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Review

Flavonoids—Chemistry, metabolism, cardioprotective effects, and dietary sources

N.C. Cook and S. Samman

Human Nutrition Unit, Department of Biochemistry, University of Sydney, Sydney, Australia

Flavonoids are a group of polyphenolic compounds, diverse in chemical structure and characteristics, found ubiquitously in plants. Therefore, flavonoids are part of the human diet. Over 4,000 different flavonoids have been identified within the major flavonoid classes which include flavonols, flavones, flavanones, catechins, anthocyanidins, isoflavones, dihydroflavonols, and chalcones. Flavonoids are absorbed from the gastrointestinal tracts of humans and animals and are excreted either unchanged or as flavonoid metabolites in the urine and feces. Flavonoids are potent antioxidants, free radical scavengers, and metal chelators and inhibit lipid peroxidation. The structural requirements for the antioxidant and free radical scavenging functions of flavonoids include a hydroxyl group in carbon position three, a double bond between carbon positions two and three, a carbonyl group in carbon position four, and polyhydroxylation of the A and B aromatic rings. Epidemiological studies show an inverse correlation between dietary flavonoid intake and mortality from coronary heart disease (CHD) which is explained in part by the inhibition of low density lipoprotein oxidation and reduced platelet aggregability. Dietary intake of flavonoids range between 23 mg/day estimated in The Netherlands and 170 mg/day estimated in the USA. Major dietary sources of flavonoids determined from studies and analyses conducted in The Netherlands include tea, onions, apples, and red wine. More research is needed for further elucidation of the mechanisms of flavonoid absorption, metabolism, biochemical action, and association with CHD. (J. Nutr. Biochem. 7:66–76, 1996.)

Keywords: flavonoids; chemical structure; metabolism; low density lipoprotein; oxidation; platelet aggregation; coronary heart disease; diet

Introduction

Flavonoids are a group of polyphenolic compounds diverse in chemical structure and characteristics. They occur naturally in fruit, vegetables, nuts, seeds, flowers, and bark and are an integral part of the human diet.[1–3] They have been reported to exhibit a wide range of biological effects, including antibacterial, antiviral,[4] anti-inflammatory, antiallergic,[1,4,5] and vasodilatory[6] actions. In addition, flavonoids inhibit lipid peroxidation (LPO)[2,7] platelet aggregation,[8–12,15] capillary permeability, and fragility,[13,14] and the activity of enzyme systems including cyclo-oxygenase and lipoxygenase.[1,5,15,16] Flavonoids exert these effects as antioxidants, free radical scavengers,[4,17–19] and chelators of divalent cations.[20]

Less is known about the absorption and metabolism of flavonoids, at the usual levels of dietary intake. They are believed to be nontoxic[1] and if absorbed and biologically active in vivo may prevent free radical mediated cytotoxicity and LPO,[21] which is associated with cell aging and chronic diseases such as atherosclerosis.[22]

Much evidence suggests that peroxidation of low density lipoproteins (LDL) is positively associated with atherogenesis.[23–25] Frankel et al.[26] reported that phenolic compounds (including flavonoids and nonflavonoid polyphenols) isolated from red wine inhibit copper catalyzed oxidation of LDL in vitro. It is postulated that the antioxidant and free

Address reprint requests to Dr. S. Samman, at the Human Nutrition Unit, University of Sydney, NSW 2006, Australia.
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radical scavenging properties of phenolic compounds, present in red wine, may partly explain the anomaly observed in the coronary heart disease (CHD) rate between the French population who consume wine regularly and have rates of CHD lower than other populations despite similar fat intakes.[26–28] The aims of this review are to evaluate the chemistry of flavonoids, their absorption, metabolism, dietary sources, and association with CHD.

Chemistry of flavonoids

Generic structure and major classifications

Flavonoids are low molecular weight polyphenolic substances based on the flavan nucleus.[29] *Figure 1* shows the generic structure of flavonoids and the numbering system used to distinguish the carbon positions around the molecule. The three phenolic rings are referred to as the A, B, and C (or pyrane) rings. The biochemical activities of flavonoids and their metabolites depend on their chemical structure and the relative orientation of various moieties on the molecule.[30] Flavonoids are classified according to their chemical structure. The major flavonoid classes include flavonols, flavones, flavanones, catechins (or flavanols), anthocyanidins, isoflavones, dihydroflavonols, and chalcones.[16,31–33]

Substitution

Tables 1–3 and *Figure 2* show the major flavonoid classes and some structural variations that have been identified. The structure of flavonoids varies widely within the major classifications, and substitutions include hydrogenation, hydroxylation, methylation, malonylation, sulphation, and glycosylation.[31,32] Many flavonoids occur naturally as flavonoid glycosides,[33,34] and carbohydrate substitutions include D-glucose, L-rhamnose, glucorhamnose, galactose, lignin, and arabinose.[34] Quercitrin, rutin, and robinin are the most common flavonoid glycosides in the diet. They are hydrolyzed by intestinal flora to produce the biologically active aglycone (sugar-free flavonoid).[33] Quercetin is the subject of many studies investigating the biological effects of flavonoids, because it is the predominant flavonoid found in foods.[35]

Polymerization

Flavonoids may be monomeric, dimeric, or oligomeric. Monomers vary greatly in size; for example flavone has a

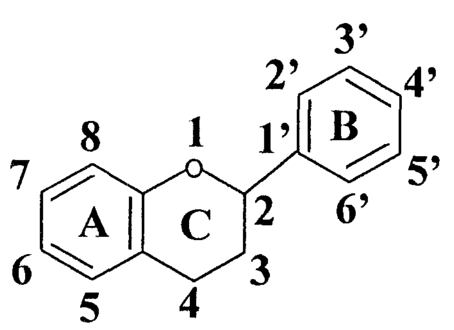

Figure 1 The generic structure of flavonoids.

molecular weight of 222 whereas blue anthocyanin has a molecular weight of 1,759.[32] Polymeric compounds, called tannins, are divided into two groups based on their structure: condensed and hydrolyzable.[36] Condensed tannins are polymers of flavonoids[1] and hydrolyzable tannins contain gallic acid (*Figure 3*), or similar compounds, esterified to a carbohydrate.[36] Galloyl groups have iron chelating properties in vitro and are believed to interfere with iron absorption in vivo.[36]

Tea tannins consist of four main catechin components: epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate.[37,38] In tea epigallocatechin gallate is the predominant catechin accounting for more than half of the total catechin content.[29,38] Enzymatic oxidation of tea catechins during fermentation of macerated tea leaves produces the dimeric theaflavins and the polymeric thearubigins of black ''Indian'' tea[29] which produce the brightness and astringency, respectively.[29,33,37] Thearubigins range widely in size between oligomers of four or five flavonoid units to molecules of up to 100 flavonoid units.[29] In contrast to black tea, the flavonoids in green ''Chinese'' tea occur mostly as monomers because green tea is not fermented during processing.[37] In red wine, tannins are formed by the polymerization of anthocyanins and other flavonoids producing the wine's characteristic colors, flavors, and astringency.[39]

Distribution and function in plants

Over 4,000 types of flavonoid compounds have been identified in vascular plants and these vary in type and quantity due to variations in plant growth, conditions, and maturity.[37] Only a small number of plant species have been examined systematically for their flavonoid content[32] and therefore the identification and quantification of all the types of flavonoids consumed by humans is incomplete.[37] Plants have evolved to produce flavonoids to protect against fungal parasites,[32] herbivores, pathogens, and oxidative cell injury.[39] Conversely, flavonoids produce stimuli to assist in pollination[31] and guide insects to their food source.[32] For example, anthocyanins produce the pink, red, mauve, violet, and blue colors of flowers, fruits, and vegetables.[29]

Absorption and metabolism

Studies in animals

Bravo et al.[40] studied the degradability of the polyphenolic compounds, catechin, and tannic acid in the intestinal tract of rats. Male Wistar rats were fed diets containing catechin or tannic acid (equivalent to 0.5 g/day, assuming a rat weighing 200 g consumes 25 g diet/day), over a 3 week period. There was little fermentation by the gut flora, and less than 5% of the ingested catechin and tannic acid were excreted unchanged in the feces suggesting that absorption of the polyphenolic compounds had occurred.

Although there were no interactions between phenolic compounds and protein digestion,[40] alterations to lipid metabolism have been reported in rats fed diets containing tannic acid and catechin.[38,40] It is postulated that catechin

Table 1 Structure of flavonoids

Flavonoid	Total No. of OH groups	Position of OH groups	Substitutions on the generic structure	Position of the substitutions
Myricetin	6	3,5,7,3′,4′,5′.		
Gossypetin	6	3,5,7,8,3′,4′.		
Quercetagen	6	3,5,6,7,3′,4′.		
Quercetin	5	3,5,7,3′,4′.		
Morin	5	3,5,7,2′,4′.		
Robinetin	5	3,7,3′,4′,5′.		
Myricetrin	5	5,7,3′,4′,5′.	O-Rh	3
Rutin	4	5,7,3′,4′.	O-Ru	3
Kaempferol	4	3,5,7,4′.		
Quercetrin	4	5,7,3′,4′.	O-Rh	3
Fisetin	4	3,7,3′,4′.		
Datiscetin	4	3,5,7,2′.		
Rhamnetin	4	3,5,3′,4′.	O-Me	7
Tamarixetin	4	3,5,7,3′	O-Me	4′
Silybin	3	3,5,7.	O-Lig-O	4′
Galangin	3	3,5,7.		
Kaempferide	3	3,5,7.	O-Me	4′
Diosmin	2	3,3′.	O-Ru, O-Me	5,4′
Robinin	2.	5,4′.	O-Gal-Rh, Rh	3,7′
Troxerutin	1	5	O-Ru, O-He, O-He, O-He	3,7,3′,4′.
3-OH-Flavone	1	3		

Rh = rhamnose = 6-deoxy-L-mannose ($C_6H_{12}O_5$); Lig = lignin; Ru = rutinose = 6-O-D-glucose ($C_{12}H_{12}O_{10}$); He = hydroxyethyl (CH_2CH_2OH); Me = methyl (CH_3); Gal = galactose ($C_6H_{12}O_6$).

influences lipid metabolism by increasing bile acid excretion leading to a hypocholesterolemic effect.[38]

Studies in humans

Despite the potentially significant effects of flavonoids on coronary heart disease,[41] information about the absorption, metabolism, and excretion of individual flavonoids in humans is scarce. Some studies report that flavonoids are absorbed after oral administration,[42] although others conclude that they are poorly absorbed and do not reach the general circulation unchanged at measurable concentrations.[43] However, most studies of flavonoid metabolism in humans have examined the metabolism of individual flavonoids taken at pharmacological doses rather than at estimated levels of dietary intake[42,43] of approximately 23[35] to 170 mg/day.[33] Therefore, extrapolation of the results of these studies may be inappropriate to explain the absorption and metabolism of dietary flavonoids.

Das[42] studied the absorption and metabolism of (+)-catechin in six healthy male volunteers following the administration of a single dose of (+)-catechin (92.3 mg/kg of body weight, mean 4.2 g). Within 6 hr, phenols were detected in plasma and returned to baseline by 96 hr. The phenolic compounds were excreted in urine in both free and conjugated forms and included sulphate conjugates. In the feces, approximately 19% of the administered dose was excreted unchanged. There were no adverse side effects reported following the large single oral dose of catechin.[42]

Gugler et al.[43] investigated the metabolism of quercetin in six volunteers (four male and two female) aged between 21 and 32 years. After the oral administration of a single dose of 4 g, no measurable concentrations of the flavonoid or its derivatives were detected in plasma or urine. However, approximately 53% of the oral dose was recovered unchanged in the feces, and it was concluded that 1% of the original 4 g dose of quercetin or approximately 40 mg was absorbed.[43] The estimated average intake of all flavonoids from dietary sources is between 23[35] and 170 mg/day.[33] Therefore, absorption of 40 mg is not discountable. However, studying the metabolism of one flavonoid, namely quercetin, at a single pharmacological dose that greatly exceeds the estimated dietary consumption of flavonoids[33,35] may not explain the metabolism of flavonoids from dietary sources. Humans are unlikely to consume dietary flavonoids individually due to the diversity and wide distribution of flavonoids in foods. Therefore, the results of the study by Gugler et al.[43] may not reflect the mechanisms of absorption and metabolism of dietary flavonoids.

Lipid peroxidation

Polyunsaturated fatty acids (PUFA) present in cell membranes are oxidized by both enzymatic and auto-oxidative peroxidation and by free radical chain reactions.[13] An overabundance of free radicals can lead to uncontrolled chain reactions and LPO[44] resulting in pathological conditions that may include atherosclerosis and cancer.[22] LPO proceeds in three stages: initiation, propagation, and termination.[13,45]

In the initiation stage of LPO, free radicals abstract hydrogen from PUFA to form the lipid radical. In the propagation stage, the lipid radical reacts with molecular oxygen to form the lipid peroxy radical which breaks down to generate more free radicals thus maintaining the chain of reactions. In the termination stage, the free radical species react

Table 2 Structure of flavones

Flavone	Total no. of OH groups	Position of OH groups	Substitutions on the generic structure	Position of the substitutions
Hypolactin	5	5,7,8,3′,4′		
Luteolin	4	5,7,3′,4′		
Scutellarein	4	5,6,7,4′		
Isoorientin	4	5,7,3′,4′	Gluc	6
Orientin	4	5,7,3′,4′	Gluc	8
Apigenin	3	5,7,4′		
Silymarin	3	4,6,3′		
Diosmetin	3	5,7,3′	O-Me	4′
Luteolin-7-glucoside	3	5,3′,4′.	O-Gluc	7
Baicalein	3	5,6,7		
Cirsiliol	3	5,3′,4′	O-Me	7
Sideritoflavone	3	5,3′,4′	O-Me	6,7,8
Pedalitin	3	5,3′,4′	O-Me	7
Vitexin	3	5,7,4′	Gluc	8
Vicenin-2	3	5,7,4′	Gluc	6,8
Pinocembrin	2	5,7		
Hispidulin	2	5,7	O-Me	4′
5,7-Dihydroxytrimethoxy- flavone	2	5,7	O-Me	3,4′,5′
Gardenin-D	2	5,3′	O-Me	6,7,8,4′
Acetetin	2	5,7	O-Me	4′
Chrysin	2	5,7		
Cirsimaritin	2	5,4′	O-Me	6,7
Xanthomicrol	2	5,4′	O-Me	6,7,8
8-Methoxycirisilincol	2	5,4′	O-Me	6,7,8,3′
5-O-Demethylnobiletin	1	5	O-Me	6,7,8,3′,4′
Techtochyrsin	1	5	O-Me	7
Flavone	0			

Me = methyl = (CH_3); Gluc = glucose.

