# Flavonoids protect LDL from oxidation and attenuate atherosclerosis

Bianca Fuhrman and Michael Aviram

Consumption of some plant-derived flavonoids results in their absorption and appearance in plasma and tissues. The inverse relationship between dietary flavonoids consumption and cardiovascular diseases may be associated with the ability of flavonoids to attenuate LDL oxidation, macrophage foam cell formation and atherosclerosis. The effect of flavonoids on arterial cell-mediated oxidation of LDL is determined by their accumulation in the lipoprotein and in arterial cells, such as macrophages. Flavonoids can reduce LDL lipid peroxidation by scavenging reactive oxygen/nitrogen species, chelation of transition metal ions and sparing of LDL-associated antioxidants. They can also reduce macrophage oxidative stress by inhibition of cellular oxygenases [such as nicotinamide adenine dinucleotide phosphate, reduced form (NADPH) oxidase] or by activating cellular antioxidants (such as the glutathione system). Thus, plant flavonoids, as potent natural antioxidants that protect against lipid peroxidation in arterial cells and lipoproteins, significantly attenuate the development of atherosclerosis. Curr Opin Lipidol 12:41–48. 

The Lipid Research Laboratory, Technion Faculty of Medicine, The Rappaport Family Institute for Research in the Medical Sciences and Rambam Medical Center, Haifa, Israel

Correspondence to Michael Aviram, The Lipid Research Laboratory, Rambam Medical Center, Haifa, Israel, 31096.
Tel: +972 4 854 2970; fax: +972 4 854 2130; e-mail: aviram@tx.technion.ac.il

**Current Opinion in Lipidology** 2001, 12:41–48

**Abbreviations**

| | |
|---|---|
| **AAPH** | 2,2′-azobis,2-amidinopropane hydrochloride |
| **NADPH** | nicotinamide adenine dinucleotide phosphate, reduced form |


0957-9672

## Introduction

The oxidative modification hypothesis of atherosclerosis proposes that LDL oxidation plays a causative role in early atherogenesis [1–8], and oxidized LDL has been shown to exist in atherosclerotic lesions [9]. The process of LDL oxidation occurs within the arterial wall, where all major cells, including endothelial cells, smooth muscle cells and monocyte-derived macrophages, can oxidize LDL [10–13]. The interaction of LDL with macrophages under oxidative stress activates cellular oxygenases, which can then produce reactive oxygen species and reactive nitrogen species, both of which are capable of oxidizing LDL [14]. Studies in cell culture identified a number of enzyme systems that could play a role in cell-mediated oxidation of LDL, including nicotinamide adenine dinucleotide phosphate, reduced form (NADPH) oxidase [14], 15-lipoxygenase [15], cytochrome P450 [16], and myeloperoxidase [17,18].

Under conditions of oxidative stress lipids in cells of the arterial wall, including arterial macrophages, are also exposed to oxidation, resulting in the formation of 'oxidized macrophages', which in turn can easily oxidize LDL [19,20]. On the other hand, LDL is protected from oxidation by human serum paraoxonase, which is an HDL-associated esterase that can hydrolyze and reduce lipid peroxides in lipoproteins and in lesions [21,22].

Because LDL oxidation in the arterial wall is a key event in early atherogenesis, agents that can prevent LDL oxidation could possibly attenuate the development of atherosclerosis. Thus, an effort is being made to identify natural dietary potent antioxidant components and also means to increase paraoxonase activity, in order to attenuate the progression of atherosclerosis. Flavonoids constitute one of the largest groups of antioxidant phytochemicals, and they are an integral part of the human diet. Flavonoid intake was shown [23] to be inversely related to morbidity and mortality from coronary heart disease, and this phenomenon could be associated with inhibition of LDL oxidation. The present review details recent studies on the antioxidative effect of dietary flavonoids, and presents a model for flavonoid-induced attenuation of atherogenesis.

## Flavonoids and LDL oxidation

Flavonoids are powerful antioxidants that act against LDL oxidation, and their antioxidant capacity is related to their localization in the LDL particle, as well as to their chemical structures [24–26]. Flavonoids, which are

mostly hydrophylic and thus not LDL-bound, can act as potent inhibitors of LDL oxidation via several mechanisms: (1) scavenging of free radicals by acting as reducing agents, as hydrogen atom donating molecules and as singlet oxygen quenchers; (2) chelation of transition metal ions, thereby reducing the metal's capacity to generate free radicals; (3) sparing of vitamin E and of carotenoids ($\beta$-carotene, lycopene) in the LDL particle, thus protecting LDL from oxidation; and (4) preserving or increasing serum paraoxonase activity, thus promoting hydrolysis of arterial cells and LDL-associated lipid peroxides.

Oxidation of LDL was inhibited *in vitro*, as well as *in vivo* (after dietary consumption), by several nutrients that are rich in flavonoids, including the following: ginger extract and its flavonoids gingerol and shogaol [27]; soy extract and its isoflavones genistein and daidzein [28]; pomegranate juice and its tannins [29••]; olive oil and its phenolics oleuropein and hydroxytyrosol [30]; and purple grape juice and its catechins [31•]. Tea polyphenols also inhibited LDL oxidation in some [32–35] but not all studies [36–38].

## Flavonoid localization and antioxidant activity

Protection of LDL against copper ion-induced or free radical-induced oxidation by flavonoids depends on their partitioning between the aqueous and the lipophylic compartments in plasma, within the LDL particle, and also on the physical localization of the generated free radicals.

Flavonoids contain both lipophylic and hydrophylic moieties, and hence they can act against free radicals that are generated in the aqueous and in the lipid milieu. The bioavailability of phenolic compounds was recently discussed by Duthie and Crozier [39•].

In humans, polyphenols from red wine were shown to be absorbed, to bind to LDL and to protect it from oxidation [40,41]. In contrast, a recent study [42•] showed that, although red wine consumption increased plasma phenolic acid concentrations, this increase was insufficient to affect resistance of LDL to oxidation. These results suggest that the absorption rate and the type of flavonoids in the red wine are important for its activity against LDL oxidation. Glabridin, the major flavonoid in licorice root extract, is an isoflavan with hydrophobic characteristics. After ingestion of licorice extract by humans, glabridin is absorbed and its hydrophobic characteristics direct its position in LDL towards the core lipid compartment [43,44]. After incubation of LDL with glabridin, catechin or quercetin, about 80% of the glabridin was found to be LDL associated, whereas quercetin and catechin, which are less lipophylic, only minimally bind to the LDL [44,45]. The soybean isoflavones daidzein and genistein in unesterified form are also incorporated into LDL to a relatively small extent.

Esterification with fatty acids at different hydroxyl groups provides lipophilicity, which is needed for flavonoid incorporation into LDL [46•]. The flavanol catechin prevented plasma lipid peroxidation that was induced by azo compounds, such as the water soluble 2,2′-azobis,2-amidinopropane hydrochloride (AAPH) and the lipid soluble 2,2′-azobis, 2,4-dimethylvaleronitrile. As expected from its hydrophylic structure, however, catechin showed a higher antioxidant capacity when the free radical reactions were initiated in the aqueous phase rather than in the lipid phase [47]. After administration of proanthocyanidin-rich extract from grape seeds, these flavonoids could be detected in plasma but not in the LDL fraction, suggesting that these antioxidants protect LDL from oxidation by their action in the aqueous milieu that surrounds the LDL particle [48].

## Protection of LDL-associated antioxidants by flavonoids

Flavonoids can also protect LDL from oxidation by their ability to spare some LDL-associated antioxidants. Glabridin enrichment of LDL prevented the consumption of $\beta$-carotene and of lycopene by 41% and 50%, respectively, after 1 h of LDL oxidation in the presence of AAPH, but failed to protect vitamin E, the major LDL-associated antioxidant, from oxidation [44]. Quercetin glycosides, as well as its aglycone form, were shown to inhibit the consumption of LDL-associated vitamin E during its oxidation [49]. Morin, fisetin, quercetin and gossypetin inhibited copper ion-induced LDL oxidation and macrophage-mediated oxidation of LDL with a 50% inhibitory concentration of 2 $\mu$mol/l, by protecting vitamin E in LDL from being consumed during oxidation [49].

## Flavonoid structure and antioxidant activity

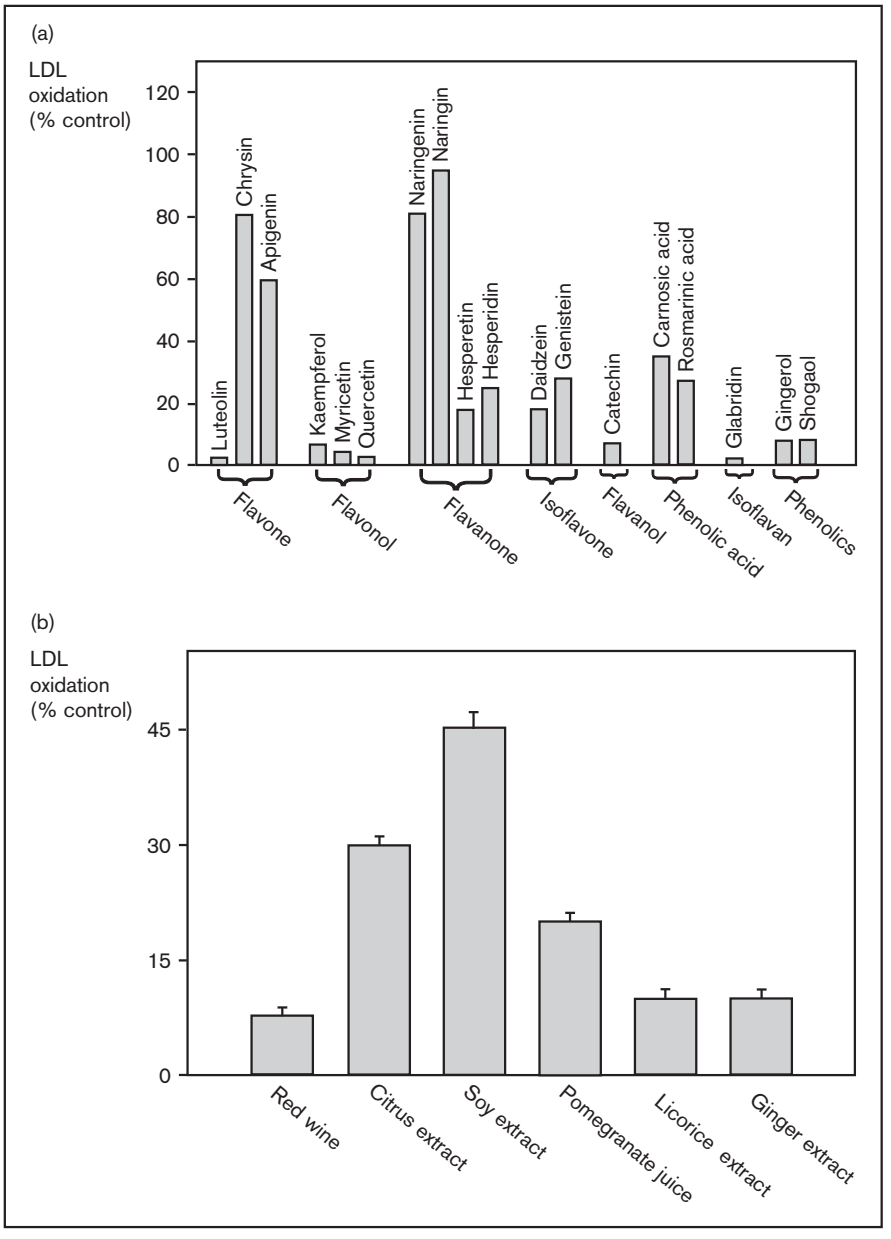
The main dietary sources and structure of flavonoids was recently reviewed by Lairon and Amiot [50]. The capacity of several groups of flavonoids and polyphenols to inhibit copper ion-induced LDL oxidation is presented in Fig. 1a.

Different groups of flavonoids exhibit an inhibitory activity against LDL oxidation at different ranges of concentration. Among the different groups of flavonoids, the flavonols, flavanols and isoflavans are most potent protectors of LDL against copper ion-induced oxidation. However, although possessing similar hydroxyl group arrangements, the flavonol quercetin is a more potent antioxidant in comparison with the flavanol catechin,

because of the 2-3 double bond and the 4-oxo structure in the quercetin ring C. Similarly, the antioxidative effect of glabridin on LDL oxidation resides mainly in the 2′-hydroxyl group of the isoflavan ring B [51]. The hydrophobic moiety of the isoflavan is also essential to obtain the inhibitory effect of glabridin on LDL oxidation.

Furthermore, within each group of flavonoids, there are differences in the antioxidant capacity of the individual flavonoids, which stems from their structure. For example, in the flavanone group, hesperetin and its glycoside hesperidin are more potent antioxidants than are naringenin and its glycoside naringin. In the phenolic acids group, rosmarinic acid is a more potent antioxidant against LDL oxidation than is carnosic acid. Quercetin, rutin, luteolin and kaempferol also inhibited copper ion-induced LDL oxidation [52]. Quercetin, rutin and luteolin were more effective inhibitors than kaempferol, probably because they were shown to chelate copper ions, in addition to their capacity to scavenge free radicals. Other flavonoids, which inhibited LDL oxidation via metal ion chelation, include hydroxycinnamic acid, caffeic acid, ferulic acids and p-coumaric acids [53].

**Figure 1. Inhibition of copper ion-induced LDL oxidation**




**(a)** Purified flavonoids. LDL (100 $\mu$g protein/ml) was incubated at 37°C for 2 h with 5 $\mu$mol/l $CuSO_4$ in the absence (control) or presence of 10 $\mu$mol/l of flavonoids [flavone (luteolin, chrysin, apigenin), flavonol (kaempferol, myricetin, quercetin), flavanone (naringenin, naringin, hesperetin, hesperidin), isoflavone (daidzein, genistein), and flavanol (catechin)], or with 75 $\mu$mol/l of phenolic acids (carnosic acid, rosmarinic acid), or 3 $\mu$mol/l of the isoflavan glabridin, or 0.3 $\mu$mol/l of the phenolics gingerol and shogaol. The extent of LDL oxidation was measured using the thiobarbituric acid reactive substances (TBARS) assay. **(b)** Flavonoid-rich nutrients. LDL (100 $\mu$g protein/ml) was incubated at 37°C for 2 h with 5 $\mu$mol/l $CuSO_4$ in absence (control) or presence of 1 ml/l red wine, 20 mg/l citrus extract, 50 mg/l soy extract, 30 $\mu$l/l pomegranate juice, 3 mg/l licorice extract, or 1 mg/l ginger extract. The control value (LDL incubated with no flavonoids) was 39 $\pm$ 6 nmol malondialdehyde equivalents/mg LDL protein. LDL oxidation is expressed as a percentage of the control value.

Natural antioxidants exist in nature in combination, and combinations of different antioxidants can act additively and even synergistically against LDL oxidation [54]. Although an individual polyphenol antioxidant inhibits lipid peroxidation via free radical scavenging or via metal ion chelation in a manner that depends on its characteristics, a mixture of several antioxidants in a natural extract can act in concert, thus increasing the efficacy of protection against oxidation. In this regard it was demonstrated that the efficiency of red wine resveratrol for protecting polyunsaturated fatty acids was low in oxidation induced by AAPH (a free radical initiator) and was high in copper ion-induced oxidation. Red wine extract, which contained monomeric and oligomeric forms of flavonoids and phenolic acids, protected LDL to a greater extent by both mechanisms [55].

Red wine, licorice extract, ginger extract and pomegranate juice remarkably inhibited LDL lipid peroxidation (by 80–90%), whereas citrus extract and soy extract demonstrated only up to 70% and 55% inhibition, respectively (Fig. 1b).

Several antioxidants were recently shown to preserve human serum paraoxonase activity, because they decrease the content of lipid peroxides, which are potent oxidants that inactivate paraoxonase [56]. Licorice root glabridin, when present during LDL oxidation together with human serum paraoxonase, preserved the activity of paraoxonase, including its ability to hydrolyze oxidated LDL cholesteryl linoleate hydroperoxides [56].

Catechin, quercetin and red wine consumption also preserved serum paraoxonase activity and prevented its inactivation in apolipoprotein E-deficient mice [45]. The most potent natural antioxidant in this respect however is pomegranate juice; consumption of pomegranate juice by humans not only preserved, but even increased serum paraoxonase activity by 18% [29••].

## Effect of flavonoids on macrophage-mediated oxidation of LDL

The capacity of macrophages to oxidize LDL depends on the oxidative status of the cells, which is determined by the balance between cellular pro-oxidants and cellular antioxidants [7,8,57,58]. Flavonoids can affect LDL oxidation by arterial cells, including macrophages, by inhibition of cellular oxygenases or activation of cellular antioxidants.

Consumption of pomegranate juice resulted in the inhibition of cellular lipid peroxidation and the formation of 'oxidized macrophages' [29••]. Consumption of nutrients that are rich in flavonoids (such as pomegranate juice or ginger extract) or of purified flavonoids (such as glabridin, catechin or quercetin) by apolipoprotein E-deficient mice resulted in a reduced capacity of the harvested macrophages to oxidize LDL [58].

Red wine was shown to inhibit macrophage-mediated oxidation of LDL *in vitro*, when added to the incubation medium [59]. Upon incubation of macrophages with the isoflavan glabridin, with the flavanol catechin, or with the flavonol quercetin, all of these flavonoids accumulated in the cells in a time-dependent and dose-dependent manner, and this phenomenon was accompanied by a substantial reduction in the capacity of the flavonoid-enriched cells to oxidize LDL [57]. Cell fractionation revealed that approximately 60% of the accumulated glabridin was localized in the macrophage plasma membrane [60••]. These results were confirmed *in vivo*. Peritoneal macrophages harvested from apolipoprotein E-deficient mice that consumed 20 $\mu$g/day per mouse of glabridin contained 1.6 $\mu$g glabridin/mg cell protein, and LDL oxidation by these glabridin-enriched cells was reduced by 88% [60••].

