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MINIREVIEW

Dietary Flavonoids and Risk of Coronary Heart Disease

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Summary

Flavonoids, a group of phenolic compounds found naturally in fruit, vegetables, nuts, flowers, seeds and bark are an integral part of the human diet. They have been reported to exhibit a wide range of biological effects, including anti-ischemic, antiplatelet, antineoplastic, antiinflammatory, antiallergic, antilipoperoxidant or gastroprotective actions. Furthermore, flavonoids are potent antioxidants, free radical scavengers and metal chelators, and inhibit lipid peroxidation. Oxidative modification of low-density lipoproteins (LDLs) is believed to play a crucial role in atherogenesis. Epidemiological studies have shown that the consumption of fruits and vegetables, and regular red wine consumption is related with a reduced risk of cardiovascular diseases.

Key words

Flavonoids • Coronary heart diseases • LDL • Free radicals • Wine • Diet

The human body generates free radicals under a number of conditions. They are by-products of the body´s normal routine of processing the oxygen we breathe and the food we eat. Exercise, smoking, exposure to pollutants and excess sunlight contribute to the production of free radicals in the body. These radicals are very unstable and, therefore, very reactive. After an attack of radicals on membranes and lipoproteins, lipid peroxidation starts and may lead to the development of vascular lesions. The human body has developed antioxidant protection against free radicals such as superoxide dismutases, glutathione peroxidases, plasma iron binding proteins, or alpha-tocopherol (Catapano 1997). When the balance between the production and elimination of free radicals is impaired, an oxidative stress develops. Free radicals frequently induce cascade reactions, the result of which may be the damage of cells, whole tissues and organs (Mojžiš *et al.* 1998). Oxygen-derived free radicals and oxidative stress also play a significant role in a large variety of cardiovascular diseases, including atherosclerosis and ischemic heart disease (Kalra and Prasad 1994, Das and Maulik 1995). Reparative processes may not fully eliminate the damage of biological macromolecules. A much more effective way is the prevention, e.g. reduction of sources of free radical formation to a minimum, and reinforcement of natural antioxidant mechanism by administration of agents with antioxidant action. For this reason, in the study of agents with antioxidant action, special attention

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is paid to the search for agents with a possible preventive action (Mojžišová *et al*. 1999).

Flavonoids are an ubiquitous group of polyphenolic compounds of variable chemical structure present in fruits, vegetables, nuts, seeds and beverages such as tea, coffee, beer and wine (Kandaswaani and Middleton 1994). More than 4000 types of flavonoid compounds have been isolated from various plants. Depending on their structural features, flavonoids can be further subdivided into flavones, flavonols, isoflavones, flavanes and flavanols (Cook and Samman 1996). The biological activities of the flavonoids have been extensively reviewed. Some were found to possess antiischemic (Rump *et al.* 1995), antiplatelet (Belinky *et al*. 1998), antineoplastic (Lin *et al*. 1997), anti-inflammatory (Read 1995), antiallergic (Yamamura *et al.* 1998), antilipoperoxidant (Terao *et al*. 1994), gastro-protective (Mojžiš 1999) properties and other effects have also been described. It is suggested that most of these biological effects are related to their antioxidant activity. Flavonoids can exert their antioxidant activity by various mechanisms, e.g. by scavenging or quenching free radicals, by chelating metal ions, or by inhibiting enzymatic systems responsible for the generation of free radicals (Belinky *et al*. 1998).

The source and metabolism of flavonoids

Food is the main source of flavonoids for the human organism. Onions, citrus fruits, apples, cabbage, broccoli, fresh black tea and coffee are examples of fruits and vegetables with a high content of flavonoids (Hertog *et al*. 1992, Mojžišová *et al.* 1999). The flavanols, particularly catechin and catechin gallate ester family and the flavonols quercetin, kaempferol, and their glycosides are constituents of the beverages such as green and black tea and red wine (Formica and Regelson 1995). Estimations of the daily intake of flavonoids in the food vary and they fluctuate from 50 mg up to 1 g, with quercetin and kaempferol being the most frequently occurring flavonoids (Kuo *et al*. 1998).

The enormous variability of flavonoids creates great difficulties in the study of their absorption and metabolism. These processes are determined primarily by their chemical structure, which depends on factors such as the degree of glycosylation/acylation, conjugation with other phenolic compounds, molecular weight and solubility (Bravo 1998). It was found that some phenolic compounds are metabolized within the gastrointestinal tract. Aglycones and free simple phenolic compounds, flavonoids (e.g. quercetin, genistein) or phenolic acids can be directly absorbed through the small intestinal mucosa (King *et al.* 1996, Manach *et al.* 1997).

On the other hand, glycosides must be hydrolyzed to their corresponding aglycones before absorption. Because mammals lack the appropriate beta-glycosidases, most of glycosides pass into the large intestine, where they are hydrolyzed by the microflora, rendering free aglycones (Griffiths and Barrow 1972). In the colon, aglycones are absorbed and then methylated or conjugated in the liver. However, the involvement of other organs, such as kidney or intestinal mucosa in the flavonoid metabolism cannot be excluded, since they contain enzymes involved in polyphenol metabolism (Hackett 1986).

The cardioprotective effects of flavonoids

It is suggested that flavonoids decrease the risk of coronary heart disease by three major actions:

1. preventing low-density lipoproteins (LDLs) from oxidizing;
2. decreasing the ability of platelets in the blood to clot;
3. improving coronary vasodilation.

Inhibition of LDL oxidation by flavonoids

Atherosclerosis is a multifactorial disease that represents the primary worldwide cause of death. Elevated plasma LDL cholesterol concentrations and low levels of HDL are associated with accelerated atherosclerosis (Witztum and Steinberg 1991). Atheromatous lesions develop in the subendothelial space due to accumulation of cholesteryl esters in monocytes/macrophages forming foam cells and atherosclerotic plaques (Witztum and Steinberg 1991).

The chemically modified (oxidized) LDLs are recognized by specific macrophagic “scavenger” receptors. These receptors have been identified and there is much evidence that oxidized LDLs are responsible for cholesterol loading of macrophages, foam cell formation and atherogenesis (Cook and Samman 1996).

During the past several years, a number of papers have reported that the oxidative modification of LDLs can be inhibited by antioxidants such as vitamin E, ascorbate and β-carotene (Jialal and Scaccini 1992, Naruszewicz *et al.* 1992, Viana *et al*. 1996). As was mentioned above, many biological effects of flavonoids are due to their antioxidant activity and they may

therefore be suitable for decreasing LDL susceptibility to oxidation and for preventing heart disease. This hypothesis was verified by numerous *in vitro* (Viana *et al.* 1996, Lim *et al*. 1998), *ex vivo* (Wang and Goodman 1999) and *in vivo* experiments (Day *et al*. 1997, Hayek *et al*. 1997).

Furthermore, another link between flavonoids and their ability to block LDL oxidation is the so-called "French paradox". It was observed that people in France eat more high-fat and high-cholesterol food (e.g. 3.8 times as much butter as Americans), smoke many cigarettes, and have higher blood-cholesterol levels and higher blood-pressure readings compared to Americans-but they have 30 % of heart attack rate (Goldberg 1995).

It has been reported that this effect may result from phenolic antioxidants found in red wine (Maxwell *et al*. 1994, Goldberg 1995, Durak *et al*. 1999). This suggestion is supported by the experimental data confirming that phenolic compounds in red wine are able to inhibit the copper-catalyzed oxidation of LDL (Frankel *et al*. 1993). Later, Fuhrman *et al*. (1995) also noted that red wine flavonoids possess the ability to reduce LDL oxidation.

According to Rifici *et al*. (1999), red wine blocked LDL oxidation by 85.7%. Serafini *et al*. (1994) showed that the consumption of polyphenol-rich beverages such as red wine and tea was associated with an increased plasma antioxidant potential. Kondo *et al.* (1994) provided direct evidence that regular and long-term consumption of red wine inhibited LDL oxidation *in vivo.*

Ingestion of red wine is associated with increased antioxidant activity in the serum. It has been further suggested that this increase in subjects frequently drinking red wine may be the primary factor inhibiting LDL oxidation, which in turn reduces atherosclerotic complications (Maxwell *et al*. 1994)

Flavonoids and platelet aggregation

In addition to their role in hemostasis and thrombosis, platelets are also key participants in atherogenesis. A number of proinflammatory mediators are derived from platelets including thromboxane A_2, PAF, serotonin, transforming growth factor, platelet-derived growth factor and lipoxygenase metabolites which may lead to the progression of atherosclerotic lesions (Ross 1993).

Many studies have investigated the effect of flavonoids on platelet activation and aggregation (Landolfi *et al*. 1984, Gryglewski *et al*. 1987, Ruf 1999, Pignatelli *et al*. 2000). Several studies have demonstrated that red wine or red wine polyphenols are also able to inhibit platelet aggregation significantly (Pace-Asciak *et al.* 1995, 1996, Rein *et al*. 2000). In addition, there have been numerous reports that red wine and flavonoids can also have beneficial effects on platelet function in animals (Tzeng *et al*. 1991, Demrow *et al*. 1995, Tsai *et al*. 1996. Osman *et al*. 1998).

Vasodilatory effect of flavonoids

Oxidation of low-density lipoproteins and platelet aggregation are known to interact in crucial mechanisms of atherogenesis. As was mentioned above, it is suggested that inhibition of LDL oxidation and platelet aggregation may be potential mechanisms by which flavonoids reduce cardiovascular risks. Current interest in the presumed benefits of flavonoids in protecting against coronary heart disease prompted a number of studies investigating other possible effects of various flavonoids which might contribute to the protective effect on the heart. It seems that flavonoid-induced vasorelaxation may be one of them.