together or with antioxidants to form inert products.[13,29,45] LPO can be suppressed by enzymatic inactivation of free radicals[22,34] and antioxidants that inhibit the initiation stage and/or accelerate the termination stage.[45] Thus, LPO can be prevented at the initiation stage by free radical scavengers and singlet oxygen quenchers, and the propagation chain reaction can be broken by peroxy-radical scavengers.[13]

The antioxidant and chelating properties of flavonoids

Flavonoids inhibit LPO in vitro at the initiation stage by acting as scavengers of superoxide anions and hydroxyl radicals.[13,20] It has been proposed that flavonoids terminate chain radical reactions by donating hydrogen atoms to the peroxy radical forming a flavonoid radical.[13,20] The flavonoid radical in turn reacts with free radicals thus terminating the propagating chain.[13,46] In addition to their antioxidative properties, some flavonoids act as metal-chelating agents and inhibit the superoxide-driven Fenton reaction, which is an important source of active oxygen radicals.[20] However, there is no clear evidence of the antioxidant and free radical scavenging effects of flavonoids in vivo.[40]

Structure-activity relationships of lipid peroxidation inhibition by flavonoids

The inhibition of LPO is influenced by a number of structural features of flavonoids:

(1) The presence of a hydroxyl group in position three (3-OH) of the C ring.[2,7,20,22,47,48] The flavonoid aglycones that have a 3-OH group such as fisetin, (+)-catechin, quercetin, myricetin, and morin are potent inhibitors of LPO compared with those that lack a 3-OH substitution such as diosmetin, apigenin (flavones), hesperetin, and naringenin (flavanones).[47]
(2) A double bond between carbons two and three (C2-C3) of the C ring.[2,19,48,49] Hydrogenation of this bond decreases the antiperoxidative effects.[22,49]
(3) The carbonyl group at C-4 of the C ring is necessary for antiperoxidant activity in some studies[2,19,48] but not others.[7,49] Catechin lacks a C-4 carbonyl and has lower hydroxyl radical scavenging potency than quercetrin which has a C-4 carbonyl group.[50]
(4) The number of hydroxyl groups.[2,7,19,49] The importance of polyhydroxylated substitutes on the A and B rings was demonstrated by comparing quercetin, quercetin, myricetin, myricetrin, phloretin, (+)-catechin, morin, and fisetin with apigenin, hesperetin, hesperidin, naringenin, naringin, chrysin, and 3-hydroxyflavone.[2] In the former group, each of the flavonoids has between four and six hydroxyl substitutions while the latter group has between one and three hydroxyl groups. The hydroxyl radical scavenging activity of flavonoids increases with the number of hydroxyl groups substituted on the B ring, especially at C-3′, and decreases rapidly as the number of hydroxyl groups decreases.[2] Myricetin (hydroxylation pattern: 3,5,7,3′,4′,5′) has greater hydroxyl

Table 3 Structure of flavanones, catechins, anthocyanidins, isoflavones, dihydroflavonols, and chalcones

Flavonoid classes	Total no. of OH groups	Position of OH groups	Substituions on the generic structure	Position of the substitutions
Flavanones				
Eriodictyol	4	5,7,3′,4′		
Hesperetin	3	5,7,3′	O-Me	4′
Naringenin	3	5,7,4′		
Hesperidin	2	5,3′	Rh-Gluc, O-Me	7,4′
Naringin	2	7,4′	O-Rh-Gluc	5
Catechins				
Leucocyanidol	6	3,4,5,7,3′,4′		
(+)-Catechin	5	3,5,7,3′,4′		
(+)-Epicatechin	5	3,5,7,3′,4′		
Anthocyanidins				
Delphinidin chloride	6	3,5,7,3′,4′,5′	Chloride	1
Delphinidin	6	3,5,7,3′,4′,5′		
Cyanidin chloride	5	3,5,7,3′,4′	Chloride	1
Cyanidin	5	3,5,7,3′,4′		
Petunidin	5	3,5,7,4′,5′	O-Me	3′
Peonidin	4	3,5,7,4′	O-Me	3′
Malvidin	4	3,5,7,4	O-Me	3′,5′
Isoflavones				
Genistein	3	5,7,4′		
Diadzein	2	7,4′		
Dihydroflavonols				
Taxifolin	5	3,5,7,3′,4′		
Fustin	4	3,7,3′,4′		
Chalcones				
Butein	4	3,4,4′,6		
Phloretin	4	4,2′,4′,6′		
Phloridzin	3	4′,2′,4	O-Gluc	6

Gluc = glucose; Me = methyl (CH_3); Rh = rhamnose = 6-deoxy-L-mannose ($C_6H_{12}O_5$).

radical scavenging activity than kaempferol (hydroxylation pattern: 3,5,7,4′).[50]

(5) The pattern of hydroxylation.[49] Hydroxyl groups on positions C-5 and C-7 of the A ring[7,41] C-3′ and C-4′ of the B ring[7,21,51]; and position C-3 of the C ring[49] appear to contribute to the inhibition of LPO. Flavonols require a C-2′ hydroxyl and the pyrogallol group (C-3′, C-4′, C-5′) for antiperoxidative activity.[49]

(6) The presence of a sugar moiety.[2,18] Flavonoid aglycones such as apigenin, naringenin, hesperetin, diosmetin, quercetin, phloretin and myricetin are more effective in inhibiting malondialdehyde (MDA) production than their corresponding glycosides.[2,18,19] The sugar moiety reduces the antiperoxidation efficiency of adjacent hydroxyl groups due to steric hindrance.[2,7,22,49] However, flavonoid glycosides, such as quercetin and rutin, are hydrolyzed to their corresponding aglycones by human intestinal flora.[3,51] Therefore, the findings that flavonoid glycosides have lower antiperoxidative potency than aglycone flavonoids in vitro may not be relevant to the in vivo effects of flavonoids.

(7) Methoxyl groups reduce antiperoxidative efficiency of flavonoids in vitro due to steric hindrance.[49]

(8) Flavonoids having both a C-4 carbonyl group and a C-3 or C-5 hydroxyl group, such as rutin and quercetin, form chelates with iron ions.[21,39,49] The ability of flavonoids to sequester metal ions may contribute to their antiperoxidative properties by preventing the formation of free radicals in the Fenton system.[20,34,39,48] Moreover, flavonoids retain their free radical scavenging activities after forming complexes with iron ions.[20] Thus the formation of metal ion chelates is one antioxidant mechanism of flavonoids.[20–22]

Flavonoids and coronary heart disease

Role of oxidized LDL in atherogenesis

Elevated plasma LDL cholesterol concentrations are associated with accelerated atherosclerosis.[24] Atheromatous lesions develop in the subendothelial space due to the accumulation of cholesteryl esters in macrophages forming foam cells. Until recently, the mechanism of foam cell formation was unclear because macrophages have few LDL receptors, and paradoxically these receptors are down-regulated as plasma LDL concentrations increase.[52] Goldstein et al.[53] were the first to demonstrate that a chemically modified (acetylated) LDL in vitro is recognized by specific receptors (scavenger receptors) on the macrophage. Consequently, modified LDL is endocytosed at a much higher rate than native LDL. Scavenger receptors that recognize oxidatively modified LDL have since been identified[25,54,55] and there is much evidence that oxidized LDL is responsible for cholesterol loading of macrophages, foam cell formation, and atherogenesis.

