTIAL CARDIOPROTECTIVE EFFECTS, BASED ON EPIDEMIOLOGICAL STUDIES

Many epidemiological studies suggest that dietary flavonoid intake is inversely related to mortality from

coronary heart disease (CHD); this association is thought to be largely due to the importance of cholesterol, especially low-density liporotein (LDL) cholesterol, in the formation and development of atherosclerotic plaques. Blood concentrations of total and LDL cholesterol are influenced by diet, and dietary strategies are commonly used to reduce LDL cholesterol levels. During recent years, a number of reports have suggested that one of the possible ways of improving blood lipid profile is via an increased intake of flavonoids.[43]

In the Zutphen elderly study[20] where eight hundred and five men from the Netherlands were studied over 5 years to observe possible associations between flavonoid consumption and reduced cardiovascular mortality, it was revealed that - after adjustment for age, weight, certain risk factors of coronary artery disease, and intake of antioxidant vitamins - the highest tertile of flavonoid intake (primarily from tea, onions, and apples) had a relative risk of heart disease of 0.32 compared with the lowest tertile. Similar associations between flavonoid intake and reduced CHD were reported in men and women from Finland,[44] and in men from the Seven Countries Study which included cohorts from Finland, Greece, Yugoslavia, Japan, Netherlands, Italy and the United States.[45] Hertog et al.[46] reported increased mortality from ischemic heart disease in Welsh men consuming high amounts of flavonols, mainly from tea. In this study, however, the tea consumption was associated with a lower social class and a less healthy lifestyle, which included cigarette smoking and a higher fat consumption. Although substantial epidemiological evidence suggests that flavonoids have a protective effect against cardiovascular disease, there are only a few studies that have investigated the exact associations between intakes of specific flavonoids or polyphenolic compounds and coronary heart disease.

ROLES IN CHOLESTEROL-LOWERING AND LIPOPROTEIN METABOLISM

Some bioflavonoid compounds have been associated with the prevention of chronic diseases such as cancer and hyperlipidemia.[47),48]. Cardioprotective effects of flavonoids appear to be largely related to their action as antioxidants and as inhibitors of platelet aggregation[43]. Some flavonoids were reported to produce cholesterol-lowering properties in animals and in cell culture system. A number of reports have suggested that polyphenolic compounds may influence atherogenesis through an effect on cholesterol and lipoprotein metabolism.[49)-57]

Among the plant flavonoids previously investigated for their possible cholestrol-lowering potential, the best known are isoflavones from soybeans, consisting mainly of genistein. Dietary soybean isoflavones caused decreases in VLDL (very low-density lipiprotein) and LDL cholesterol in some animal models.[58),59] These beneficial changes were not also confirmed in other animal and human studies.[60)-62] The principal citrus flavonoids, hesperetin from oranges and naringenin from grapefruit, are structurally similar to genestein. Hesperidin and a mixture of flavonoids containing mainly hesperidin and naringin were also reported to produce hypolipidemic effects in cholesteol-fed rats.[48),63] This suggests that citrus flavonoids, and the juices from which they originate, could have cholesterol-lowering potential.

Investigations of naturally-occurring compounds as regulators of triglyceride and cholesterol metabolism can potentially have substantial therapeutic significance, as evidenced by the discovery of the HMG-CoA reductase inhibitors derived from fungal fermentation products, which are now widely used for the treatment of hyperlipidemia. Flavonoids might represent another beneficial group of naturally-occurring hypolipidemic compounds. Our studies of rats, and other studies, have shown that the flavonoids quercetin,[50),64] hesperetin,[48),65] naringin,[66] hesperedin,[67] naringenin,[68] gallate,[64] tannic acid,[69] cinnamate,[70] rutin,[69] marsupin,[54] pterosupin,[54] liquiritigenin,[54] biochanin A,[55] formononetin,[55] and pratensein[55] cause significant reductions in serum or plasma lipids. In non-human primates, dietary genistein, the isoflavone analog of naringenin, significantly reduced plasma LDL and VLDL cholesterol levels.[49]