The mechanism by which flavonoids produce vasodilatation in not well understood. Because an undamaged endothelium layer is essential for the propagation of vasodilatory effects of flavonoids, it was hypothesized that nitric oxide may play an important role in this biological action of flavonoids (Fitzpatrick *et al*. 1993). Recently, it was found that dioclein, a flavonoid isolated from *Dioclea grandiflora*, induced a concentration-dependent relaxation in vessels precontracted with phenylephrine. This effect was associated with the production of cyclic GMP and was inhibited by N^G-nitro-L-arginine-methylester, a nitric oxide synthase inhibitor (Lemos *et al.* 1999). The authors suggest that this vasorelaxation likely results from the enhanced synthesis of NO rather than enhanced biological activity of NO. Later, Kim *et al*. (2000) observed similar results with procyanidins extracted form hawthorn. On the other hand, Girard *et al.* (1995) suggested that the vasodilatory effect of flavonoids can also be mediated by their scavenging activity against the superoxide anion and may thus prevent nitric oxide degradation by free radicals.

Furthermore, there is mounting evidence that red wine and red wine polyphenols also provide significant vasorelaxation (Fitzpatrick *et al.* 1993, Cishek *et al.* 1997). Moreover, flavonoids found in purple grape juice improved endothelial function in patients with coronary artery disease (Stein *et al.* 1999).

Epidemiological studies

At present, there are several prospective epidemiological studies from Europe and USA investigating the relationship between coronary heart disease (CHD) and flavonoid intake. Subjects with a low intake of flavonoids had a higher death rate from CHD than those who consumed more flavonoids (Hertog *et al.* 1993, 1995). However, the intake of flavonoids was inversely associated with subsequent CHD in most but not all prospective epidemiological studies.

The Zutphen Elderly Study in Netherlands (Hertog *et al*. 1993) is the best-known epidemiological study that examined the relationship between dietary flavonoid intake and the risk of CHD, with the majority of dietary flavonoids coming from tea, onions and apples. This study assessed the flavonoid intake of 805 men aged 65 to 84 years. Flavonoid intake and tea consumption were correlated, and both were inversely associated with death from CHD. Later, these results were confirmed in a "Seven Countries Study" in which Hertog *et al.* (1995) found an inverse correlation between flavonoid intake and coronary heart disease. Similar results were reported in a Finnish study in which the participants were examined for 5 years and followed up during the subsequent 20 years. It was found in this study of 5133 men and women that women with the highest intake of flavonoids (mostly from onions and apples) had a significant inverse association between flavonoid intake and coronary mortality (Knekt *et al*. 1996). Furthermore, Yochum *et al*. (1999) demonstrated an association between flavonoid intake (mainly from broccoli) and reduced risk of CHD in 34 492 postmenopausal women in Iowa. There are also other studies which have evaluated the relationship between fruit and vegetable intake and cardiovascular diseases (Ness and Powles 1997). The results suggest that people with a very low intake of flavonoids have a higher risk of coronary diseases. Furthermore, the Rotterdam studies also supported the hypothesis that tea flavonoids, through their antioxidant effects, may inhibit LDL oxidation and thus reduce the risk of atherosclerosis (Hofman *et al*. 1991, Geleijnse *et al*. 1999).

Other studies correlating flavonoid-rich beverages intake such as red wine or green tea with coronary morbidity and mortality have also indicated that these biologically active compounds may partly contribute to differences in coronary heart disease mortality in large populations (Gronbaek *et al.* 1995, Sesso *et al*. 1999).

Contrary to previously mentioned studies, some data do not support the important inverse association between flavonoids and the incidence of coronary heart disease. The Caerphilly Study demonstrated that the incidence of ischemic heart disease was associated neither with flavonoid nor quercetin intake (Hertog *et al.* 1997). Similar results did not find a significant inverse association between flavonoid intake and CHD in male health professionals (*Rimm et al.* 1996). Furthermore, in the United States, the Health Professionals Study did not find any significant inverse association between flavonoid intake and coronary mortality in men with previous coronary heart disease (Hollman and Katan 1997).

Conclusions

Dietary flavonoids represent a family of polyphenolic compounds found in common food constituents derived from plants. The inhibition of LDL oxidation and platelet aggregation by flavonoids suggests that regular consumption of food or beverages containing flavonoids may protect against atherosclerosis and a tendency to thrombosis. The large contribution of flavonoids to the diet from tea, onions, fruits and vegetables suggest their greater nutritional benefit than has previously been recognized, as they appear to constitute a major source of dietary antioxidants.

Numerous epidemiological studies have demonstrated an inverse correlation between flavonoid intake and the risk of death from coronary heart disease. On the other hand, some authors suggest that there is low or even no relationship between flavonoid intake and coronary heart disease. It is being suggested that these contradictory findings may be due to various factors such as the lifestyle in different populations and social classes, the possible effect of other dietary substances, the impossibility of establishing the accurate intake of flavonoids, etc.

The benefit to cardiovascular health, suggested by some epidemiological data, however, still remains unproved in the absence of randomized clinical trials. Thus, the possibility that dietary flavonoids reduce the risk of coronary heart disease remains open and further longitudinal studies are needed to confirm the importance of flavonoids in the prevention of coronary heart disease.

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