Flavonoids—dietary perspectives and health benefits

PM Lyons Wall, S Samman

Human Nutrition Unit, Department of Biochemistry, University of Sydney NSW 2006

Summary

The flavonoids are a family of phytochemicals that occur naturally in fruits, vegetables, legumes, nuts and seeds of vascular plants. They are an integral part of the human diet, yet only a small number of plant species have been examined systematically for their flavonoid content and identification of the range of flavonoids consumed by humans is incomplete (1). Little is known about their absorption and metabolism in humans but they are believed to be non-toxic at usual levels of dietary intake (1). If absorbed and biologically active in vivo, they may act as antioxidants to inhibit free-radical mediated cytotoxicity and lipid peroxidation; as antiproliferative agents to inhibit tumour growth; or as weak oestrogen agonists or antagonists to modulate endogenous hormone activity. In these ways they may confer protection against chronic diseases such as atherosclerosis, cancer and osteoporosis, and assist in management of menopausal symptoms. The aims of this paper are to discuss sources of flavonoids in the human diet and to evaluate their protective role in terms of epidemiological and clinical evidence.

Introduction

The basic structural unit of the flavonoid family comprises two benzene rings (A and B) linked through a heterocyclic pyran or pyrone ring (C); variations in the C ring and hydroxylation pattern on the A and B rings define the major classes (1) (Figure 1). These include the widely distributed flavonols and flavones; the relatively rare chalcones, flavanones (found in citrus fruit), and flavanols (found in green tea); the anthocyanidins which produce the red, blue and purple colours in flowers and fruits; and the isoflavones which are restricted to legumes—by the limited occurrence of the enzyme chalcone isomerase needed to convert flavone to isoflavone precursors. (1). Over 4,000 flavonoid compounds derived from the basic groups have been identified in vascular plants (1). Discussion in this paper will focus on those reported to be commonest in the human diet, including the flavonols (kaempferol, quercetin and myricetin), flavones (apigenin and luteolin) and isoflavones (daidzein, genistein and glycitein) (2).

Dietary intake, absorption and metabolism

The main sources of flavonols and flavones in the human diet are shown in Table 1. It is notable that the richest sources, which include onions, celery, green beans and apples, do not correspond to more often cited nutrient-rich foods such as green leafy vegetables and citrus fruits. Average intakes of flavonols and flavones have ranged from 6 mg/day in Finland to 64 mg/day in Japan, with intermediate intakes in the United States (13 mg/day), Italy (27 mg/day) and the Netherlands (33 mg/day). These estimates were based on recent analyses of five flavonoids (quercetin, kaempferol, myricetin, luteolin and apigenin) in composite food samples for populations in the Seven Countries Study (3).

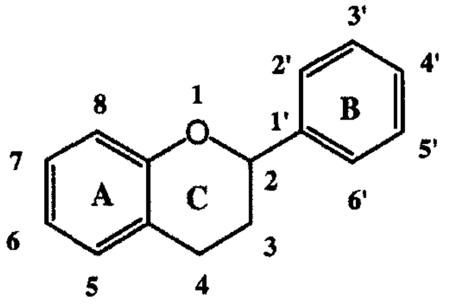
Table 1: Dietary sources* of flavonols and flavones# (4,5)

High: 5 - 35 mg/100g	Medium: 1 - 4 mg/100g:	Low: <1 mg/100g
onion, kale, celery, broccoli, French bean, broad bean	apple, black tea, leek, apricot, red wine, grape, strawberry, lettuce, cherry, red currant, red capsicum, plum, tomato	cabbage, white cabbage, cauliflower, mushroom, pea, spinach, beetroot, cucumber, peach, carrot, citrus juices, coffee, white wine

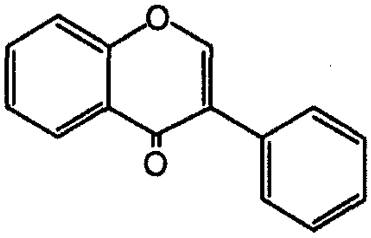
* listed from highest to lowest content within each group; # Σ quercetin, kaempferol, myricetin, luteolin, apigenin

Dietary sources of the flavonols and flavones varied between different countries with major contributions from tea in Japan (90%) and the Netherlands (64%), red wine in Italy (46%), and vegetables and fruits in Finland (100%) and the United States (80%). Quercetin was the most abundant flavonoid with onion providing the richest source - between 28 and 49 mg/100g.

