Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants

Garry G. Duthie*, Susan J. Duthie and Janet A. M. Kyle

Division of Cellular Integrity, Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB, UK

Abstract

Certain dietary antioxidants such as vitamin E and vitamin C are important for maintaining optimum health. There is now much interest in polyphenolic products of the plant phenylpropanoid pathway as they have considerable antioxidant activity *in vitro* and are ubiquitous in our diet. Rich sources include tea, wine, fruits and vegetables although levels are affected by species, light, degree of ripeness, processing and storage. This confounds the formulation of databases for the estimation of dietary intakes. Most attention to date has focused on the flavonoids, a generic term which includes chalcones, flavones, flavanones, flavanols and anthocyanins. There is little convincing epidemiological evidence that intakes of polyphenols are inversely related to the incidence of cancer whereas a number of studies suggest that high intakes of flavonoids may be protective against CHD. In contrast, numerous cell culture and animal models indicate potent anticarcinogenic activity by certain polyphenols mediated through a range of mechanisms including antioxidant activity, enzyme modulation, gene expression, apoptosis, upregulation of gap junction communication and P-glycoprotein activation. Possible protective effects against heart disease may be due to the ability of some polyphenols to prevent the oxidation of LDL to an atherogenic form although anti-platelet aggregation activity and vasodilatory properties are also reported. However, some polyphenols are toxic in mammalian cells. Thus, until more is known about their bioavailability, metabolism and intracellular location, increasing intakes of polyphenols by supplements or food fortification may be unwise.

Polyphenols: Cancer: Heart disease: Antioxidants: Bioavailability

Introduction

Living organisms are exposed to a range of oxidizing species which have the potential to damage bio-molecules such as proteins, lipids and DNA (Slater, 1984). Such damage is

Abbreviations: DMBA, dimethylbenz[*a*]anthracene; EGCG, epigallocatechin gallate; EROD, 7-ethoxyresorufin-O-deethylase; GST, glutathione transferase; NMU, *N*-nitroso-*N*-methylurea; PROD, pentoxyresorufin-O-dealkylase; Trp-P-2, 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole.

***Corresponding author:** Dr Garry Duthie, fax +44 (0) 1224 716622, email ggd@rri.sari.ac.uk

implicated in a wide range of diseases including CHD and certain cancers (Halliwell, 1987). From a biological perspective, antioxidants are compounds that protect cellular systems from the potentially harmful effects of processes that can cause excessive oxidations (Krinsky, 1992). By implication they may inhibit the pathogenesis of the many diseases which involve oxidative reactions (Diplock *et al*. 1998). Antioxidants can be of endogenous or exogenous origin and contribute to the complex and integrated biological antioxidant defence system, which normally protects cells from the injurious effects of oxidation. This is achieved by directly scavenging reactive O and N free radical species, by metabolizing peroxides to non-radical products and by chelating metal ions to prevent the generation of oxidizing species.

The recognized antioxidants we require from the diet are vitamin E, vitamin C and possibly certain carotenoids. In addition, a number of enzymes with antioxidant functions require trace elements such as Se, Cu, Zn and Fe from the diet as cofactors. In general these micronutrients are recognized as being essential because: (1) a deficiency causes a defined disease to develop, e.g. severe vitamin C deficiency causes scurvy and combined vitamin E and Se deficiencies cause myopathies and neuropathies (Combs, 1992); moreover, such conditions can be reversed by repletion with the appropriate micronutrients; (2) they are readily absorbed and are likely to be near the biomolecules in the cell where oxidative damage is to occur; (3) in fulfilling their role as antioxidants, they do not cause marked damage to cellular processes *in vivo*; (4) in nutritionally relevant amounts, they moderate markers of oxidative stress and/or disease risk (Duthie, 1999).

Recently, it has become clear that certain polyphenolic products of the phenylpropanoid biosynthetic pathway in plants have considerable antioxidant ability *in vitro* (e.g. RiceEvans *et al*. 1997). There are no known deficiency states for these 'phytochemicals' and therefore, even though dietary intakes may exceed 1 g/d (Formica & Regelson, 1995), they have been generally regarded as non-nutritive. However, epidemiological studies inversely relating dietary intakes of some polyphenols with the incidence of heart disease (Hertog *et al*. 1993*c*, 1995; Knekt *et al*. 1996; Geleijnse *et al*. 1999; Yochum *et al*. 1999) may indicate a putative role in the prevention of chronic diseases. Consequently, this present review considers whether plant polyphenols have important health benefits. There are a number of recent reviews on diverse aspects of the chemistry and biological effects of polyphenols (Bravo, 1998; Croft, 1998; Peterson & Dwyer, 1998; Di Carlo *et al*. 1999; Hollman & Katan, 1999; King & Young, 1999; Lairon & Amiot, 1999; Puddey & Croft, 1999; Ursini *et al*. 1999; Duthie & Crozier, 2000). Therefore, this present review not only focuses on the antioxidant activity of polyphenols but also discusses potential anticarcinogenic and anti-atherogenic effects that do not necessarily involve antioxidant activity.