Studies in hyperlipidemic rats[52] fed high-fat diets showed that intraperitoneal (i.p.) administration for three days of a methanolic extract from *Prunus davidiana* containing the flavonoid components catechin, naringenin 7-O-glucoside (prunin) and hesperetin 5-O-glucoside resulted in significant reductions in blood triglycerides (TG) and total cholesterol (TC). Furthermore, naringenin 7-O-glucoside or hesperetin 5-O-glucoside, when administered alone at doses of 20 mg/kg or 10 mg/kg, respectively, showed significant hypocholesterolemic effects. In a related study by the same investigators,[53] the effects of naringenin 7-O-glucoside (prunin) in streptozotocin-diabetic rats were examined; a single i.p. administration of prunin (10 mg/kg) caused significant decreases in plasma glucose, TG, and TC. In a recent study of rabbits fed a semi-purified, cholesterol-free, casein-containing diet, Kurowska et al.[71] reported that replacing drinking water with either grapefruit or orange juice resulted in significant reductions in the elevation of serum LDL cholesterol (by 43 and 32% respectively) and of hepatic cholesteryl ester (CE) levels. This rabbit model is very useful for the cholesterol study since it is characterized by an overproduction of hepatic apoB (apolipoprotein B-containing lipoproteins).[72] It was hypothesized that the hypocholesterolemic effects of the

juices were due to their flavonoid components (naringenin in grapefruit juice and hesperetin in orange juice). Subsequently, the effects of these flavonoids on the secretion of apoB-containing lipoproteins by the human hepatoma cell line, HepG2, were examined. Naringenin and hesperetin dose-dependently reduced the accumulation of apoB in culture media; for example, reductions of 76-81% were achieved at doses of 200 μM over 24 hours[52]. Reduced hepatic secretion of apoB-containing lipoproteins would be expected to be attributed to the hypocholesterolemic effects of naringenin *in vivo*.

Recent studies by Wilcox et al.[56] have further investigated the effects of naringenin on lipid and lipoprotein metabolism in HepG2 cells. Significant reductions in apoB secretion were observed (83% reduction at doses of 200 μM, $p<0.002$) after a 24-hour incubation with naringenin. Importantly, Wilcox et al.[56] demonstrated that naringenin (at 200 μM) reduced the secretion of newly-synthesized CE, TG, and phospholipid (PL). Naringenin significantly reduced intracellular CE mass, primarily due to a dose-dependent inhibition of CE synthesis (up to a reduction of 89% at doses of 200 μM of naringenin). The mass of free cholesterol and levels of TG were unaffected. The observed effect on hepatic CE synthesis may be explained, in part, by an inhibition of hepatic ACAT. Inhibition of ACAT probably reduces the hepatic production of apoB-containing lipoproteins,[73] possibly by limiting the availability of newly-synthesized CE for association with apoB during assembly of the lipoprotein. Inhibition of hepatic ACAT has been demonstrated for the flavonoid, baicalein (IC_{50} at a dose of approximately 100 μM in isolated rat hepatic microsomes)[57] that is structurally different from naringenin. Whether flavonoids in general can reduce apoB secretion and inhibit ACAT activity remains to be determined. In further studies from Wilcox et al.,[56] naringenin (at 200 μM) caused a significant 50% decrease in the mRNA for the microsomal triglyceride transfer protein (MTP), a protein known to be essential for the assembly and hepatic secretion of apoB-containing lipoproteins.[74] Together with the inhibition of ACAT, these results suggest potential mechanisms whereby naringenin may reduce the plasma concentrations of apoB-containing lipoproteins such as VLDL and LDL.

In vivo studies of animals investigated whether flavonoids could produce similar effects to the *in vitro* studies. It is important to reduce excess cholesterol to a level that is adequate for the maintenance of normal body functions. The regulation of plasma cholesterol levels involves factors that influence both extracellular and intracellular cholesterol metabolism. The two key enzymes involved are 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase and acyl CoA : cholesterol O-acyltransferase (ACAT). HMG-CoA reductase inhibitors (Fig 2)[75] are very effective in lowering plasma cholesterol in most animal species including humans,[76] and these inhibitors are now widely used in hypocholesterolemic drugs.[77] ACAT catalyzes the intracellular esterification of cholesterol. ACAT is also involved in cholesterol absorption, hepatic VLDL-cholesterol secretion, and cholesterol accumulation in the vascular wall[78] by catalyzing cholesterol esterification. For these reasons, ACAT inhibitors were used in test drugs as cholesterol-lowering agents as well as antiatherosclerotic agents. In recent years the use of HMG-CoA reductase inhibitors for lowering plasma cholesterol levels has increased.