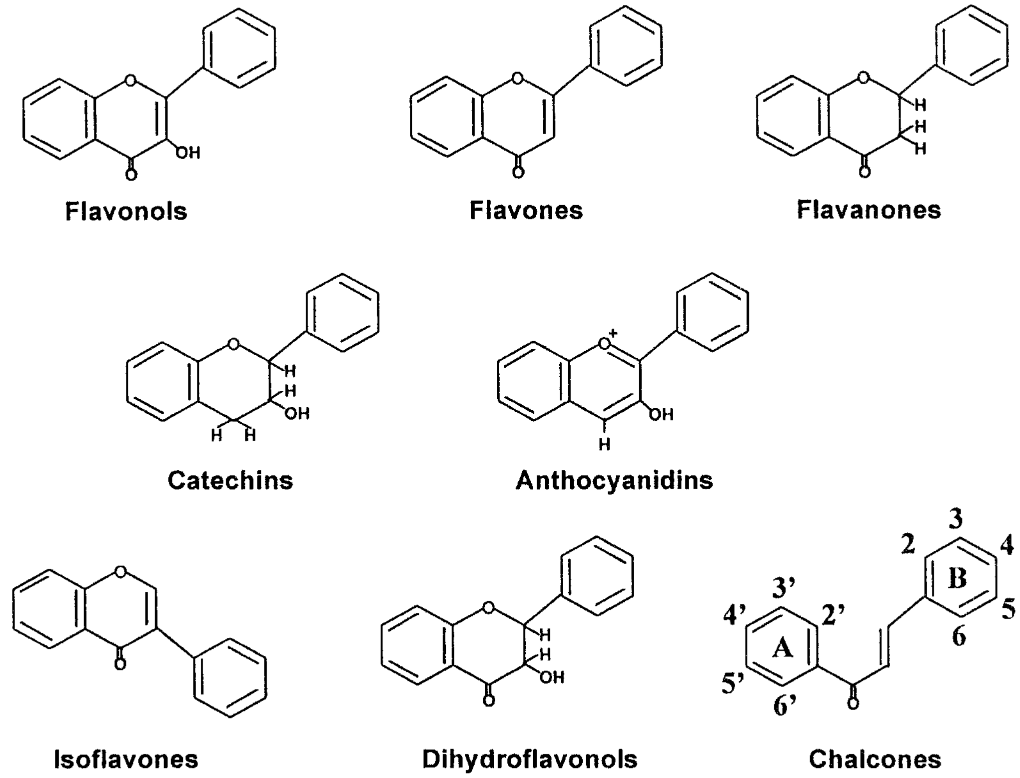

Figure 2 The structures of the major classes of flavonoids.

LDL is oxidized by free radicals generated from endothelial cells, monocyte-derived macrophages, and smooth muscle cells, resulting in several chemical and physical changes of LDL.[24,56,57] Oxidized LDL is chemotactic for macrophages promoting their residence in the intima, cytotoxic to the endothelium, chemoattractant for monocytes, and rapidly accumulated by resident macrophages.[24,57,58] Therefore, it has been hypothesized that oxidized LDL initiates and promotes atherogenesis in several ways.

LDL particles contain endogenous antioxidants including α- and γ-tocopherols, β-carotene, lycopine, and retinyl stearate.[41,59–61] LDL oxidation in vitro exhibits a lag phase corresponding to the time required for the endogenous antioxidants in LDL to be consumed.[41,59] Exogenous antioxidants, such as α-tocopherol, butylhydroxytoluene (BHT)[62] urate, ascorbic acid, and probucol, and metal chelators, such as EDTA, can protract the lag phase or even prevent LDL oxidation in vitro.[63–65] Recent reports show an inverse association between dietary intake of phenolic antioxidants, including α-tocopherol, and CHD.[66] It is postulated that the antioxidant effects of dietary α-tocopherol in a similar manner to flavonoids may in part explain the French paradox.[26,67] Hence, a range of minor dietary factors, including flavonoids and α-tocopherol, may collectively act as effective antioxidants in the prevention of CHD.

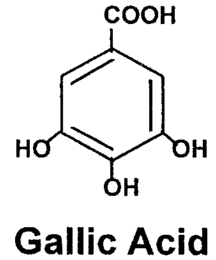

Figure 3 The structure of gallic acid.

Epidemiological evidence for the cardioprotective effects of flavonoids

The Zutphen Elderly Study[68] is the only published epidemiological study that examines the relationship between dietary flavonoid intake and the risk of CHD. The Zutphen study assessed the flavonoid intake of 805 men aged 65 to 84 years. There was a significant inverse association between dietary flavonoid intake and mortality from CHD and an inverse but weaker relation with the incidence of myocardial infarction. These findings were significant after adjusting for known major confounders.[68]

The average flavonoid intake in this population was estimated to be 26 mg/day, and the major contributors to these estimates were tea, 61%, onions, 13%, and apples, 10%.[68] Flavonoid intake and tea consumption were highly correlated, and both were inversely associated with death from CHD.[68] However, the total flavonoid intake had a greater effect on CHD mortality than tea itself, suggesting that flavonoids rather than other substances in tea were responsible for the protection against CHD.

The flavonoid content of many foods commonly eaten in The Netherlands has been analyzed.[69,70] However, more research is required to identify the flavonoid content of foods consumed in other countries. Also, further epidemiological studies are needed to confirm these findings and identify other foods that may, due to their high flavonoid content, have potential cardioprotective properties.

Inhibition of LDL oxidation in vitro by flavonoids

A number of aglycone flavonoids are potent inhibitors of oxidative modification of LDL in vitro by macrophages or copper ions.[41] Phenolic compounds isolated from red wine inhibit the copper catalyzed oxidation of LDL in vitro significantly more than α-tocopherol.[26] However, the ability of flavonoids to protect LDL from oxidative modification in vivo depends on their absorption, metabolism, and in particular the association of flavonoids with lipoproteins. Recent studies in humans suggest that polyphenols obtained through drinking red wine associate with plasma LDL[71] and are significantly more effective than white wine in reducing the oxidizability of whole plasma[71,72] and of plasma LDL.[71]

The exact mechanisms by which flavonoids inhibit LDL oxidation are uncertain. Flavonoids may reduce the formation of free radicals[2,20,21,41,46,48–50] or protect the α-tocopherol in LDL from oxidation by being oxidized themselves in preference to α-tocopherol, thus delaying the start of LPO.[41] Alternatively, flavonoids may regenerate α-tocopherol by donating a hydrogen atom to the α-tocopherol radical.[13,26] Also, flavonoids may inhibit LDL oxidation by chelating divalent metal ions and thus reducing the formation of free radicals induced by Fenton reactions.[20,39,41,49]

There have been insufficient tests of the protective effects of flavonoids against LDL oxidation to make definitive statements about their structure-activity relationships. However, hydroxylation of the flavone nucleus appears to be advantageous because flavone itself is a poor inhibitor of LDL oxidation, whereas polyhydroxylated aglycone flavonoids such as quercetin, morin, hypoleatin, fisetin, gossypetin, and galangin are potent inhibitors of LDL oxidation.[41] These findings are consistent with previous studies of the structure-activity relationships of flavonoids in the inhibition of LPO.[2,7,18,19,21,49]

The ability of (+)-catechin to inhibit LDL oxidation induced by copper and several cell lines including mouse macrophages, human monocyte-derived macrophages, and vascular endothelial cells isolated from human umbilical cords have been investigated.[57] As expected, LDL modified by cells or copper-induced oxidation was endocytosed and degraded by human macrophages more quickly than native LDL. However, in the presence of (+)-catechin, the rate of endocytosis and degradation by macrophages was similar to that of native LDL.[57] This provides further evidence that flavonoids may protect LDL from oxidative modification and therefore protect against atherosclerosis if they are delivered to the subendothelial space where LDL oxidation occurs.