The mechanisms by which flavonoids inhibit cell-mediated oxidation of LDL include inhibition of cellular oxygenases, and hence the production of reactive oxygen and nitrogen species. Pretreatment of macrophages with tea-derived flavonoids such as theaflavin reduced cell-mediated oxidation of LDL and decreased superoxide anion production by macrophages [61].

Resveratrol, a polyphenol that is present in red wine was shown to inhibit superoxide radicals and hydrogen peroxide production and to decrease the release of arachidonic acid from stimulated macrophages [62]. Glabridin that accumulated in macrophages inhibited cell-mediated oxidation of LDL via inhibition of superoxide anion release because of the inhibition of the macrophage NADPH oxidase machinery [60••]. Glabridin inhibited the activation of NADPH oxidase, secondary to its inhibitory effect on the translocation of the cytosolic component P-47 to the plasma membrane, and this effect was related to inhibition of the macrophage protein kinase C [60••]. Other cellular oxygenases were also shown to be inhibited by flavonoids.

Quercetin glycoside and its aglycone were both shown to inhibit lipoxygenase-induced LDL oxidation more efficiently than ascorbic acid or vitamin E [63]. Similarly, catechin, epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate inhibited free radical production by inhibiting the liver enzyme xanthine oxidase [64].

## Effect of flavonoid consumption on the development of atherosclerosis

The effect of flavonoid consumption on the development of atherosclerotic lesions was studied in animal

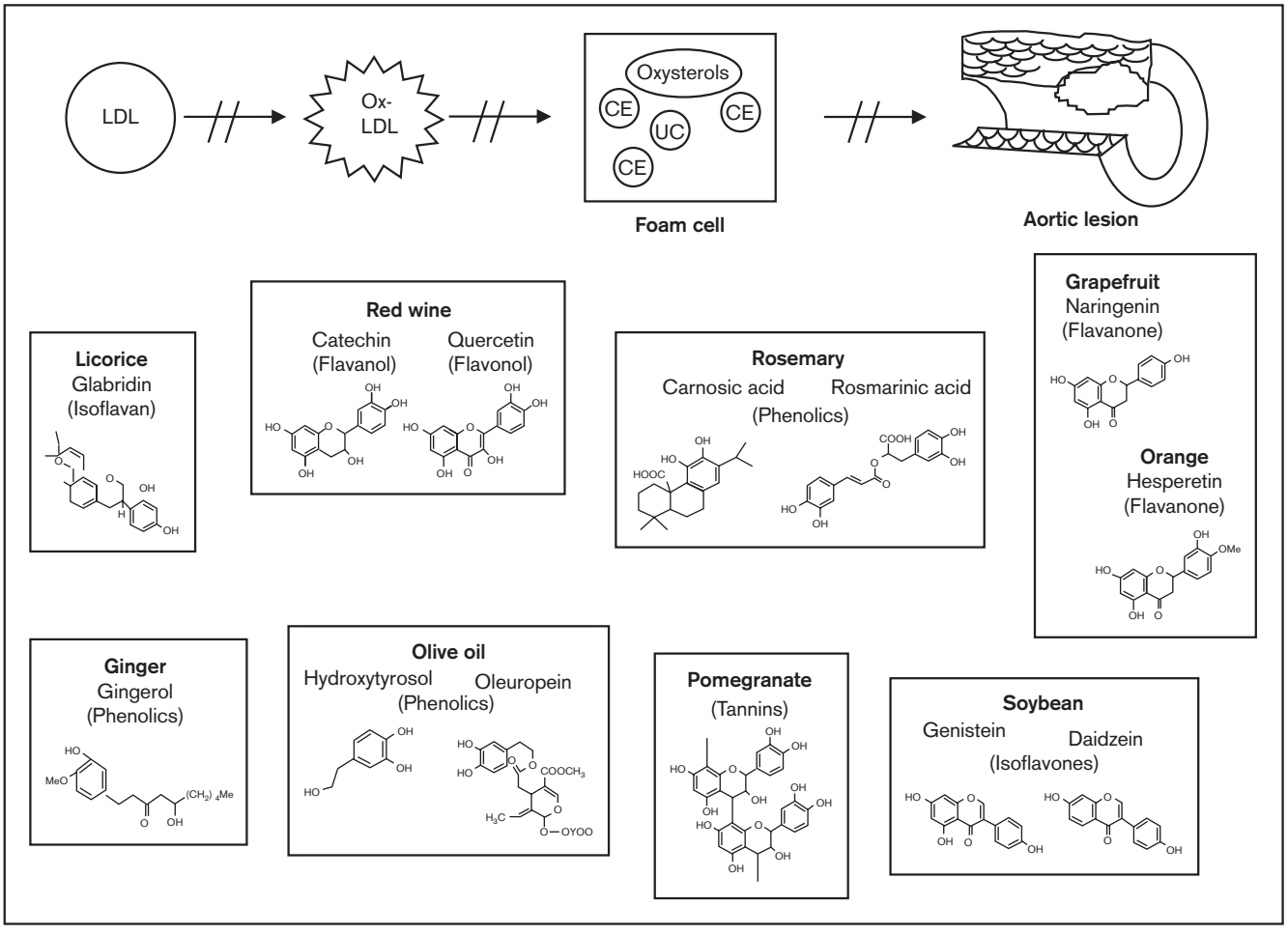
models. Apolipoprotein E-deficient mice are used as an animal model for the investigation of antiatherosclerotic effects of antioxidants. These mice are hypercholesterolaemic, and under conditions of increased oxidative stress they develop atherosclerotic lesions at 3–4 months of age [65]. These mice therefore offer the possibility of studying the effect of flavonoid consumption not only on induced oxidation (by copper ion or by free radical generators), but also on the basal oxidative state in the plasma and lipoproteins, because those are already