Generic structure

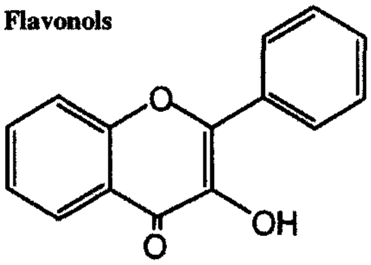


Isoflavones



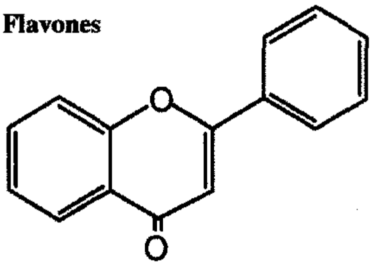
	5	6	7	4'
Genistein	OH	-	OH	OH
Daidzein	-	-	OH	OH
Glycitein	-	O-Me	OH	OH

Flavonols



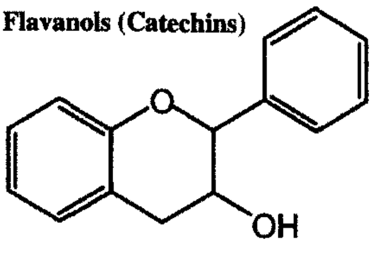
	5	7	3'	4'	5'
Myricetin	OH	OH	OH	OH	OH
Quercetin	OH	OH	OH	OH	-
Kaempferol	OH	OH	-	OH	-

Flavones



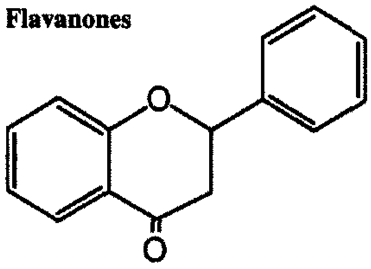
	5	7	3'	4'
Luteolin	OH	OH	OH	OH
Apigenin	OH	OH	-	OH

Flavanols (Catechins)



	5	7	3'	4'	5'
(+) Catechin	OH	OH	OH	OH	-
(-) Epicatechin *(cis)*	OH	OH	OH	OH	-
(-) Epigallocatechin	OH	OH	OH	OH	OH

Flavanones



	5	7	3'	4'
Naringenin	OH	OH	-	OH
Hesperetin	OH	OH	OH	O-Me

Figure 1. Structures of the major classes of food flavonoids. Positions of hydroxyl groups on A and B rings are listed for selected examples within each class.

Isoflavones are found in the legume family and the main dietary source in humans is the soybean. A comprehensive analysis of 49 varieties of dried legumes (6) reported that concentrations in the soybean varied from 38 to 140 mg/100g between different varieties. These levels were 50 fold higher than in chick peas (1.1-3.6 mg/100g) and up to 1000 fold higher than in other legumes including pigeon peas, pinto and haricot beans, kidney beans and lentils (0.08-0.7 mg/100g). In this study, two major isoflavones were estimated genistein and daidzein, which occurred mainly in the form of the glycosides genistin and daidzin, with smaller quantities of the methylated derivatives biochanin A and formononetin, respectively. Concentrations of genistin were higher than those of daidzin in all legumes tested with the exception of the Japanese kudzu root, in which relatively high total levels were observed (206 mg/100g), predominantly in the form of daidzin. Concentrations of glycitein, a third isoflavone present in legumes were not measured.

Another analysis reported that the isoflavone content of the soybean varied with different climatic conditions. Levels in the dry bean ranged from 47 to 171 mg/100g for the same variety grown at four different geographical sites and from 255 to 363 mg/100g for another variety grown in two consecutive seasons at the same location (7). In this analysis, isoflavones were estimated as the total content of glycones—genistin, daidzin and glycitin and their respective aglycones—genistein, daidzein and glycitein. Genistein was the predominant isoflavone accounting for over 50% of the total; glycitein was the least abundant, less than 10%, reflecting the fact that it is mainly confined to the hypocotyl or germinal region which is only 2% of the whole bean. Isoflavones are stable during processing with significant levels retained in soy products such as defatted flour, protein isolates and protein concentrates (8). However the form of isoflavone—whether glycoside, malonyl or acetyl glycoside, or aglycone—may be altered during heat treatment, or fermentation in the preparation of traditional soy items. Major losses can occur due to their high solubility in organic solvents discarded during processing. For example the isoflavone content of soy protein concentrates depends on the solvent used to prepare them from soy flour; washing with water does not alter the content but alcohol washing results in anomalously low concentrations. Ranges of isoflavones reported in traditional Asian foods and commercial soy products are shown in Table 2.