Plant polyphenols

Origins and types

Plants produce thousands of phenolic compounds as secondary metabolites. The majority are synthesized by the highly branched phenylpropanoid pathway (Fig. 1), the initial compound being 4-hydroxy-cinnamic acid (*p*-coumaric acid) which derives from phenylalanine (Chesson *et al*. 1997). Substitution of the cinnamic acid with hydroxyl or methoxyl groups at the 3- and 5-positions yields caffeic, ferulic and sinapic acids. In addition, compounds such as benzoic acid and derivatives, styrenes, acetophones and gingerols are formed from hydroxycinnamic acid by chain shortening or lengthening without ring formation. Addition of cyclic esters at the side-chain produces hydroxycoumarins and chromonones and various condensation reactions

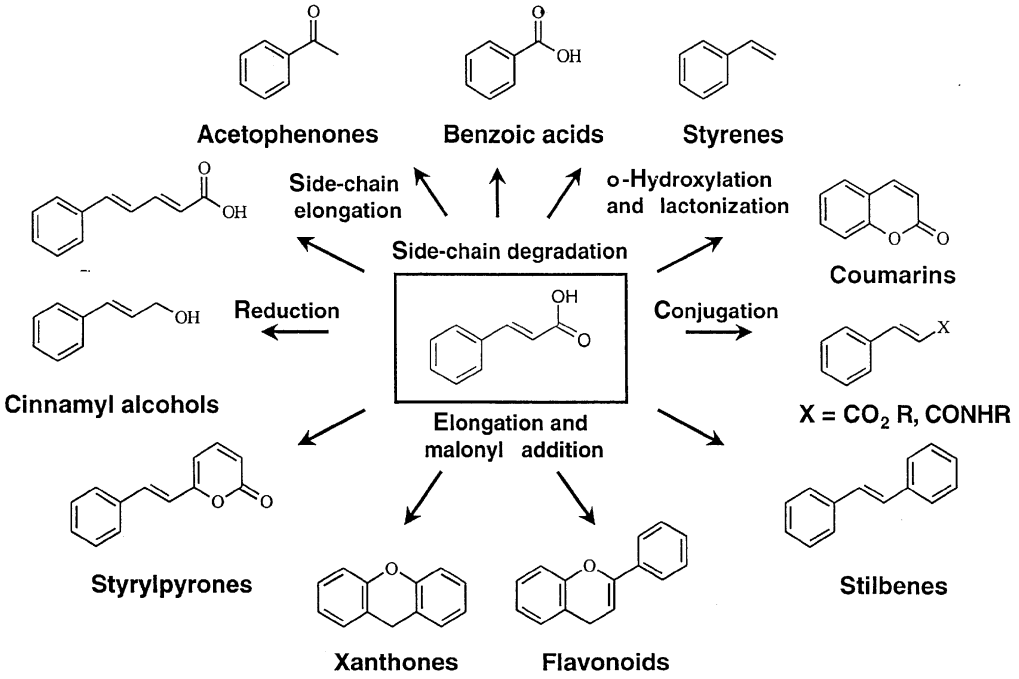


Fig. 1. Phenolic products of the plant phenylpropanoid pathway (with permission of A Chesson, Rowett Research Institute, Aberdeen, UK).

with malonyl residues produce xanthones, stilbenes and flavonoids. They are essential to the plant's physiology being involved in diverse functions such as structure, pigmentation, pollination, pathogen and predator resistance, and growth and development (Dewick, 1997).

Most research to date on the possible nutritional role of polyphenols has focused on the flavonoids. Over 5000 have been described and the thirteen subclasses (Harborne, 1994) have a common C6–C3–C6 structure in that they consist of two aromatic rings linked through an oxygenated heterocycle. Major classes include flavonol, flavones, flavanones, flavanols, anthocyanidins and isoflavones (Fig. 2). Structural differences between these major classes of flavonoid are primarily based on degree of hydroxylation and presence of a C2–C3 double bond in the heterocyclic pyrine ring. However, there are numerous structural variations within the different flavonoid classes; for example more than 380 flavonol and flavone glycosides have been described (Harborne, 1994). Differences include variations in the level of hydrogenation and hydroxylation, methylation and sulfation reactions in addition to conjugation to monosaccharides and disaccharides and formation of complexes with oligosaccharides, lipids, amines and carboxylic and organic acids (Harborne, 1994). These different classes and forms of flavonoid are present in edible plants in widely varying combinations. For example, onions contain quercetin as the aglycone as well as in the form of quercetin glucosides, diglucosides, rhamnosides, glucuronides and malonyl esters.

Food sources

Polyphenols are ubiquitous in foods of plant origin. However, accurate determination of dietary intakes is problematical owing to their immense diversity of form and to variations in analytical

https://doi.org/10.1079/095442200108729016 Published online by Cambridge University Press

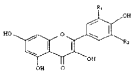
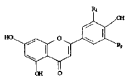
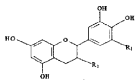
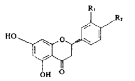
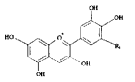
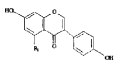
Flavonoid	Subclass	Common food source (total flavonoid subclass content, mg aglycone/kg food item)
Flavonols 	Quercetin (R_1=OH, R_2=H) