

## Dietary flavonoids and cardiovascular disease

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### Introduction

*Flavonoids are secondary plant metabolites that occur in foods, and that might have effects on atherogenesis. What are secondary plant metabolites, and what is the evidence that flavonoids affect the risk of cardiovascular disease?*

The primary components of most organisms and therefore of our diet are proteins, lipids, and carbohydrates. In addition plants contain so-called secondary metabolites that differ from one species or strain to another, and that determine plant characteristics such as colour, taste, smell, and ability to attract or repel insects and micro-organisms. The effects of such secondary metabolites on the human body have been known and exploited since time immemorial for pharmaceutical purposes; digitoxin is just one well-known example. However, interest in secondary compounds has recently widened beyond pharmaceuticals and toxicology. The stimulus for this has come both from cancer epidemiology, which suggested that eating vegetables and fruits may prevent cancer, and from cardiovascular research, which suggested that dietary anti-oxidants may prevent atherosclerosis. Dietary flavonoids can retard tumor growth in animal models, and they are also strong anti-oxidants. Both the cancer research field and the cardiovascular field have thus contributed to the recent interest in flavonoids.

### Structure

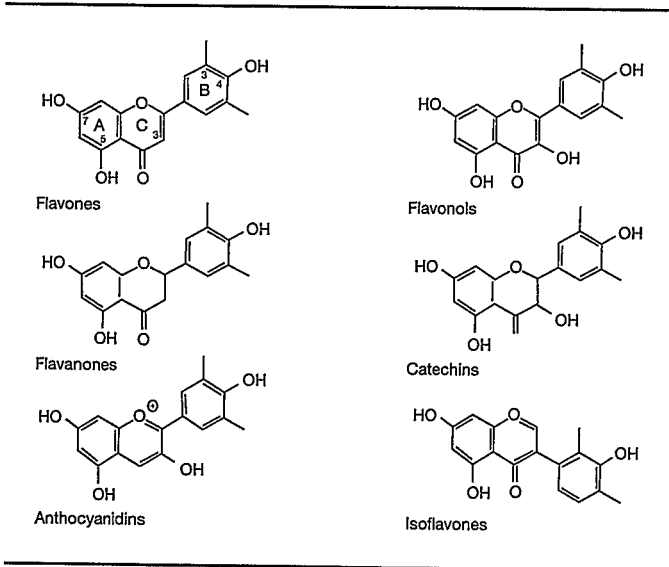
Flavonoids are polyphenolic compounds that occur ubiquitously in foods of plant origin. They comprise 2-

phenylbenzo- $\gamma$ -pyrones, -dihydropyrones, -dihydropyrans, and -pyryliums. Variations in the oxygen-containing heterocyclic ring give rise to catechins (dihydropyrans), flavonols and flavones ( $\gamma$ -pyrones), flavanones (dihydropyrones), and anthocyanidins (pyryliums) (Fig. 1). Attachment of the second benzene ring to the 3 instead of the 2 position creates isoflavonoids (Fig. 1). In addition, the basic structure of flavonoids allows a multitude of substitution patterns in the two benzene rings within each class of flavonoids. Most flavonoids contain multiple hydroxyl groups, and they are therefore known as polyphenols. In plants one or more of these hydroxyl groups are usually linked to a sugar, and flavonoids thus occur in foods largely in the form of glycosides. The free compound without its sugar attachment is known as the aglycone. Over 4000 naturally occurring flavonoids have been described (1) and this list is still growing.

### Dietary intake

Major dietary sources of flavonoids are vegetables, fruits, and beverages such as tea and red wine (2-5). Kühnau (4) estimated that the total flavonoid intake in the U.S.A. was 1 g/day expressed as glycosides or 650 mg/day expressed as aglycones, but most likely this estimate is too high. It also grouped all flavonoids together, which is probably unjustified because properties such as anti-oxidant activity and pharmacological effects differ widely from one flavonoid species to another. New, more specific food analyses focused on separate flavonoid compounds. These studies (2, 3) suggested that the Dutch intake of five major

**FIGURE 1**  
Structure of the major classes of flavonoids found in foods



flavonols and flavones was 23 mg/day (expressed as aglycones) as opposed to Kühnau's estimate of 115 mg/day (expressed as aglycones) in the USA (4).

### Health effects

In 1936 Rusznyák from the group of Szent-Györgyi, the discoverer of vitamin C, reported that consumption of a mixture of two flavanones decreased capillary permeability and fragility in humans (6). This gave rise to a claim for vitamin action of flavonoids ("Vitamin P"). Doubts about the evidence for these claims arose fairly soon, and flavonoids were stripped of vitamin status around 1950. However, the oxidation hypothesis of atherosclerosis has given a new stimulus to the flavonoid field; for instance, the low incidence of coronary heart disease in France has been ascribed to a high intake of flavonoids from red wine.