**Table 1. Antioxidative and antiatherosclerotic effects of flavonoids**

| | Inhibitory effect (% of control) | | | | |
|---|---|---|---|---|---|
| Nutrient/flavonoid | LDL oxidative state | LDL oxidation (copper ion-induced) | LDL aggregation (vortexing) | Atherosclerotic lesion area | Ref. |
| Red wine: | 49 | 43 | 50 | 48 | [40,45] |
| Catechin | 39 | 39 | 33 | 39 | [45] |
| Quercetin | 48 | 54 | 48 | 46 | [45] |
| Licorice | 54 | 68 | 29 | 35 | [43] |
| Glabridin | 51 | 37 | 25 | 30 | [43,60] |
| Pomegranate juice | 30 | 60 | 43 | 44 | [29••] |
| Ginger (gingerol) | 62 | 50 | 33 | 44 | [27] |

Control values are the following: LDL oxidative state, 3.0 $\pm$ 1.1 nmol thiobarbituric acid reactive substances (TBARS)/mg LDL protein; LDL oxidation (copper ion-induced), 30 $\pm$ 7 nmol TBARS/mg LDL protein; LDL aggregation, 0.45 $\pm$ 0.04 Optical Density at 680 nm; atherosclerosis lesion area, 17 000 $\pm$ 1500 $\mu m^2$.

**Figure 2. Anti-atherogenic effects of dietary polyphenolic flavonoids**




Dietary consumption of nutrients rich in flavonoids inhibit LDL oxidation, foam cell formation and the development of aortic atherosclerotic lesions. Major flavonoid-rich nutrients are shown, along with the chemical structure of their flavonoids. CE, cholesteryl ester; Ox-LDL, oxidized LDL; UC, unesterified cholesterol.

minimally oxidized in apolipoprotein E-deficient mice. On the contrary, human plasma lipids in most cases are not oxidized, and thus oxidation studies in humans involve analyses of susceptibility to oxidation, rather than the oxidative status.

Reduced susceptibility of LDL to oxidation, decreased basal LDL oxidative state and decreased development of atherosclerotic lesions in apolipoprotein E-deficient mice was demonstrated after supplementation of red wine or its flavonoids quercetin and catechin [45], licorice or its derived isoflavan glabridin [43], ginger extract [27] and pomegranate juice [29••] (Table 1). Ingestion of proanthocyanidin-rich extract from grape seeds reduced severe atherosclerosis in the aorta of cholesterol-fed rabbits [48].

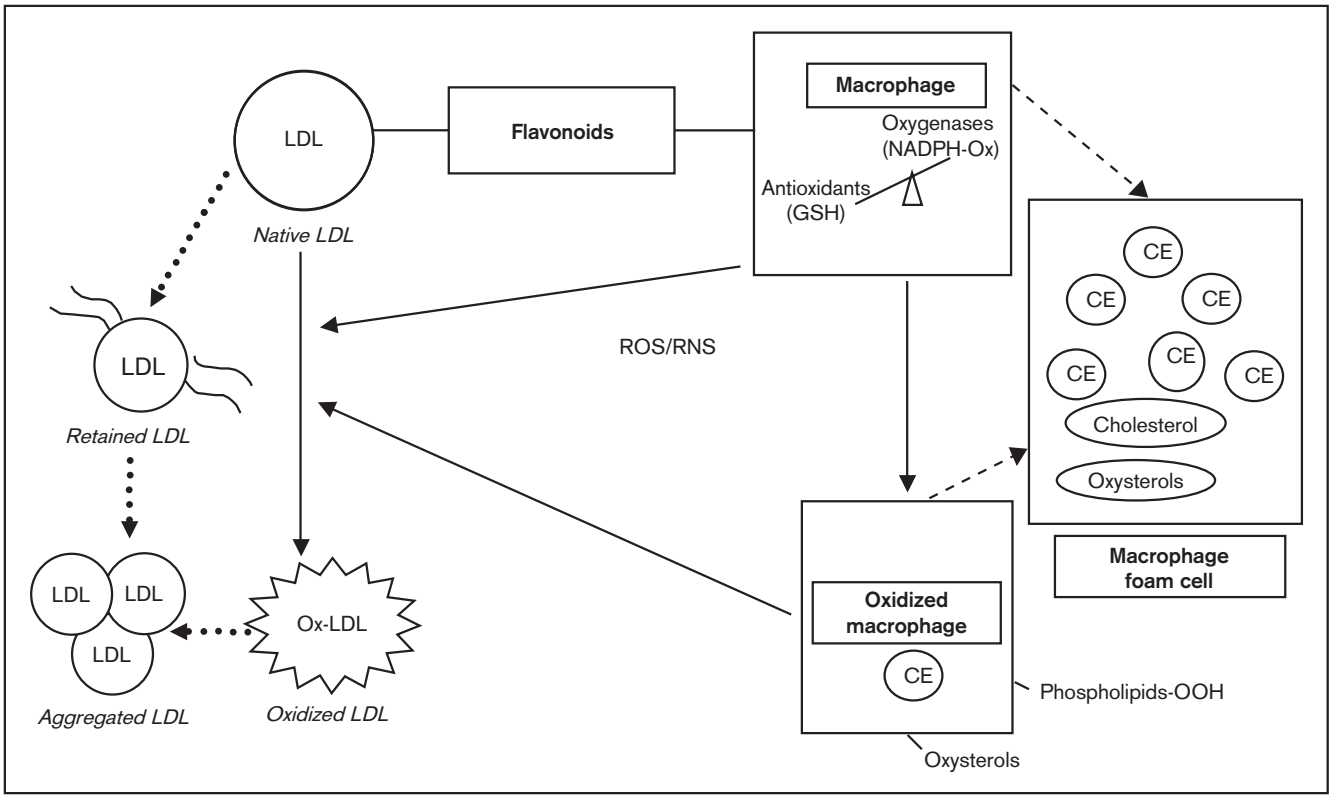
Red wine consumption by the Watanabe heritable hyperlipidemic rabbits (another animal model of atherosclerosis) failed to prevent the progression of atherosclerotic lesions, despite reduction in the susceptibility of LDL to oxidation [66]. Figure 2 shows several nutrients and their derived flavonoids which were shown to possess antioxidative and antiatherosclerotic properties.

