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## Role of dietary flavonoids in protection against cancer and coronary heart disease

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### Introduction

The weight of the epidemiological evidence for a protective effect of vegetables and fruits against cancer is impressive [1,2]. Various hypotheses have been proposed to explain this very consistent beneficial effect of an increased consumption of vegetables and fruits. An attractive hypothesis is that vegetables and fruits contain compounds that have a protective effect independent of that of known nutrients and micronutrients. This is

supported by *in vitro* and *in vivo* studies which show that naturally occurring plant compounds may inhibit various stages in the cancer process [3]. In these studies flavonoids have also been studied extensively.

Flavonoids are polyphenolic compounds that occur ubiquitously in foods of plant origin. The basic structure of flavonoids allows a multitude of substitution patterns in the benzene rings A and B: phenolic hydroxyls, *O*-sugars, methoxy groups and sulphates. Variations can also occur in the heterocyclic ring C, giving rise to flavonols, flavones, catechins, flavanones, anthocyanidins

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Abbreviation used: LDL, low-density lipoprotein.

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and isoflavonoids (Figure 1). Over 4000 different naturally occurring flavonoids have been described [4] and this list is still growing. Table 1 gives an overview of the occurrence of flavonoids in foods [5-7].

A multitude of *in vitro* studies has shown that flavonoids can inhibit, and sometimes induce, a large variety of mammalian enzyme systems. The effects of mainly flavones and flavonols on 24 different enzymes or enzyme systems have been reviewed by Middleton and Kandaswami [4]. Some of these enzymes are involved in important pathways that regulate cell division and proliferation, platelet aggregation, detoxification and inflammatory and immune response. Thus it is not surprising that effects of flavonoids have been found in cell systems and animals, on different stages in the cancer process, on the immune system and on haemostasis [4]. Anticarcinogenic and antiproliferative effects of flavonoid properties have been described in several animal models and mammalian cells [8].

Recently, much attention has been paid to their antioxidant properties [9], which may protect tissues against oxygen free radicals and lipid peroxidation. Oxygen free radicals and lipid per-

oxidation may be involved in several pathological conditions such as atherosclerosis, cancer and chronic inflammation [10]. Quercetin, the major representative of the flavonol subclass of flavonoids, prevents oxidation of low-density lipoproteins (LDLs) *in vitro* [11]. Oxidized LDLs have been found in atherosclerotic lesions of humans [12], and increased plasma concentrations of autoantibodies against oxidized LDLs occur in patients with atherosclerosis [13,14]. Quercetin may therefore contribute to the prevention of atherosclerosis [15].

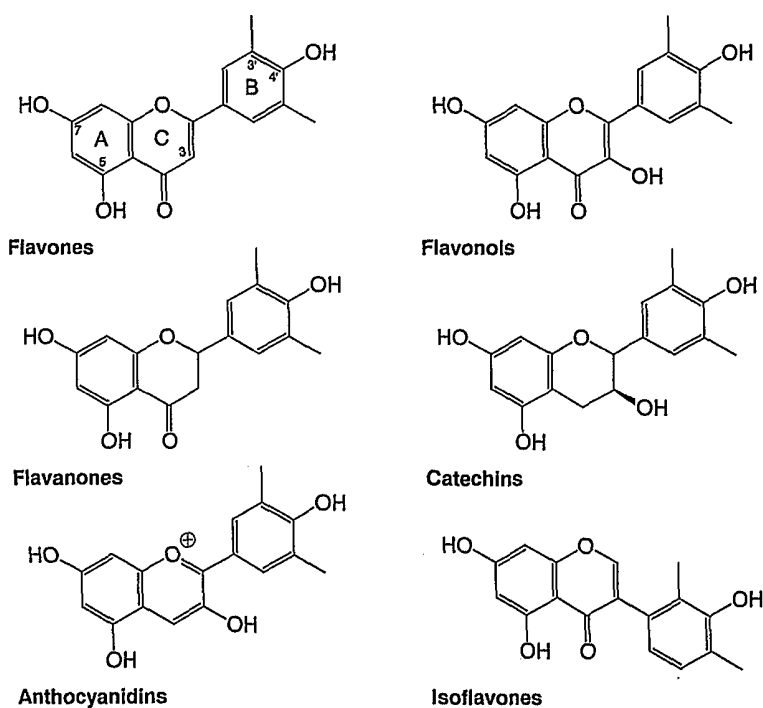
### Flavonols, flavones and cancer and cardiovascular disease

In order to study the potential role of dietary flavonoids in prevention of cancer and coronary heart disease, we needed reliable data on flavonoid contents of common Dutch vegetables and fruits. Such a database did not exist for the Netherlands, and data produced in other countries were fragmentary. In addition the quality of these data was questionable, because they were obtained with methods now considered obsolete. We decided to focus on flavonols and flavones,

**Figure 1**

#### Subclasses of flavonoids

Classification is based on variations in the heterocyclic ring.