### Flavonoids and cardiovascular epidemiology

The hypothesis that flavonoids prevent heart disease received a boost when Hertog *et al* (7) reported an inverse association between the intake of the flavonol quercetin and coronary mortality in men in Zutphen, The Netherlands. The major sources of quercetin and related

flavonols turned out to be tea and onions rather than fruits or green vegetables. Subsequent epidemiological studies on the association between flavonoid intake and cardiovascular disease have produced divergent results (Table 1). Inverse and thus protective associations were seen in Zutphen (7, 8) and in elderly persons in Rotterdam, The Netherlands, where high tea consumption was associated inversely with non-invasively assessed measures of atherosclerosis (JCM Witteman, personal communication, 1997). A modest protective effect was also seen in Finland (9). However, in a large cohort of US Health Professionals there was no association of flavonol intake with incidence of non-fatal coronary heart disease (10), and in men in Great Britain coronary and total mortality even rose with the intake of the major flavonol source, tea (11).

The most likely explanation for the latter observation is that in this study tea consumption merely acted as a marker for a lifestyle that favours the development of cardiovascular disease. Indeed, the men with the highest intake of tea and flavonols tended to be manual workers, they smoked more, they consumed less alcohol, and they ate more fat. The authors corrected for these confounders, but confounders that have been measured with less than perfect precision cannot be completely eliminated by multivariate analysis. Measurements of diet, smoking, and alcohol intake were necessarily crude, and no data were available on physical activity, or on psychosocial factors. Therefore the most plausible explanation for the higher mortality in tea drinkers is confounding by unmeasured or imperfectly measured coronary risk factors which clustered with tea intake.

However, a similar reasoning may also be applied to the studies that found a protective effect of flavonoids. In Great Britain, high intakes of flavonoids are often associated with a less healthy lifestyle. In continental Europe the opposite may occur, and subjects with a high consumption of tea, onions or berries - an important source of flavonoids in the Finnish study (9) - may have been protected by other lifestyle aspects that occurred simultaneously.

### Where does this leave flavonoids?

Metabolic studies suggest that an *in vivo* anti-oxidant activity of dietary quercetin remains feasible: quercetin is well absorbed from foods, and plasma concentrations of up to 0.5  $\mu\text{mol/L}$  can be reached (12). Addition of flavonols to blood plasma *in vitro* reduces the oxidizability of LDL, although the effects of administration *in vivo* are less clear.

**TABLE 1**  
Prospective studies of flavonol intake and cardiovascular disease

Reference	Location	Years of follow-up	Number and type of events	Relative Risk <sup>1</sup>
Hertog <i>et al</i> (7)	Zutphen, The Netherlands	5	43 deaths from CHD 38 first myocardial infarctions	0.3 0.5
Keli <i>et al</i> (8)	Zutphen, The Netherlands	15	42 strokes	0.3
Knekt <i>et al</i> (9)	Finland	20	473 deaths from CHD	0.7
Rimm <i>et al</i> (10)	USA	6	486 nonfatal myocardial infarctions 140 deaths from CHD	1.1 0.8
Hertog <i>et al</i> (11)	Caerphilly, Wales, UK	10 14	186 cases of CHD 131 deaths from CHD	1.0 1.6

<sup>1</sup>Relative risk in the highest versus lowest tertile (7), quarter (8,9), or quintile (10) of intake of quercetin and related flavonols and flavones; risks were corrected for age, diet, and conventional risk factors for coronary heart disease

A recent study from Japan suggested that a high intake of tea increased the resistance of isolated LDL to oxidation (13). This is in fact surprising, because flavonols and catechins, the major anti-oxidant flavonoids in tea, probably do not accumulate inside LDL particles. Indeed, in other studies consumption of tea by human volunteers had only minimal effects on the oxidizability of LDL isolated from plasma. Addition of red wine to blood plasma reduced the oxidizability of low density lipoproteins (LDL), but *in vivo* data are again conflicting (14,15).

The problems involved in detecting an association of a dietary factor with a disease that develops over the course of decades are huge, and failure to observe such an association in epidemiological studies does not prove that none exists. Thus saturated fat intake does not correlate with coronary heart disease within populations, even though metabolic studies and clinical trials provide proof for a causal relation. The question whether flavonoids protect against atherosclerosis could be answered definitively by a randomized clinical trial, but unfortunately such a trial is unlikely to be performed in the foreseeable future.

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