## Conclusion

Dietary consumption of certain flavonoid-rich nutrients such as red wine, tea, pomegranate juice, licorice and ginger extract has been shown to inhibit LDL oxidation (and in some cases also LDL aggregation and retention) and to attenuate the progression of atherosclerosis in laboratory animals. Figure 3 summarizes our current view on the antioxidative effects of flavonoids against atherogenic modifications of LDL.

Flavonoids can protect LDL from oxidation via a direct interaction with the lipoprotein and, through their accumulation in arterial macrophages, they can reduce cell-mediated oxidation of LDL. Dietary supplementation of flavonoid-rich nutrients to atherosclerosis-prone animals has been shown to protect LDL against

**Figure 3. Flavonoids inhibit macrophage-mediated oxidation of LDL and foam cell formation**




Flavonoids can associate directly with LDL, resulting in the inhibition of LDL oxidation. Flavonoids can also associate with arterial cells such as macrophages, resulting in the inhibition of cellular oxygenases such as nicotinamide adenine dinucleotide phosphate, reduced form oxidase (NADPH-Ox) system, or in the activation of cellular antioxidants such as the glutathione (GSH) system. Reduction in the formation and release of macrophage-reactive oxygen species/nitrogen species (ROS/RNS) by flavonoids, inhibit the formation of 'oxidized macrophages', and hence reduce cell-mediated oxidation of LDL. Together, these effects lead to a reduced formation of macrophage-foam cells, and thus attenuate the development of atherosclerosis.

atherogenic modification, along with a significant inhibition of cholesterol and oxysterol accumulation in macrophages, and reduction in foam cell formation and the development of complicated atherosclerotic lesions. However, data on the antiatherosclerotic properties of dietary flavonoids in humans are still not available, though a beneficial effect of pomegranate juice consumption on carotid lesion size was recently found in our laboratory. Clinical and nutritional studies in humans should be also directed towards the use of combinations of different flavonoids, as well as combinations of flavonoids together with other nutritional antioxidants, such as vitamin E and carotenoids.

## References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest


**1** Steinberg D. Low density lipoprotein oxidation and its pathobiological significance. J Biol Chem 1997; 272:20963–20966.

**2** Berliner JA, Heinecke JW. The role of oxidized lipoproteins in atherosclerosis. Free Radic Biol Med 1996; 20:707–727.

**3** Aviram M. Review of human studies on oxidative damage and antioxidant protection related to cardiovascular diseases. Free Radic Res 2000; (in press).

**4** Aviram M. Interaction of oxidized low density lipoprotein with macrophages in atherosclerosis and the antiatherogenicity of antioxidants. Eur J Clin Chem Clin Biochem 1996; 34:599–608.

**5** Parthasarathy S, Santanam N, Auge N. Oxidized low-density lipoprotein, a two-faced janus in coronary artery disease? Biochem Pharmacol 1998; 56:279–284.

**6** Kaplan M, Aviram M. Oxidized low density lipoprotein: atherogenic and proinflammatory characteristics during macrophage foam cell formation. An inhibitory role for nutritional antioxidants and serum paraoxonase. Clin Chem Lab Med 1999; 37:777–787.

**7** Aviram M. Antioxidants in restenosis and atherosclerosis. Curr Intervent Cardiol Rep 1999; 1:66–78.

**8** Aviram M. Macrophage foam cell formation during early atherogenesis is determined by the balance between pro-oxidants and antioxidants in arterial cells and in blood lipoproteins: a need for a dual approach. Antiox Redox Signal 1999; 1:585–594.

**9** Aviram M, Maor I, Keidar S, *et al.* Lesioned low density lipoprotein in atherosclerotic apolipoprotein E-deficient transgenic mice and in humans is oxidized and aggregated. Biochem Biophys Res Commun 1995; 216:501–513.

**10** Witztum JL, Steinberg D. Modification of low density lipoprotein by endothelial cells involves lipid peroxidation and degradation of low density lipoprotein phospholipids. Proc Natl Acad Sci USA 1984; 81:3883–3887.

**11** Heinecke JW, Rosen H, Chait A. Iron and copper promote modification of low density lipoprotein by human arterial smooth muscle cells in culture. J Clin Invest 1984; 74:1980–1984.

**12** Parthasarathy S, Printz DJ, Boyd D, *et al.* Macrophage oxidation of low density lipoprotein generates a modified form recognized by the scavenger receptor. Arteriosclerosis 1986; 6:505–510.

**13** Aviram M, Rosenblat M. Macrophage mediated oxidation of extracellular low density lipoprotein requires an initial binding of the lipoprotein to its receptor. J Lipid Res 1994; 35:385–398.

**14** Aviram M, Rosenblat M, Etzioni A, Levy R. Activation of NADPH oxidase is required for macrophage-mediated oxidation of low density lipoprotein. Metabolism 1996; 45:1069–1079.

**15** Yla-Herttuala S, Rosenfeld ME, Parthasarathy S, *et al.* Colocalization of 15-lipoxygenase mRNA and protein with epitopes of oxidized low density lipoprotein in macrophage-rich areas of atherosclerotic lesions. Proc Natl Acad Sci USA 1990; 87:6959–6963.

**16** Aviram M, Kent UM, Hollenberg PF. Microsomal cytochromes P450 catalyze the oxidation of low density lipoprotein. Atherosclerosis 1999; 143:253–260.

**17** Heinecke JW. Mechanism of oxidative damage of low density lipoprotein in human atherosclerosis. Curr Opin Lipidol 1997; 8:268–274.

**18** Heinecke JW. Pathways for oxidation of low density lipoprotein by myeloperoxidase: tyrosyl radical, reactive aldehydes, hypochlorous acid and molecular chlorine. Biofactors 1997; 6:145–155.