**Table 1****Occurrence of flavonoids in common foods**

Flavonoid subgroup	Major food sources
Flavonols	Onions, kale, broccoli Apples, cherries, berries Tea, red wine
Flavones	Parsley, thyme
Flavanones	Citrus
Catechins	Apples Tea
Anthocyanidins	Cherries, grapes
Isoflavones	Soya beans, legumes

because these flavonoids, including the flavonol quercetin (3,5,7,3',4'-pentahydroxyflavone), occur ubiquitously in plant foods and were the ones most frequently studied in model systems.

We measured the concentration of the flavonols, quercetin, kaempferol and myricetin and the flavones luteolin and apigenin in 28 vegetables, nine fruits and ten beverages commonly consumed in the Netherlands [6,7]. Quercetin was by far the most abundant flavonol, followed by kaempferol (3,5,7,4'-tetrahydroxyflavone). Flavones were found in only a few products.

With these data we were able to calculate the intake of flavonols and flavones in a population of elderly men in the Dutch town of Zutphen (the Zutphen Elderly Study). In 1985 their food consumption was assessed using a dietary history method. A total number of 805 men aged 65–84 years entered the study. The intake of flavonols and flavones was on average 26 mg/day. Major sources of flavonols and flavones were tea (61%), onions (13%) and apples (10%). After 5 years, in 1990, their health records were collected, and morbidity and mortality data were studied. Differences in baseline characteristics of these men between tertiles of flavonol and flavone intake were evaluated, and relative risks were calculated. No associations were found between flavonol and flavone intake and total cancer mortality. Also specific forms of cancer, such as lung cancer, were not associated with flavonols and flavones [16]. In a large cohort study (The Netherlands Cohort study) consisting of 120 850 men and women aged 55–69 years, again no association was found between flavonol and flavone intake and stomach cancer, colon cancer and lung cancer over 4.3 years of follow-

up [17]. In contrast, mortality from coronary heart disease was strongly and inversely associated with flavonol and flavone intake in the Zutphen Elderly Study [18]; a reduction in mortality risk of more than 50% was found. These results were not confounded by known risk factors for coronary heart disease and antioxidant vitamins. The association between flavonol and flavone intake and risk of stroke was studied in a cohort of 550 middle-aged men [19]. These men were followed for 15 years, and the men in the highest quartile of flavonol and flavone intake (>30 mg/day) had a considerable reduction in disease risk of about 60%. Coronary heart disease mortality was inversely associated with flavonol and flavone intake in a cohort of 5130 Finnish men and women aged 30–69 years [20]. These subjects were followed for over 20 years. The relative risks for coronary heart disease mortality between the highest (>5 mg/day) and lowest (<2.5 mg/day) quartiles of flavonol and flavone intake were 0.73 for women and 0.67 for men. These results were adjusted for known risk factors for coronary heart disease and intake of antioxidant vitamins and fatty acids.

The Zutphen Study cohort is one of the cohorts of the Seven Countries Study, a cross-cultural study of diet, lifestyle and disease. In 1987 the foods that represented the baseline diet as per 1960 of each cohort were bought locally. The foods were combined into food composites that represented the average daily food intake of each cohort. In these food composites flavonols and flavones were determined. The intake of flavonols and flavones ranged from 3 mg/day in a Finnish cohort to 70 mg/day in a Japanese cohort. The major dietary sources of flavonols and flavones varied substantially between cohorts. In the Japanese and Dutch cohorts the major source was tea, while red wine was the major source in Italy. Onions and apples were the predominant sources in the United States, Finland, Greece and former Yugoslavia. We found a strong inverse association ( $r = -0.50$ ,  $n = 16$ ) between flavonol and flavone intake and mortality from coronary heart disease [21]. Again, no association with cancer mortality was found.