Our own recent research data suggest that the two citrus bioflavonoids (naringin and hesperidin) and their aglycones (naringenin and hesperetin) are effective in altering cholesterol-regulating enzymes. We first identified that a mixture of naringin and hesperidin (0.5g/100g body weight), and tangerine-peel extract that contained the equivalent of the total flavonoids in the naringin and hesperidin mixed diet, lowered plasma and hepatic cholesterol levels in rats fed a high-cholesterol diet through the inhibition of hepatic HMG-CoA reductase and possibly ACAT.[63] When doses of naringin were reduced to 0.1 % (wt/wt) in high cholesterol diets fed to rats, plasma cholesterol levels were also lowered.[79] Further, we showed that naringenin and hesperetin (aglycones of naringin and hesperidin) exhibited the same cholesterol-lowering effect as their glycones when used as supplements at a dose of 0.1% (wt/wt) in high cholesterol diets.[65),68] Moreover, when the dose of naringin was further reduced (to 0.02g or 0.05g/1000g) and supplemented to a high fat (15g lard/100g) and high-cholesterol (1g/100g) diet in rats,[66] the concentrations of plasma total cholesterol were significantly lowered. In later studies, we confirmed that low doses of naringin (0.02% in high cholesterol and/or high fat diets) are very effective in lowering cholesterol and in inhibiting HMG-CoA reductase in rats. Two cinnamate derivatives, 3,4-di(OH)-cinnamate and 3,4-di(OH)-hydrocinnamate, also lowered HMG-CoA reductase activity with a simultaneous decrease in plasma cholesterol in rats fed a high cholesterol diet.[70] Furthermore, rutin and tannic acid significantly lowered hepatic HMG-CoA reductase and ACAT activity, and plasma cholesterol concentration when supplemented with a high cholesterol diet in rats.[69]

Reduced ACAT activity may lead to less cholesteryl ester (Fig 2)[75] being available for VLDL packing, thereby resulting in a reduction of VLDL secretion from the liver. Another hypothesis is that the potential increase of hepatic cholesterol uptake by LDL receptors could accelerate cholesterol catabolism in citrus bioflavonoid-supplemented rats. Naringin and hesperidin can be converted to narigenin and hesperetin in the intestine.

Interestingly, neither naringin, hesperidin nor their respective alglycones inhibited HMG-CoA reductase and ACAT *in vitro* ($IC_{50} > 1$ mM),[65),68)] suggesting that both flavonoids may undergo some structural changes to become active metabolites either in the liver or other organs.

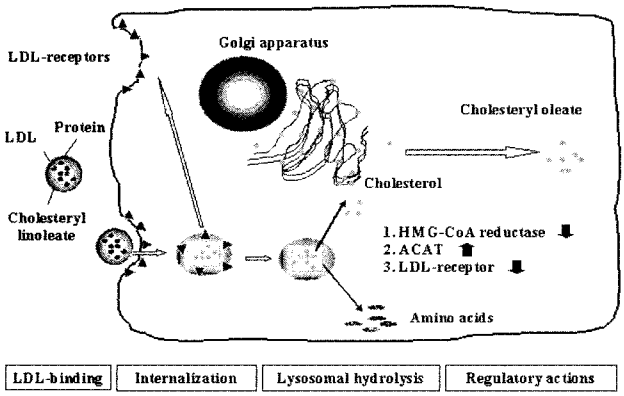

Fig 2. Diagrammatic respresentation of uptake and partial degradation of LDL via the LDL receptor pathway[79)]

EFFECTS ON ATHEROSCLEROSIS AND RELEVANCE TO ANTIOXIDANT ACTIVITY

Due to the strong evidence that active oxygen species are involved in a variety of disorders, the role of antioxidants in limiting oxidative stress recently increased attention. It is now hypothesized that LDL cholesterol is linked to atherosclerosis and the risk of ischemic heart disease[80)]. Although the mechanisms through which high plasma LDL concentrations increase the risk of CVD are not completely understood, evidence is emerging to implicate the oxidation of LDL by free radical by-products or via the process of oxidative injury. The LDL oxidation hypothesis in cardiovascular disease suggests that oxidized-LDL contributes to all stages of the atherosclerotic process, including activation of inflammatory events, endothelial damage, recruitment of macrophages, and the unregulated uptake of oxLDL by macrophages to form foam cells (Fig 3), which are the marks of early atheorsclerotic lesions.[81)].