Table 2. Isoflavone content in traditional and commercial soy items (adapted from references 8-11).

Traditional foods		Commercial soy products	
soybean (dry)	91 - 160	defatted soy flour, soy gifts	178 - 306
miso	32 - 92	textured vegetable protein	104 - 118
tempeh	36 - 43	soy protein isolates	103 - 145
tofu	21 - 49	soy protein concentrates - water washed	247 - 317
soy drink	25	- alcohol washed	21 - 43
soy sauce	2	soy hot dogs, soy bacon, tofu yoghurt	10 - 13
soybean oil	0	soy cheeses	1 - 7
soybean sprouts	42		

Isoflavone intakes are highest in communities consuming a traditional soy staple. A dietary study conducted in a rural Japanese village reported a mean (± SD) intake of 39±36 g and 54±34 g/day soy products in the men and women, respectively, derived from soybean curd (tofu), fermented soybean paste (miso), and fermented (tempeh) or boiled whole soybeans (12). This corresponds to an average isoflavone intake of about 50 mg/day (10). By comparison, intake from a typical Western diet is low, about 1-5 mg/day, although increasing use of soy ingredients in common items such as breads, drinks, sausages and soups, suggests that intake is rising.

Little is understood about the absorption and metabolism of flavonoids in humans (13). Hydrolysis of the glycone to the aglycone can occur in the lumen of the large bowel by the action of glycosidases produced by colonic microflora. In animal models, extensive metabolism and degradation can also occur by colonic microflora to yield a variety of phenolic acids and other derivatives. Aglycone flavonoids can be absorbed from the large intestine and transferred via the portal vein to the liver where they are further metabolised by methylation, or by conjugation with glucuronate or sulfate and excretion in bile to undergo enterohepatic circulation. It is the aglycone flavonoids that appear to exert greatest physiological activity. Flavonoid conjugates are polar and are finally excreted in urine. In humans, urinary concentrations have increased after supplementation with flavonoid-rich foods, but wide unexplained variation is seen between individuals. Kelly et al (14) observed that excretion following a 2-day soy challenge varied 4 to 12 fold for parent isoflavones daidzein, genistein and glycitein, and up to 1000 fold for three major metabolites. It is not clear whether this variation reflected true differences in absorption. For example low excretion may indicate minimal absorption; alternatively it could indicate extensive enterohepatic recycling, or rapid uptake from circulation into different tissues, or degradation to metabolites that were not actually measured. Diet, particularly content of dietary fibre, could also contribute to the variability—either directly by binding the isoflavone to retard its absorption (15), or indirectly by influencing the composition of colonic bacteria (16). The variable response to dietary flavonoids, as yet largely uncharacterised in humans, could have important physiological consequences since individual flavonoids and their metabolites have differing effects and potencies within the body.

Health benefits: epidemiological and clinical evidence

Coronary heart disease

The association between flavonoid intake and coronary heart disease (CHD) has been examined in several epidemiological studies using estimated intakes of five major flavonoids: quercetin, kaempferol, myricetin, luteolin and apigenin (Table 3).

Table 3. Relative risk of cardiovascular disease for high versus low flavonoid intake#.

	Hertog et al (17)	Keli et al (18)	Knekt et al (19)	Rimm et al (20)
Subjects	805 males; 65-84 y Zutfen Elderly Study	552 males; 50-69 y Zutfen Study	5133; 30-69 y i. male; ii. female	34789 males; 40-75 y Health Professionals
Country	Netherlands	Netherlands	Finland	United States
Study design	Cohort 5 y follow up	Cohort 15 y follow up	Cohort 20-25 y follow up	Cohort 6 y follow up
Mean flavonoid intake	25.9 mg/d	23.8 mg/d	3.4 mg/d	20.1 mg/d
Outcome	i. CHD mortality ii. MI incidence	Stroke incidence	CHD mortality	CHD mortality
Relative risk+	i. 0.32 [0.15-0.71]* ii. 0.52 [0.22-1.23]	0.27 [0.11-0.70]*	i. 0.67 [0.44-1.00] ii. 0.73 [0.41-1.32]	1.08 [0.81-1.40]

Σ quercetin, kaempferol, myricetin, luteolin, apigenin; + mean [95% CI]; * statistically significant
CHD: coronary heart disease, MI: myocardial infarction

In the Zutphen Elderly Study (17), a flavonoid intake of greater than 30 mg/day was associated with a 68% reduction in mortality from CHD after adjustment for other dietary and non-dietary variables; an inverse but weaker relationship was seen with the incidence of myocardial infarction. The major source of flavonoids in this Dutch population was black tea, followed by onions and apples. Keli et al (18) found a dose-dependent inverse association between the mean intake of flavonoids over 15 years and the risk of stroke, after adjustment for known confounders. In this cohort, tea was again a major contributor to flavonoid intake and men who consumed 4.7 or more cups of tea had a lower incidence of stroke than men who drank less than 2.6 cups per day.

Further support for the cardioprotective effect of flavonoids is obtained from a re-examination of the food records from sixteen cohorts in the Seven Countries Study (3). During a 25-year follow-up period, an inverse association was observed between CHD mortality and flavonoid intake, which explained a small (8%) but significant portion of the variance in CHD deaths, independently of intake of alcohol and antioxidant vitamins. CHD mortality was lowest in Japan with an average flavonoid intake of 61 mg/d, mainly derived from green tea.

Intake of other unmeasured antioxidant polyphenols such as the catechins in green tea or isoflavones in the traditional soy staple, as well as a diet low in saturated fat may have also contributed to the low rates in Japan, despite their high percentage of smokers. Overall, the intake of saturated fat remained the most significant dietary constituent in relation to cardiovascular disease, explaining 73% of the total variance.

In contrast, other epidemiological studies conducted in Finland and the United States have shown no significant effects of flavonoid intake despite large sample sizes (19,20). Possible reasons for the discrepancy include relatively low flavonoid intakes in the Finnish population (19), or measurement error in the American Health Professionals Study due to limited assessment of onions, a major source of flavonoids in their diet (20). Another limitation is that analyses were confined to five major flavonoids in the Dutch diet (4,5). Further studies are needed to identify the range of flavonoids that may have cardioprotective effects in countries with different dietary patterns.

Based on in vitro evidence, several mechanisms have been proposed to explain the protective effect of flavonoids on CHD (reviewed in (1)). Flavonoids could act as antioxidants to protect LDL from oxidation and inhibit atherogenesis. The antioxidant effect appears to increase with increasing number of hydroxyl groups, especially on positions C-5 and C-7 of the A ring and C-3' and C-4' of the B ring, as seen with the polyhydroxylated flavonoids: myricetin, quercetin and the catechins (Figure 1). Flavonoids could also exert protection via their similarity to endogenous oestrogens. Miksicek (21) showed significant oestrogenic activity in 11 of 38 food flavonoids tested, with greatest activity in genistein followed by kaempferol > apigenin > daidzein >> luteolin. The oestrogenic effect required a minimum of two OH groups, in position C-5, 6 or 7 on the A ring and position C-4' on the B ring—a configuration similar to that of oestradiol-17β that permits binding to the oestrogen receptor. Polyhydroxylated flavonoids, such as myricetin, quercetin and the catechins, showed no oestrogenic activity possibly due to steric hindrance of the additional substitutions.

Cancer

Epidemiological studies on diet and cancer are limited by the fact that cancer involves a complex series of initiation and promotional events developing over several decades. In the Seven Countries Study after 25 years of follow up, average flavonoid intakes determined at baseline were not associated with differences in mortality from lung, colorectal or stomach cancers, or cancer from all causes (3). Protective effects on cancer risk of soy beans, a uniquely rich source of isoflavones, have also been investigated. However relatively few countries have high soy intakes and until recently soy foods were not a focus of research, and therefore dietary data do not encompass the range of soy products consumed. Messina et al (22) reviewed 26 studies in which soy consumption was mentioned in relation to a range of cancers, including breast and prostate cancers, stomach lung, colon, oesophageal, liver and pancreatic cancers. Ten studies showed a protective effect of non-fermented soy products, mainly tofu, with relative risks between 0.12 and 0.69; 15 showed no significant effects while only one study showed an increase in risk. These results tentatively suggest that the soy products may be protective. Of the different cancer sites studied, the most consistent trends were seen with the hormone-dependent breast cancer, in which three of four epidemiological studies showed beneficial effects of soy; all showed adverse effects of meat consumption (Table 4).