### Absorption and metabolism of flavonoids

These epidemiological data support a role for flavonoids as antioxidants in coronary heart disease prevention. However, absorption from the

diet is a prerequisite for a causal relation between flavonols and coronary heart disease. In addition, the metabolism of flavonols after absorption should not substantially inhibit their antioxidant capacity. The absorption and subsequent distribution, metabolism and excretion of flavonoids in humans has been little studied. Animal studies [5] show that flavonoids present in foods are unable to pass across the small-intestinal wall, because they are bound to sugars as glycosides (with the exception of catechins). Only free flavonoids without a sugar molecule, the so-called aglycones, are considered to be able to pass through the gut wall, and no enzymes that can split these predominantly  $\beta$ -glycosidic bonds are secreted into the gut or are present in the intestinal wall [5,22]. Hydrolysis only occurs in the colon by micro-organisms, which at the same time degrade dietary flavonoids extensively [5]. Thus only a marginal absorption of dietary flavonoids is to be expected. However, research on the mechanisms for aglycone transfer across the gut wall is lacking. Although only flavonoid aglycones are considered to be able to pass through the gut wall, poor absorption (<1%) of the orally administered aglycone of quercetin was found in a human trial [23]. In contrast, Ueno et al. [24] showed that the absorption of orally administered quercetin aglycone was about 20% in rats. We recently confirmed these results in a human study with ileostomy subjects: absorption of orally administered quercetin aglycone was 24% [25]. The absorption of quercetin glycosides from onions in this study was 52%, and 17% for pure quercetin rutinoside, a common glycoside in foods [25]. These data show that humans absorb appreciable amounts of quercetin and that absorption of glycosides in the small intestine is possible.

After absorption of flavonoids, the subsequent metabolism of flavonoids is quite well known from animal studies [22,26], but practically no data for humans are available. Hydroxy groups are conjugated with glucuronic acid or sulphate in the liver. In addition, methylation may occur. Excretion in bile of glucuronides and sulphates seems to be important. Micro-organisms in the colon hydrolyse conjugates and glycosides, which is supposed to enable absorption of the liberated aglycones. Thus conjugates can be reabsorbed and enter an enterohepatic cycle. However, these micro-organisms also substantially degrade the flavonoid moiety by cleavage of the heterocyclic ring. Three main types of

ring scission, depending on the ring structure, each leading to different phenolic acids or their lactones, have been postulated. These primarily produced phenolic acids are prone to secondary reactions such as  $\beta$ -oxidation, demethylation and dehydroxylation. The phenolic acids are absorbed and excreted in urine. The significance of these results for humans is not clear, because a wide range of mammalian species has shown considerable species variation in this secondary metabolism. Ring cleavage of flavonoids seems to be wholly mediated by the colonic micro-organisms. Thus far, enzyme systems in mammalian tissue capable of ring fission have not been found.

### Absorption and disposition of kinetics of quercetin from onions

We studied the time course of the plasma quercetin concentration in two subjects after ingestion of fried onions containing quercetin glucosides equivalent to 64 mg of quercetin aglycone [27]. The mean peak plasma level of quercetin was 200 ng/ml and was reached 2.9 h after ingestion of the onions, with an average half-life of absorption of 0.87 h. The half-life of the elimination phase was 17 h. We could still detect quercetin 48 h after ingestion of the onions; at that time the plasma concentration was about 10 ng/ml.

The elimination half-life of 17 h implies that repeated intake of quercetin glucosides would lead to a build-up of the concentration in plasma. A peak plasma concentration of 200 ng/ml or 0.6  $\mu$ M was measured after administration of a single high dose of dietary quercetin, equivalent to 4 times the average Dutch daily intake [28]. Concentrations of the dietary antioxidant  $\beta$ -carotene in human plasma are similar to this value [29]. Thus plasma quercetin levels in subjects who regularly eat onions may approach those of  $\beta$ -carotene.

### Conclusions

Flavonoids have many effects on mammalian biology. Animal studies and *in vitro* studies suggest that dietary flavonols could inhibit cancer in humans. However, so far no association with cancer mortality has been found in three epidemiological studies. Intake of flavonols and flavones was inversely associated with coronary heart disease in two prospective cohort studies and in a prospective cross-cultural study. An inverse association with stroke was also found. Antioxidant and/or antithrombotic effects of

flavonoids possibly explain these results. Dietary quercetin, the major flavonol in foods, is absorbed in humans and is only slowly eliminated throughout the day. Quercetin could thus contribute significantly to the antioxidant defences present in blood plasma.

The role of dietary flavonols and flavones in disease prevention is promising. Epidemiological research in other countries and cultures, studies on biological mechanisms and bioavailability, and clinical trials are needed to evaluate fully their role in human health.

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