The extent to which flavonoids can affect endogenous antioxidant concentrations is not yet determined. This is true from a number of perspectives including the range of flavonoids that can exert these effects, dose-response relationships for the effects, the range of body sites that can be affected, and the circumstances under which the effects will occur. Antioxidant defense mechanisms also include enzymes such as superoxide dismutase (which removes superoxide), glutathione peroxidase (which converts hydrogen peroxide to water and also converts various hydroperoxides to less harmful hydroxides) and catalase (which can also break down hydrogen peroxide). In mice, inclusion of green tea in the drinking water increases colon levels of superoxide dismutase (Cu-Zn SOD).[82)] Flavonoids can also affect concentrations of the antioxidant glutathione. In rodent studies, there is considerable specificity for flavonoid types and affected tissues. A number of different flavonoids are reported either to increase gasterointestinal glutathione concentrations or to prevent their depletion by inflammatory stress.[83)-85)] Catechin ingestion partially protected against lung lipid peroxidation caused by diethylmaleate, a chemical that depletes lung glutathione.[86)] Activities of glutathione peroxidase and glutathione reductase are increased in livers of rats fed isoflavone-containing soy protein isolate[87)] and in mouse skin by feeding the isoflavone genestein.[88)] Consumption of a green tea extract by mice induces moderately-high activity levels for liver glutathione reductase and very high activity levels for glutathione peroxidase in three tissues.[89)] In humans, plasma glutathione peroxidase activity increases after 10 days of consumption of quercetin-rich juices.[90)] There are some studies of flavonoids and catalase, another antioxidant enzyme. Some of these report that flavonoid supplementation does not affect rat liver catalase activity.[91)] Genestein[92)] and green tea[89)] were reported to increase catalase activity in many tissues.

More research is needed into the whole area of how flavonoids affect endogenous antioxidants. In particular, more research is needed to elucidate the full biological importance of the polyphenolics-endogenous antioxidant relationship. Much of the observed association between flavonoid intake and reduced CHD mortality has been attributed to the antioxidant properties of the flavonoids.[93)-95)] There is some evidence to suggest that flavonoids can be incorporated into lipoprotein particles.[96),97)] Naringenin has also been shown to associate with and penetrate lipid membranes[95)]; as a result, flavonoids, including naringenin, may be ideally located for protecting LDL from oxidation.

While there are numerous *in vitro* examples of dietary phenolic compounds acting as antioxidants, there are only limited studies examining *in vivo* effects. Most *in vivo* studies have been undertaken due to increased interest in the potential antioxidant effects of beverages such as red wine and tea. These beverages contain a wide range of phenolic compounds, including flavonoids and phenolic acids. Of the *in vivo* studies conducted to date, most have used indirect measures of lipid and lipoprotein damage, or non-specific measures of plasma antioxidant capacity. We reported that curcumin, the yellow pigment in turmeric, curry and mustard, not only exhibits cholesterol-lowering properties in cholesterol-fed rabbits, but also antioxidative and anti-inflammatory activities.[98)]

Curcumin also significantly decreased the formation of fatty streaks and atheromatous plaques on the intima of rabbit aortas.[98] Another study was designed to test the lipid-lowering and antioxidative activities of rutin and tannic acid (supplemented at 1g/kg diet) in high-cholesterol fed rats; the rutin and tannic acid significantly lowered plasma lipid and hepatic cholesterol levels.[69] The overall potential of the antioxidant system was significantly enhanced by the rutin and tannic acid supplements, as plasma and hepatic TBARS levels were lowered while hepatic SOD and GSH-Px activities were increased in the high-cholesterol fed rats.[69]