In addition to the inhibition of LDL oxidation, flavonoids such as catechin, rutin, and quercetin strongly inhibit LPO and the subsequent cytotoxicity of oxidized LDL.[63,65] Moreover, cells preincubated with these flavonoids were resistant to the cytotoxic effects of previously oxidized LDL.[63,65] The postulated mechanisms by which flavonoids guard against cytotoxicity of oxidized LDL are consistent with the antioxidant and free radical scavenging properties.[2,20,21,41,46,48–50]

Antithrombotic and vasoprotective effects of flavonoids

Platelet-blood vessel interactions are implicated in the development of thrombosis and atherosclerosis. Particular flavonoids inhibit platelet aggregation and adhesion thus reducing thrombotic tendencies.[8,10–12,15,22,73,74] However, the antiaggregatory effects of flavonoids cannot be attributed to a single biochemical mechanism because they appear to influence several pathways involved in platelet function[12,75,76] such as the inhibition of the enzymes cyclo-oxygenase and lipoxygenase involved in arachidonic acid metabolism in platelets. Also flavonoids inhibit platelet aggregation by antagonizing thromboxane formation and thromboxane receptor function.[12] One of the most potent mechanisms by which flavonoids appear to inhibit platelet aggregation is by mediating increases in platelet cyclic AMP (cAMP) levels by either stimulation of adenylate cyclase or inhibition of cAMP phosphodiesterase (PDE) activity.[6,9–11,73,76–78]

The antioxidant actions of flavonoids appear to participate in their antithrombotic action.[8,15,73] The antithrombotic and vasoprotective actions of quercetin, rutin, and other flavonoids have been attributed to their ability to bind to platelet membranes and scavenge free radicals.[8] By their antioxidant actions, flavonoids restore the biosynthesis and action of endothelial prostacyclin and endothelial derived relaxing factor (EDRF) both of which are inhibited by free radicals.[8,10,46] However, the lack of antioxidant actions of sideritoflavone and cirsiliol, which are potent LPO inhibitors, suggests that some flavonoids may inhibit arachidonic acid metabolism and platelet function by flavonoid-enzyme interactions rather than by antioxidant effects.[22]

The structural features required for flavonoids to inhibit human platelet aggregation and adhesion are similar to those associated with the antioxidant function of flavonoids and the inhibition of cAMP PDE and include a double bond between C-2 and C-3, a 3-OH group, and a carbonyl group at C-4.[73] The inhibitory effect of flavonoids on platelet function is diminished by glycosylation at C-3,[15] saturation of the double bond between C-2 and C-3, and polyhydroxylation.[73] Thus flavonoid glycosides and flavanone derivatives do not appear to affect platelet function.

Regular consumption of red wine is linked to decreased platelet aggregation and the prevention of CHD.[27] However, withdrawal from beer or spirits for at least 12 hr by people who regularly consume these beverages is associated with rebound platelet reactivity and an increased risk of thrombosis.[79] In rats withdrawal from red wine resulted in a 59% decrease in rebound platelet reactivity compared with increases following withdrawal from ethanol or white wine.[79] It is postulated that the antioxidant properties of phenolic compounds in red wine reduce platelet aggregation and inhibit LPO in vitro.[26,79] If reproduced in humans, the protective effects of red wine against platelet aggregation may partly explain the long-term advantages of consuming moderate amounts of red wine over other alcoholic beverages.[80]

In addition to their antiaggregatory effects, flavonoids appear to increase vasodilation by inducing vascular smooth muscle relaxation which may be mediated by the inhibition of protein kinase C, PDEs, or by decreased cellular uptake of calcium.[6]

Dietary intake and food sources of flavonoids

Until recently, data on human flavonoid intake were obtained from Kühnau[33] who estimated the average intake of all dietary flavonoids in the USA to be approximately 1 g/day (expressed as glycosides) of which about 170 mg (expressed as aglycones) consisted of flavonols, flavanones, and flavones. These values have been widely quoted[1,2,4,51]; however, they are based on food analysis techniques now considered inappropriate.[81] Furthermore, estimates of flavonoid intake were based on analysis of whole foods and estimates of the average American diet extrapolated from the Organization for Economic Cooperation and Development (OECD) food consumption statistics[81] thus overestimating food intake and consequently the average flavonoid intake.

The content of the flavonols quercetin, kaempferol, and myricetin and the flavones luteolin and apigenin in 28 vegetables, 9 fruits, and beverages commonly consumed in The Netherlands was analyzed using more recent and advanced methodologies.[35,69,70] Based on these analyses and using data from the Dutch National Food Consumption Survey 1987–88, the average dietary flavonoid intake in The Netherlands was estimated to be approximately 23 mg/day (expressed as aglycones).[35]

Quercetin was the major dietary flavonoid (mean intake 16 mg/day), followed by kaempferol (4 mg/day), myricetin (1.4 mg/day), luteolin (0.92 mg/day), and apigenin (0.69 mg/day). The greatest dietary sources of flavonoids were: tea, 48% of total intake; onions, 29%; and apples, 7%. The average consumption was: tea, 2 cups/day (294 ± 310 mL); onions, 16 ± 32 g/day; and apples, 45 ± 71 g/day. Thus, these levels of flavonoid intake were achieved without unusually high consumption of these foods. Red wine is also a rich sources of flavonoids and contains approximately 22.5 mg/L (3.8 mg/170 mL glass).[69]

The estimated flavonoid intake of 23 mg/day was based on the content of five flavonoids in Dutch foods; therefore, the total flavonoid intake in this population may be higher. Moreover, this estimation is based on analysis of foods commonly consumed in The Netherlands and thus may not represent the flavonoid content of foods consumed in other countries. A systematic analysis of the flavonoid content of foods consumed in other countries is required to estimate flavonoid intakes in other populations.

Therapeutic potential of flavonoids

Concentrated forms of flavonoids, such as propolis (a resinous substance obtained by bees from plants for use as glue in their hives) has been used for centuries to treat a wide variety of human conditions including inflammation, allergy, headache, cancer, viral infections, the common cold, bee stings, and gastric and duodenal ulcers.[34] Flavonoid preparations have been used widely in medical practice for over 40 years to treat disorders of peripheral circulation.[82] Over 100 preparations containing flavonoids, including cianidanol, diosmetin, hesperidin, leucocianidin, rutin, and troxerutin, are marketed in France and Switzerland.[82] Many of the alleged effects of pharmacological doses of flavonoids have been linked to their known functions as strong antioxidants (including vitamin C–sparing properties), free radical scavengers, metal chelators, and enzyme-flavonoid interactions. However, therapeutic preparations of flavonoids have yet to pass controlled clinical trials.

Red wine is a rich source of flavonoids, and regular red wine consumption is associated with a decreased risk of CHD and may partly explain the French paradox. However, an indirect adverse effect of encouraging the consumption of red wine[83] is the potential to increase the risk of cirrhosis associated with alcohol consumption. While the risk/benefit ratio may vary for individuals, the use of alcohol for cardioprotective purposes should not be encouraged as a public health measure.

Most research conducted on the biochemical effects of flavonoids has focused on the potential of flavonoids as pharmaceuticals[18,20,34] rather than the possible health benefits of obtaining flavonoids in the diet. The reported benefits of flavonoids have mostly been inferred from results at pharmacological concentrations. Consequently, the reported effects of pharmacological doses of flavonoids are primarily of pharmacological rather than dietary significance. Thus more research is needed to elucidate the biochemical effects of flavonoids in the diet.