**19** Fuhrman B, Oiknine J, Aviram M. Iron induces lipid peroxidation in cultured macrophages, increases their ability to oxidatively modify LDL and affect their secretory properties. Atherosclerosis 1994; 111:65–78.

**20** Fuhrman B, Oiknine J, Keidar S, *et al.* Increased uptake of low density lipoprotein (LDL) by oxidized macrophages is the result of enhanced LDL receptor activity and of progressive LDL oxidation. Free Radic Biol Med 1997; 23:34–46.

**21** Aviram M, Billecke S, Sorenson R, *et al.* Paraoxonase active site required for protection against LDL oxidation involves its free sulfhydryl group and is different from that required for its arylesterase/paraoxonase activities: selective action of human paraoxonase allozymes Q and R. Arterioscler Thromb Vasc Biol 1998; 18:1617–1624.

**22** Aviram M, Rosenblat M, Bisgaier CL, *et al.* Paraoxonase inhibits high-density lipoprotein oxidation and preserves its functions. A possible peroxidative role for paraoxonase. J Clin Invest 1998; 101:1581–1590.

**23** Hertog MG, Kromhout D, Aravanis C, *et al.* Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. Arch Intern Med 1995; 155:381–386.

**24** Rice-Evans CA, Miller NJ, Bolwell PG, *et al.* The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Radic Res 1995; 22:375–383.

**25** Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med 1996; 20:933–956.

**26** Van Acker SABE, Van-den Berg DJ, Tromp MNJL, *et al.* Structural aspects of antioxidants activity of flavonoids. Free Radic Biol Med 1996; 20:331–342.

**27** Fuhrman B, Rosenblat M, Hayek T, *et al.* Dietary consumption of ginger extract attenuates development of atherosclerosis in the atherosclerotic apolipoprotein E deficient mice: hypocholesterolemic and antioxidative effects. J Nutr 2000; 130:1124–1131.

**28** Tikkanen MJ, Wahala K, Ojala S, *et al.* Effect of soybean phytoestrogen intake on low density lipoprotein oxidation resistance. Proc Natl Acad Sci USA 1998; 95:3106–3110.

**29** •• Aviram M, Dorenfeld L, Rosenblat M, *et al.* Pomegranate juice consumption reduces oxidative stress, atherogenic modifications to LDL and platelet aggregation: studies in humans and in the atherosclerotic apolipoprotein E deficient mice. Am J Clin Nutr 2000; 71:1062–1076.
This study demonstrates that pomegranate juice has potent antiatherogenic effects in healthy humans and in atherosclerotic mice. Pomegranate juice consumption inhibited LDL atherogenic modifications, including LDL oxidation, aggregation and retention; reduced macrophage atherogenicity, including cell-mediated oxidation of LDL and cellular uptake of oxidized LDL; and reduced aortic atherosclerotic lesion formation.

**30** Aviram M, Eias K. Dietary olive oil reduces low-density lipoprotein uptake by macrophages and decreases the susceptibility of the lipoprotein to undergo lipid peroxidation. Ann Nutr Metab 1993; 37:75–84.

**31** • Stein JH, Keevil JG, Wiebe DA, *et al.* Purple grape juice improves endothelial function and reduces the susceptibility of LDL cholesterol to oxidation in patients with coronary artery disease. Circulation 1999; 100:1050–1055.
This study shows that ingestion of purple grape juice improved endothelial function and reduced LDL susceptibility to oxidation in patients with coronary artery disease. Both of these processes represent potential mechanisms by which purple grape juice may prevent cardiovascular events.

**32** Ishikawa T, Suzukawa M, Ito T, *et al.* Effect of tea flavonoid supplementation on the susceptibility of low density lipoprotein to oxidative modification. Am J Clin Nutr 1997; 66:261–266.

**33** Weisburger JH. Tea and health: the underlying mechanisms. Proc Soc Exp Biol Med 1999; 220:271–275.

**34** Serafini M, Ghiselli A, Ferro-Luzzi A. In vivo antioxidant effect of green and black tea in man. Eur J Clin Nutr 1996; 50:28–32.

**35** Benzie IFF, Szeto YT, Tomlinson B, Strain JJ. Drinking green tea leads to a rapid increase in plasma antioxidant potential. In: Natural antioxidants and anticarcinogens in nutrition, health and disease. Kumpulainen JT, Salonen JT (editors). Cambridge, UK: The Royal Society of Chemistry; 1999. pp. 280–282.

**36** Van het Hof KH, de Boer HS, Wiseman SA, *et al.* Consumption of green or black tea does not increase resistance of low density lipoprotein to oxidation in humans. Am J Clin Nutr 1997; 66:1125–1132.

**37** McAnlis GT, McEneny J, Pearce J, Young IS. Black tea consumption does not protect low density lipoprotein from oxidative modification. Eur J Clin Nutr 1998; 52:202–206.

**38** Princen HM, van Duyvennvoorde W, Buytenhek R, *et al.* No effect of consumption of green and black tea on plasma lipid and antioxidant levels and on LDL oxidation in smokers. Arterioscler Thromb Vasc Biol 1998; 18:833–841.

**39** • Duthie G, Crozier A. Plant-derived phenolic antioxidants. Curr Opin Lipidol 2000; 11:43–47.
This is an important review on the bioavailability of phenolic compounds.

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

**41** Nigdikar SV, Williams NR, Griffin BA, Howard AN. Consumption of red wine polyphenols reduces the susceptibility of low density lipoproteins to oxidation in vivo. Am J Clin Nutr 1998; 68:258–265.

**42** • Caccetta RA, Croft KD, Beilin LJ, Puddey IB. Ingestion of red wine significantly increases plasma phenolic acid concentrations but does not acutely affect ex vivo lipoprotein oxidizability. Am J Clin Nutr 2000; 71:67–74.
This study shows that, although polyphenols concentration in plasma increased significantly after red wine consumption, the increase was insufficient to influence LDL oxidation *ex vivo*.