Recently, some bioflavonoids have clearly been shown to be potent antioxidants and to have pharmacokinetic properties similar to those of vitamin E.[99] Several studies suggest an inverse correlation between the dietary intake of bioflavonoids or plasma concentrations of antioxidants, and cardiovascular disease.[100] The interactive effects of naringin and vitamin E[101], and hesperidin and vitamin E,[67] were studied with respect to cholesterol metabolism and antioxidant status. The usual vitamin E requirement for most of the frequently-used strains of rats is a 27 IU/kg diet when lipids comprise less than 10% of the diet.[102] When two different levels of dietary vitamin E (5 IU and 50 IU/kg diet), along with 0.1% (wt/wt) of naringin, were given with a high cholesterol diet for 5 weeks, the naringin supplementation significantly lowered plasma cholesterol and triglyceride concentrations in low vitamin E groups compared to the naringin-free group.[101] Naringin supplementation appeared to have a vitamin E-sparing effect as it resulted in a higher plasma vitamin E concentration in each naringin-supplemented group compared to the naringin-free group. This suggests that the plasma cholesterol-lowering effect of naringin is significant only when the dietary vitamin E level is low. In contrast, supplementation using 0.1% hesperidin significantly lowered the plasma cholesterol concentrations in both the 5 IU and 50 IU/kg of vitamin E dietary groups, while no changes of plasma triglyceride concentration were found.[67]

The consumption of a cholesterol-enriched diet increases the degree of lipid peroxidation, which is one of the early processes of atherosclerosis. A cholesterol-rich diet would appear to induce free radical production, followed by oxidative stress and hypercholesterolemia,[103),104] which is a major risk factor for atherosclerosis and is related to occlusive vascular disease.[105] The antioxidative effects of naringin (0.5g/kg diet) were compared with probucol, which is known as an antioxidative cholesterol-lowering drug, in rabbits fed a high cholesterol diet.[106] Probucol and naringin supplementation led to an increase in the hepatic SOD and catalase activities, and a decrease in the hepatic mitochondrial hydrogen peroxide content, compared to rats fed a high cholesterol diet only. Both naringin and probucol supplements significantly increased plasma vitamin E concentrations, compared to rats fed a high cholesterol diet only.[106] Accordingly, it would appear that naringin is effective in altering antioxidative metabolism and in sparing plasma vitamin E. Another study with rabbits undertaken by the current author indicated that naringin acts as a superoxide scavenger and hydroperoxide counteragent while still maintaining a vitamin E sparing effect[107]. With regards to the hepatic antioxidant enzyme system, the current author showed that two cinnamate derivatives, 3,4-di(OH)-cinnamate and 3,4-di(OH)-hydrocinnamate, enhanced hepatic GSH-Px and catalase activity while lowering plasma cholesterol concentration in rats fed high cholesterol diets.[70] Thus, polyphenols appear to have antioxidant activities and may function to lower lipid levels.

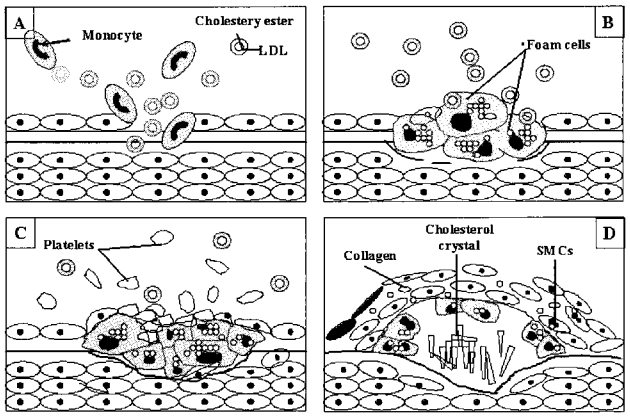

Fig 3. Response to injury hypothesis of atherosclerosis.

(A) Several different source of injury to the endothelium can lead to endothelial cell dysfunction. One of the parameters associated with endothelial cell dysfunction that results from exposure to agents, such oxLDL, is increased adherence of monocyte/macrophage and T lymphocyte (A). These cells then migrate between the endothelium and localize subendothelially. The macrophages become large foam cells because of lipid accumulation (B) and, with the T cells and smooth muscle cells, form a fatty streak (C). The fatty streak can then progress to an stages of lesion formation is potentially reversible. Thus, lesion regression can occur if the injurious agents are removed, or when protective factors intervene to reverse the inflammatory and fibroproliferative processes (D).

In conclusion, the results of studies outlined in the present review provide a rationale for continuing research to determine whether fruit-derived phenolic compounds have net nutritional benefits. In particular, the specific mechanisms of how these compounds may affect cardiovascular health need to be defined before general and robust recommendations about dietary intake can be made. Further research needs to focus on at least two major areas: (i) the development of reliable and specific methods to measure individual phenolic compounds or their metabolites, as well as classes of these compounds,

in body fluids and tissue; and (ii) the application of specific and unequivocal methods to measure biological actions in vivo.

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