Possible adverse effects of flavonoids

Adverse reactions from flavonoids have been reported following administration of chronic pharmacological doses[82] that exceed the estimated dietary intake of 23[35] to 170 mg/day.[33] Toxic effects that have been documented from doses of 1 to 1.5 g/day of flavonoid drugs such as cianidanol include acute renal failure, hemolytic anemia, thrombocytopenia, hepatitis, fever, and skin reactions.[80] In one study, quercetin is reported to have induced bladder cancer in rats when consumed at the level of 2% in the diet.[80] However, these results were not confirmed in another study where quercetin was administered at doses up to 10% of the diet.[84] Importantly, in a diet containing a wide variety of foods flavonoids are unlikely to be consumed in toxic quantities because foods originating from plants contain many diverse types of flavonoids in varying quantities.

Tea is a rich source of flavonoids[69] but black tea due to the presence of hydrolyzable tannins (tannic acid) is a well known inhibitor of iron absorption. Phenolic compounds, such as phenolic monomers, polyphenols, and tannins are considered to interfere with iron absorption by forming insoluble complexes in the gastrointestinal lumen thus reducing iron bioavailability.[36] Phenolic molecules with aromatic rings bearing two hydroxyls (catechol group) or three hydroxyls (galloyl group) on adjacent carbons have iron binding properties in vitro. However, the inhibition of iron absorption by phenolic compounds in vivo has been positively correlated with the presence of galloyl groups but not catechol groups.[36] Research is needed to elucidate the relationship between iron absorption and the chelation of iron by the C-4 carbonyl group of flavonols, flavones, and flavanones.

Conclusion

Epidemiological and in vitro evidence of antioxidant and cardioprotective effects support the hypothesis that flavonoids benefit health. The inhibition of LDL oxidation and platelet aggregation by flavonoids suggests that regular consumption of foods containing flavonoids and moderate consumption of red wine may protect against atherosclerosis and thrombotic tendency. The large contribution of flavonoids to the diet from tea, onions, and apples suggests that these foods may have greater nutritional benefits than previously recognized as they appear to constitute a major source of dietary antioxidants. More research is required for further elucidation of the mechanisms of flavonoid absorption, metabolism, and biochemical action and interaction with other nutrients in vivo. Furthermore, research is needed to identify the mechanisms by which flavonoids contribute to the amelioration of atherosclerosis and reduce the risk of morbidity and mortality from CHD.

References

1 Middleton, E., Jr. and Kandaswami, C. (1993). The impact of plant flavonoids on mammalian biology: Implications for immunity, inflammation and cancer. In *The Flavonoids: Advances in Research Since 1986.* (J.B. Harborne, ed.), p. 619–652, Chapman and Hall, London, UK

2 Ratty, A.K. and Das, N.P. (1988). Effects of flavonoids on nonenzymic lipid peroxidation: Structure activity relationship. *Biochem. Med. Metabol. Biol.* **39,** 69–79

3 Hackett, A.M. (1986). The metabolism of flavonoid compounds in mammals. In *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological, and Structural-Activity Relationships,* p. 177–194, Alan R. Liss, New York, NY USA

4 Hanasaki, Y., Ogawa, S., and Fukui, S. (1994). The correlation between active oxygens scavenging and antioxidative effects of flavonoids. *Free Radical Biol. Med.* **16,** 845–850

5 Hope, W.C., Welton, A.F., Fielder-Nagy, C., Batula-Bernardo, C., and Coffey, J.W. (1983). In vitro inhibition of the biosynthesis of slow reacting substances of anaphylaxis (SRS-A) and lipoxygenase activity of quercetin. *Biochem. Pharmacol.* **32,** 367–371

6 Duarte, J., Vizcaino, F.P., Utrilla, P., Jimenez, J., Tamargo, J., and Zarzuelo, A. (1993). Vasodilatory effects of flavonoids in rat aortic smooth muscle. Structure activity relationships. *Biochem. Pharmacol.* **24,** 857–862

7 Salvayre, R., Negre, A., Affany, A., Lenoble, M., and Douste-Blazy, L. (1988). Protective effect of plant flavonoids, analogs and vitamin E against lipid peroxidation of membranes. In *Plant Flavonoids in Biology and Medicine II. Biochemical, Cellular and Medicinal Properties,* p. 313–316, Alan R. Liss, New York, NY USA

8 Gryglewski, R.J., Korbut, R., Robak, J., and Swies, J. (1987). On the mechanism of antithrombotic action of flavonoids. *Biochem. Pharmacol.* **36,** 317–322

9 Bourdillat, B. Delautier, D., Labat, J., Benveniste, J., Potier, P., and Brink, C. (1988). Mechanism of action of hispidulin, a natural flavone, on human platelets. In *Plant Flavonoids in Biology and Medicine II. Biochemical, Cellular and Medicinal Properties,* p. 211–214, Alan R. Liss, New York, NY USA

10 Beretz, A. and Cazenave, J. (1988). The effect of flavonoids on blood-vessel wall interactions. In *Plant Flavonoids in Biology and Medicine II: Biochemical, Cellular, and Medicinal Properties,* p. 187–200, Alan R. Liss, New York, NY USA

11 Beretz, A., Anton, R., and Cazenave, J. (1986). The effect of flavonoids on cyclic nucleotide phosphodiesterase. In *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological and Structure-Activity Relationships,* p. 281–296, Alan R. Liss, New York, NY USA

12 Tzeng, S.H., Ko, W.-C., Ko, F.-N. and Teng, C.-M. (1991). Inhibition of platelet aggregation by some flavonoids. *Thromb. Res.* **64,** 91–100

13 Torel, J., Cillard, J., and Cillard, P. (1986). Antioxidant activity of flavonoids and reactivity with peroxy radical. *Phytochemistry* **25,** 383–385

14 Budavari, S., O'Neil, M.J., Smith, A., and Heckelman, P.E. (eds.). (1989). *The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals,* 11th ed., Merck & Co., Inc., USA

15 Robak, J., Korbut, R., Shridi, F., Swies, J., and Rzadkowska-Bodalska, H. (1988). On the mechanism of antiaggregatory effect of myricetin. *Pol. J. Pharmacol. Pharm.* **40,** 337–340

16 Hodnick, W.F., Milosavljevic, E.B., Nelson, J.H., and Pardini, R.S. (1988). Electrochemistry of flavonoids: Relationships between redox potentials, inhibition of mitochondrial respiration and production of oxygen radicals by flavonoids. *Biochem. Pharmacol.* **37,** 2607–2611

17 Pignol, B., Etienne, A., Crastes de Paulet, A., Deby, C., Mencia-Huerta, J.M., and Braquet, P. (1988). Role of flavonoids in the oxygen-free radical modulation of the immune response. In *Plant Flavonoids in Biology and Medicine II. Biochemical, Cellular and Medicinal Properties,* p. 173–182, Alan R. Liss, New York, NY USA

18 Fraga, C.G., Martino, V.S., Ferraro, G.E., Coussio, J.D., and Boveris, A. (1987). Flavonoids as antioxidants evaluated by in vitro and in situ liver chemiluminescence. *Biochem. Pharmacol.* **36,** 717–720

19 Cavallini, L., Bindoli, A., and Siliprandi, N. (1978). Comparative evaluation of antiperoxidative action of silymarin and other flavonoids. *Pharmacol. Res. Commun.* **10,** 133–136

20 Afanas'ev, I.B., Dorozhko, A.I., Brodskii, A.V., Kostyuk, V.A., and Potapovitch, A.I. (1989). Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. *Biochem. Pharmacol.* **38,** 1763–1769

21 Yuting, C., Rongliang, Z., Zhongjian, J., and Yong, J. (1990). Flavonoids as superoxide scavengers and antioxidants. *Free Radical Biol. Med.* **9,** 19–21

22 Mora, A., Paya, M., Rios, J.L., and Alcaraz, M.J. (1990). Structure-activity relationships of polymethoxyflavones and other flavonoids as inhibitors of non-enzymic lipid peroxidation. *Biochem. Pharmacol.* **40,** 793–797

23 Rankin, S.M., De Whalley, C.V., Hoult, J.R.S., Jessup, W., Wilkins, G.M., Collard, J., and Leake, D.S. (1993). The modification of low density lipoprotein by the flavonoids myricetin and gossypetin. *Biochem. Pharmacol.* **45,** 67–75

24 Steinberg, D., Parthasarathy, S., Carew, T.E., Khoo, J.C., and Witztum, J.L. (1989). Beyond cholesterol: Modification of low-density

lipoprotein that increases its atherogenicity. *New Engl. J. Med.* **320,** 915–924
25 Henriksen, T., Mahoney, E.M., and Steinberg, D. (1981). Enhanced macrophage degradation of low density lipoprotein previously incubated with cultured endothelial cell: Recognition by receptors for acetylated low density lipoprotein. *Proc. Natl. Acad. Sci. USA* **78,** 6499–6507
26 Frankel, E.N., Kanner, J., German, J.B., Parks, E., and Kinsella, J.E. (1993). Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet* **341,** 454–457
27 Renaud, S. and De Lorgeril, M. (1992). Wine, alcohol, platelets, and the French paradox for cononary heart disease. *Lancet* **339,** 1523–1526
28 Rimm, E.B., Giovannucci, E.L., Willet, W.C. Colditz, G.A., Ascherio, A., Rosner, B., and Stampfer, M.J. (1991). Prospective study of alcohol consumption and risk of cononary disease in men. *Lancet* **338,** 464–467
29 Coultate, T.P. (1990). *Food: The Chemistry of Its Components,* 2nd ed. The Royal Society of Chemistry, p. 137–149
30 Cody, V. (1988). Crystal and molecular structure of flavonoids. In *Plant Flavonoids in Biology and Medicine II: Biochemical, Cellular, and Medicinal Properties,* p. 29–44, Alan R. Liss, New York, NY USA
31 Harborne, J.B. (1986). Nature, distribution and function of plant flavonoids. In *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological, and Structure-Activity Relationships,* p. 15–24, Alan R. Liss, New York, NY USA
32 Harborne, J.B. (1988). Flavonoids in the environment: Structure-activity relationships. In *Plant Flavonoids in Biology and Medicine II: Biochemical, Cellular and Medicinal Properties,* p. 17–27, Alan R. Liss, New York, NY USA
33 Kühnau, J. (1976). The Flavonoids. A class of semi-essential food components: Their role in human nutrition. *Wld. Rev. Nutr. Diet.* **24,** 117–191
34 Havsteen, B. (1983). Flavonoids, a class of natural products of high pharmacological potency. *Biochem. Pharmacol.* **32,** 1141–1148
35 Hertog, M.G.L., Hollman, P.C.H., Katan, M.B., and Kromhout, D. (1993). Intake of potentially anticarcinogenic flavonoids and their determinants in adults in The Netherlands. *Nutr. Cancer* **20,** 21–29
36 Brune, M., Rossander, L., and Hallberg, L. (1989). Iron absorption and phenolic compound: Importance of different phenolic structures. *Eur. J. Clin. Nutr.* **43,** 547–558
37 Pierpoint, W.S. (1986). Flavonoids in the human diet. In *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological and Structure-Activity Relationships,* p. 125–140, Alan R. Liss, New York, NY USA
38 Muramatsu, K., Fukuyo, M., and Hara, Y. (1986). Effect of green tea catechins on plasma cholesterol level in cholesterol-fed rats. *J. Nutr. Sci. Vitaminol.* **32,** 613–622
39 Swain, T. (1986). The evolution of flavonoids. In *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological, and Structure-Activity Relationships,* p. 1–14, Alan R. Liss, New York, NY USA
40 Bravo, L., Abia, R., Eastwood, M.A., and Saura-Calixto, F. (1994). Degradation of polyphenols (catechin and tannic acid) in the rat intestinal tract. Effect on colonic fermentation and faecal output. *Brit. J. Nutr.* **71,** 933–946
41 De Whalley, C.V., Rankin, S.M., Hoult, J.R.S., Jessup, W., and Leake, D.S. (1990). Flavonoids inhibit the oxidative modification of low density lipoproteins by macrophages. *Biochem. Pharmacol.* **39,** 1743–1750
42 Das, N.P. (1971). Studies on flavonoid metabolism: Absorption and metabolism of (+)-catechin in Man. *Biochem. Pharmacol.* **20,** 3435–3445
43 Gugler, R., Leschik, M., and Dengler, H.J. (1975). Disposition of quercetin in Man after single oral and intravenous doses. *Eur. J. Clin. Pharmacol.* **9,** 229–234
44 Kaul, N., Siveski-Iliskovic, N., Hill, M., Slezak, J., and Singal, P.K. (1993). Free radicals and the heart. *J. Pharmacol. Toxicol. Meth.* **30,** 55–67
45 Niki, E. (1987). Antioxidants in relation to lipid peroxidation. *Chem. Phys. Lipids* **44,** 227–253
46 Robak, J. and Gryglewski, R.J. (1988). Flavonoids are scavengers of superoxide anions. *Biochem. Pharmacol.* **37,** 837–841
47 Das, N.P. and Ratty, A.K. (1986). Effect of flavonoids on induced non-enzymic lipid peroxidation. In *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological and Structure-Activity Relationships,* p. 243–247, Alan R. Liss, New York, NY USA
48 Morel, I., Lescoat, G., Cogrel, P., Sergent, O., Pasdeloup, N., Brissot, P., Cillard, P. and Cillard, J. (1993). Antioxidant and iron-chelating activities of the flavonoids catechin, quercetin and diosmetin on iron-loaded rat hepatocyte cultures. *Biochem. Pharmacol.* **45,** 13–19
49 Cholbi, M.R., Paya, M., and Alcaraz, M.J. (1991). Inhibitory effects of phenolic compounds on CCl_4-induced microsomal lipid peroxidation. *Experientia* **47,** 195–199
50 Husain, S.R., Cillard, J., and Cillard, P. (1987). Hydroxyl radical scavenging activity of flavonoids. *Phytochemistry* **26,** 2489–2491
51 Bokkenheuser, V.D. and Winter, J. (1988). Hydrolysis of flavonoids by human bacteria. In *Plant Flavonoids in Biology and Medicine II: Biochemical, Cellular, and Medical Properties,* p. 143–145, Alan R. Liss, New York, NY USA
52 Goldstein, J.L. and Brown, M.S. (1983). Lipid metabolism in the macrophage. *Ann. Rev. Biochem.* **52,** 223–262
53 Goldstein, J.L., Ho, Y.K., Basu, S.K., and Brown, M.S. (1979). Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition. *Proc. Natl. Acad. Sci. USA* **76,** 333–337
54 Sparrow, C.P., Parthasarathy, S., and Steinberg, D. (1989). A macrophage receptor that recognizes oxidized low density lipoprotein but not acetylated low density lipoprotein. *J. Biol. Chem.* **264,** 2599–2604
55 Kodama, T., Freeman, M., Rohrer, L., Zadrecky, J., Matsudaira, P., and Krieger, M. (1990). Type I macrophage scavenger receptor contains alpha-helical and collagen-like coiled coils. *Nature* **343,** 531–535
56 Jurgens, G., Hoff, H.F., Chisolm, G.M., and Esterbauer, H. (1987). Modification of human serum low density lipoprotein by oxidation. Characterization and pathophysiological implications. *Chem. Phys. Lipids.* **45,** 315–336
57 Mangiapane, H., Thomson, J., Salter, A., Brown, S., Bell, G.D., and White, D.A. (1992). The inhibition of the oxidation of low density lipoprotein by (+)-catechin, a naturally occurring flavonoid. *Biochem. Pharmacol.* **43,** 445–450
58 Palinsky, W., Rosenfeld, M.E., Yla-Herttula, S., Grutner, G.C., Socher, S.S., Butler, S.W., Parthasarathy, S., Carew, T.E., Steinberg, D., and Witzum, J.L. (1989). Low density lipoprotein undergoes oxidative modification in vivo. *Proc. Natl. Acad. Sci. USA* **86,** 1372–1376
59 Jessup, W., Rankin, S.M., de Whalley, C.V., Hoult, J.R.S., Scott, J., and Leake, D.S. (1990). Tocopherol consumption during LDL oxidation. *Biochem. J.* **265,** 399–405
60 Stocker, R. (1993). Natural antioxidants and atherosclerosis. *Asia Pac. J. Clin. Nutr.* **2** (Suppl 1), 15–20
61 Frei, B. and Gaziano, J.M. (1993). Content of antioxidants, preformed lipid hydroperoxides, and cholesterol as predictors of the susceptibility of human LDL to metal ion-dependent and independent oxidation. *J. Lipid Res.* **34,** 2135–2145
62 Steinbrecher, U.P., Parthasarathy, S., Leake, D.S., and Witztum, J.L. (1984). Modification of low density lipoprotein by endothelial cells involves lipid peroxidation and degradation of low density lipoprotein phospholipids. *Proc. Natl. Acad. Sci. USA* **81,** 3883–3887
63 Negre-Salvayre, A. and Salvayre, R. (1992). Quercetin prevents the cytotoxicity of oxidized LDL on lymphoid cell lines. *Free Radical Biol. Med.* **12,** 101–106
64 Parthasarathy, S., Young, S.G., Witztum, J.L., Pittman, R.C., and Steinberg, D. (1986). Probucol inhibits oxidative modification of low density lipoprotein. *J. Clin. Invest.* **77,** 641–644
65 Negre-Salvayre, A., Alomar, Y., Troly, M., and Salvayre, R. (1991). Ultraviolet-treated lipoproteins as a model system for the study of the biological effects of lipid peroxides on cultured cells. III. The protective effect of antioxidants (probucol, catechin, vitamin E) against the cytotoxicity of oxidized LDL occurs in two different ways. *Biochim. Biophys. Acta.* **1096,** 291–300
66 Gaziano, J.M. and Hennekens, C.H. (1993). The epidemiology of dietary antioxidants and atherosclerotic disease. *Asia Pac. J. Clin. Nutr.* **2,** (Suppl 1), 27–31
67 Bellizzi, M.C., Franklin, M.F., Duthie, G.G., and James, W.P.T.