In the study by Lee et al (23), the protective effect of soy was observed in premenopausal subjects, who were undergoing rapid dietary change and in whom breast cancer incidence was rapidly rising; no effects were seen in the postmenopausal women who retained more stable, traditional dietary patterns. Nomura et al (24) studied breast cancer risk in a group of Japanese immigrants to Hawaii; rates were lower in those who consumed traditional Japanese foods and higher in those who had adopted Western food habits including consumption of beef, ham and frankfurt sausages. In a third study conducted by Hirayama in Japan, breast cancer rate was halved in those who consumed miso soup daily, compared to non consumers (25).

Table 4. Epidemiological studies on soy consumption and breast cancer risk

	Lee et al (23)	Nomura et al (24)	Hirayama (25)	Yuan et al (26)
Subjects	200 cases; 420 controls 24-88 y	6860$^\#$ 46-68 y	142857 >40 y	834 cases, 834 controls; 20-69 y
Country	Singapore (Chinese)	Hawaii (Japanese)	Japan	China
Study design	Case control	Cohort 10 y follow up	Cohort 17 y follow up	Case control
Results	Relative risk$^+$ in high versus low consumers (premenopausal only): Soy protein: 0.43 [0.23-0.79]* Total soy products: 0.44 [0.24-0.81]* Red meat: 2.57 [1.36-4.87]*	Cases compared to non cases consumed: ⇓ miso soup, seaweed & green tea* ⇓ tofu (trend) ⇑ meat* [beef, wieners]	Relative risk$^+$ in high versus low consumers: Miso soup: 0.46* Meat: 3.03*	Relative risk$^+$ in high versus low consumers: Soy protein: 1.0 [0.7-1.4] Cases compared to non cases consumed: ⇓ green vegetables* ⇑ pork*

$^+$ mean [95% CI]; $^\#$ cases were males married to women with breast cancer; * statistically significant

An important feature of these studies is the diversity of dietary habits within each population—in particular, high and low consumers of soy products: two populations were in a state of transition from their traditional Asian soy-based diet to a Western meat-based diet (23,24), while subjects in the large prospective study in Japan represented 95% of the census population from areas in southern, central and northern Japan, including rural communities consuming the traditional soy staple and industrialised cities with a more Western lifestyle (25).

No protective effect of soy was seen in a Chinese study conducted in two large urban centres (26). Although soy products, mainly tofu and soy milk, were an integral part of the diet, breast cancer rates were low and stable in these populations which could have contributed to the lack of effect. Meat intake was higher in cases than controls but was derived largely from pork consumed in traditional dishes such as pork spare ribs, pig trotters, salted pork and pork liver, and unlike in the other studies, was not an index of change to a more Western style of consumption.

Several mechanisms have been proposed to explain the anticancer properties of soy, in particular the constituent isoflavones (22,27). As antioxidants, isoflavones could prevent conversion of precarcinogens to carcinogens in cells or reduce the damaging effect of free radicals on cellular DNA (1). In vitro studies have shown that genistein, a major isoflavone in soy, can inhibit several steps necessary for the proliferation, transformation or metastasis of cancer cells, including inhibition of protein tyrosine kinases and angiogenesis; genistein has also inhibited growth of human breast cancer cell lines and suppressed growth of chemically-induced mammary cancer in animals. As weak oestrogen agonists, the isoflavones could also inhibit development of breast cancer by competing at the oestrogen receptor to reduce endogenous oestrogen activity. In these studies, other biologically active substances consumed as part of the traditional Asian cuisine such as weakly oestrogenic lignans present in seaweed added to the miso soup, or antioxidant catechins in the green tea, could also have contributed protective effects (24).

Bone health

Age-related bone loss is greater in women than men and is exacerbated by the hormonal changes that occur at menopause, particularly lowered oestrogens. Losses have been estimated at about 1% per year in lumber spine bone mineral density (BMD), with accelerated losses in the immediate two years after menopause. Hormone replacement therapy (HRT) or use of the synthetic anti-oestrogen, Tamoxifen, are associated with preservation of BMD. Preliminary studies have tested the possibility that isoflavones could exert similar protection against oestrogen-responsive bone loss at menopause.