**43** Fuhrman B, Buch S, Vaya J, *et al.* Licorice extract and its major polyphenol glabridin protect low-density lipoprotein against lipid peroxidation: in vitro and ex vivo studies in humans and in atherosclerotic apolipoprotein E-deficient mice. Am J Clin Nutr 1997; 66:267–275.

**44** Belinky PA, Aviram M, Fuhrman B, *et al.* The antioxidative effects of the isoflavan glabridin on endogenous constituents of LDL during its oxidation. Atherosclerosis 1998; 137:49–61.

**45** Hayek T, Fuhrman B, Vaya J, *et al.* Reduced progression of atherosclerosis in the apolipoprotein E deficient mice following consumption of red wine, or its polyphenols quercetin or catechin, is associated with reduced susceptibility of LDL to oxidation and aggregation. Arterioscler Thromb Vasc Biol 1997; 17:2744–2752.

**46** • Meng QH, Lewis P, Wahala K, *et al.* Incorporation of esterified soybean isoflavones with antioxidant activity into low density lipoprotein. Biochim Biophys Acta 1999; 1438:369–376.
This study shows that soybean-isoflavone protect LDL from oxidation after incorporation into the LDL particle. Lipophilicity of the flavonoid is needed for the incorporation of flavonoids into LDL.

**47** Lotito SB, Fraga CG. (+)-Catechin prevents human plasma oxidation. Free Radic Biol Med 1998; 24:435–441.

**48** Yamakoshi J, Kataoka S, Koga T, Ariga T. Proanthocyanidin-rich extract from grape seeds attenuates the development of aortic atherosclerosis in cholesterol-fed rabbits. Atherosclerosis 1999; 142:139–149.

**49** De Whalley CV, Rankin SM, Hoult RS, *et al.* Flavonoids inhibit the oxidative modification of low density lipoproteins by macrophages. Biochem Pharmacol 1990; 39:1743–1750.

**50** Lairon D, Amiot MJ. Flavonoids in food and natural antioxidants in wine. Curr Opin Lipidol 1999; 10:23–28.

**51** Belinky P, Aviram M, Mahmood S, Vaya J. Structural aspects of the inhibitory effect of glabridin on LDL oxidation. Free Radic Biol Med 1998; 25:1419–1429.

**52** Brown JA, Khodr H, Hider RC, Rice-Evans C. Structural dependence of flavonoid interactions with $Cu^{2+}$ ions: implications for their antioxidant properties. Biochem J 1998; 330:1173–1178.

**53** Nardini M, D'Aquino M, Tamassi G, *et al.* Inhibition of human low density lipoprotein oxidation by caffeic acid and other hydroxycinnamic acid derivatives. Free Radic Biol Med 1995; 19:541–552.

**54** Fuhrman B, Volkova N, Rosenblat M, Aviram M. Lycopene synergistically inhibits LDL oxidation in combination with vitamin E, glabridin, rosmarinic acid, carnosic acid, or garlic. Antiox Redox Signal 2000; 2:491-506.

**55** Fremon L, Belguendouz L, Delpal S. Antioxidant activity of resveratrol and alcohol-free wine polyphenols related to LDL oxidation and polyunsaturated fatty acids. Life Sci 1999; 64:2511–2521.

**56** Aviram M, Rosenblat M, Billecke S, *et al.* Human serum paraoxonase (PON 1) is inactivated by oxidized low density lipoprotein and preserved by antioxidants. Free Radic Biol Med 1999; 26:892–904.

**57** Aviram M, Fuhrman B. LDL oxidation by arterial wall macrophages depends on the antioxidative status in the lipoprotein and in the cells: role of prooxidants vs. antioxidants. Mol Cell Biochem 1998; 188:149–159.

**58** Aviram M, Fuhrman B. Polyphenolic flavonoids inhibit macrophage-mediated oxidation of LDL and attenuate atherogenesis. Atherosclerosis 1998; 137(suppl):S45–S50.

**59** Rifici VA, Stephan EM, Schneider SH, Khachadurian AK. Red wine inhibits the cell-mediated oxidation of LDL and HDL. J Am Coll Nutr 1999; 18:137–143.

**60** •• Rosenblat M, Belinky P, Vaya J, *et al.* Macrophage enrichment with the isoflavan glabridin inhibits NADPH oxidase-induced cell mediated oxidation of low density lipoprotein. J Biol Chem 1999; 274:13790–13799.
This study investigated the mechanism by which glabridin, an isoflavan derived from licorice root, inhibits macrophage-mediated oxidation of LDL. Glabridin is taken up and accumulates in macrophages, resulting in reduced capacity of these cells to oxidize LDL due to glabridin-inhibition of NADPH oxidase, via inhibition of protein kinase C activity.

**61** Yoshida H, Ishikawa T, Hosoai H, *et al.* Inhibitory effect of tea flavonoids on the ability of cells to oxidize low density lipoprotein. Biochem Pharmacol 1999; 58:1695–1703.

**62** Martinez J, Moreno JJ. Effect of resveratrol, a natural polyphenolic compound, on reactive oxygen species and prostaglandin production. Biochem Pharmacol 2000; 59:865–870.

**63** Luiz da Silva E, Tsushida T, Terao J. Inhibition of mammalian 15-lipoxygenase-dependent lipid peroxidation in low-density lipoprotein by quercetin and quercetin monoglucosides. Arch Biochem Biophys 1998; 349:313–320.

**64** Aucamp J, Gaspar A, Hara Y, Apostolides Z. Inhibition of xanthine oxidase by catechins from tea (*Camelia sinensis*). Anticancer Res 1997; 17:4381–4385.

**65** Hayek T, Oiknine J, Brook JG, Aviram M. Increased plasma lipoprotein lipid peroxidation in apo E-deficient mice. Biochem Biophys Res Commun 1994; 201:1567–1574.

**66** Wakabayashi Y. Effect of red wine consumption on low density lipoprotein oxidation and atherosclerosis in aorta and coronary artery in Watanabe heritable hyperlipidemic rabbits. J Agric Food Chem 1999; 47:4724–4730.