(1994). Vitamin E and coronary heart disease: the European paradox. *Eur. J. Clin. Nutr.* **48,** 822–831

68 Hertog, M.G.L., Feskens, E.J.M., Hollman, P.C.H., Katan, M.B., and Kromhout, D. (1993). Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen Elderly Study. *Lancet* **342,** 1007–1011

69 Hertog, M.G.L., Hollman, P.C.H., and Van de Putte, B. (1993). Content of potentially anticarcinogenic flavonoids of tea infusions, wines and fruit juices. *J. Agric. Food Chem.* **41,** 1242–1246

70 Hertog, M.G.L., Hollman, P.C.H., and Katan, M.B. (1992). Content of potentially anticarcinogenic flavonoid of 28 vegetables and 9 fruits commonly consumed in The Netherlands. *J. Agric. Food Chem.* **40,** 2379–2383

71 Fuhrman, B., Lavy, A., and Aviram, M. (1995). Consumption of red wine with meals reduces the susceptibility of human plasma and LDL to lipid peroxidation. *Am. J. Clin. Nutr.* **61,** 549–554

72 Whitehead, T.P., Robinson, D., Allaway, S., Syms, J., and Hale, A. (1995). Effect of red wine ingestion on the antioxidant capacity of serum. *Clin. Chem.* **41,** 32–35

73 Beretz, A., Cazenave, J.-P., and Anton, A. (1982). Inhibition of aggregation and secretion of human platelets by quercetin and other flavonoids: Structure-activity relationships. *Agent. Actio.* **12,** 382–387

74 Swies, J., Robak, J., Dabrowski, L., Duniec, Z., Michalska, Z., and Gryglewski, R.J. (1984). Antiaggregatory effects of flavonoids in vivo and their influence on lipoxygenase and cyclooxygenase in vitro. *Pol. J. Pharmacol. Pharm.* **36,** 455–463

75 Elliott, A.J., Scheiber, S.A., Thomas, C., and Pardini, R.S. (1992). Inhibition of glutathione reductase by flavonoids. A structure-activity study. *Biochem. Pharmacol.* **44,** 1603–1608

76 Landolfi, R., Mower, R.L., and Steiner, M. (1984). Modification of platelet function and arachidonic acid metabolism by bioflavonoids: Structure-activity relations. *Biochem. Pharmacol.* **33,** 1525–1530

77 Ferrell, J.E., Chang Sing, P.D.G., Loew, G., King, R., Mansour, J.M., and Mansour, T.E. (1979). Structure-activity studies of flavonoids as inhibitors of cyclic AMP phosphodiesterase and relationship to quantum indices. *Mol. Pharmacol.* **16,** 556–568

78 Kuppusamy, U.R. and Das, N.P. (1992). Effects of flavonoids on cyclic AMP phosphodiesterase and lipid mobilization in rat adipocytes. *Biochem. Pharmacol.* **44,** 1307–1315

79 Ruf, J.-C., Berger, J.-L., and Renaud, S. (1995). Platelet rebound effect of alcohol withdrawal and wine drinking in rats: Relation to tannins and lipid peroxidation. *Atherioscler. Thromb. Vasc. Biol.* **15,** 140–144

80 Criqui, M.H. and Ringel, B.L. (1994). Does diet or alcohol explain the French paradox? *Lancet* **344,** 1719–1723

81 Hertog, M.G.L., Hollman, P.C.H., and Venema, D.P. (1992). Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. *J. Agric. Food Chem.* **40,** 1591–1598

82 Jaeger, A., Walti, M., and Neftel, K. (1988). Side effects of flavonoids in medical practice. In *Plant Flavonoids in Biology and Medicine II: Biochemical, Cellular, and Medical Properties,* p. 379–394, Alan R. Liss, New York, NY USA

83 Goldberg, D.M. (1995). Does wine work? *Clin. Chem.* **41,** 14–16

84 Hirono, I., Ueno, I., Hosaka, S., Takanashi, H., Matsushima, T., Sugimura, T., and Natori, S. (1981). Carcinogenicity examination of quercetin and rutin in ACI rats. *Cancer Lett.* **13,** 15–21