In a series of experiments using the ovariectomised female rat model and rat osteoblast cell lines, Anderson et al (28) concluded that genistein could act as an agonist on oestrogen receptors in bone tissue to retain mineral mass. The response was biphasic and tissue-specific, with optimal effects observed at lower rather than higher doses and in trabecular rather than cortical bone—possibly because the former is more metabolically active. In a 6-month trial with 66 postmenopausal women given either soy protein, providing 90 mg isoflavones, or casein as a control, soy significantly increased BMD although the effect was still less than the 1% per year loss that normally occurs in postmenopausal women (29). While these studies suggest that isoflavone supplements could have short-term beneficial effects on BMD, epidemiological studies do not provide clear evidence of long-term benefits, at least not directly attributable to soy or the hypothesised presence of isoflavones. Lower rates of bone fracture have been observed in Asian women consuming a traditional soy staple, although other studies have shown that their bone density is actually less than in Caucasian women (30,31). It was proposed that non-dietary factors such as greater physical activity, shorter stature or shorter hip axis length in Oriental women may contribute to their lower fracture rates by resulting in fewer or less serious falls. Longer term prospective studies and controlled dietary trials are required before firm conclusions can be drawn about a protective role of isoflavones on postmenopausal bone loss.

Hormonal effects on menopause

Menopause or the cessation of ovarian function is accompanied by a range of symptoms that are highly variable in intensity and duration for different women. Traditional HRT is successful in treating hypo-oestrogenic symptoms such as vaginal dryness, and is usually beneficial in stabilising vasomotor symptoms such as hot flushes. However compliance with HRT has ranged from only ten to fifty percent (32) and alternative treatments have been sought. Of interest in this regard is the inverse association observed between incidence of hot flushes and soy consumption in different populations, with lower rates of hot flushes, 14-19% reported in Asian women from Singapore, China or Japan, and higher rates of 65-80% in Canada or Europe (32,33). These observations led to the hypothesis that isoflavones in the soybean could be acting as weak replacement oestrogens to ameliorate symptoms. Several clinical trials have been conducted to test this hypothesis in postmenopausal women experiencing moderate to severe flushing—between 4 and 18 flushes daily. In these studies the dose of isoflavones, either in tablet form or from soy products, varied from 40 to 165 mg/day, which is comparable to dietary intakes reported by Asian women.

Although small 25-40% reductions were reported in all four studies that measured hot flushes (34-37) these could not be attributed specifically to the isoflavone intervention, since similar decreases were observed in the control groups (35,36) or during parallel treatment with wheat flour in which isoflavone content was low (34,37). Wide variation in flush symptoms was also observed between different women and within the same woman on a daily or monthly basis, which may have precluded any significant group effect. Innovative work by Wilcox et al (38) showed that sequential supplementation with three weakly-oestrogenic plant foods, including soy flour, could increase maturation of vaginal epithelium in postmenopausal women—a more objective clinical index of oestrogenic stimulation than self-reported hot flushes which are prone to placebo effects and tend to resolve over time without treatment. However, of three subsequent studies that measured the vaginal maturation index (34,36,37) only one showed an improvement (37) although this was not conclusive since no placebo control group was included.

Isoflavones appear to differ from current hormone replacement therapies in that they have more subtle and varying effects in different women. Further research is needed to identify which women are most likely to be responders and to clarify the levels of isoflavones that have beneficial effects, not only in relief of the more immediate symptoms such as hot flushes, but in the areas of chronic diseases associated with the menopause and ageing, namely coronary heart disease, cancer and osteoporosis.

Flavonoids in perspective

Diets that are rich in vegetables and fruits have well established protective effects against a range of chronic diseases including coronary heart disease and cancer. Of the dietary factors that may contribute, several nutrients including dietary fibre, the unsaturated fatty acids and the antioxidant vitamins and minerals, have been extensively researched and characterised. Less well understood is the contribution from non-nutrient components such as the flavonoids, which form part of an abundant array of phytochemicals that co-exist with the nutrient components in vegetables and fruits. While persuasive evidence from in vitro studies indicates that individual flavonoids can exert antioxidant, antiproliferative and weak oestrogen agonist activity, evidence in humans to support health benefits that could arise from these activities is still fragmentary. In this regard, long term prospective studies and clinical trials would be useful in clarifying the range of potential benefits. Several research issues will need to be addressed including the optimal intake of flavonoids; the role and possible safety concerns of individual foods such as the soybean, tea and wine, that are especially rich sources of flavonoids; and possible interactions of different flavonoids consumed in a mixed diet.

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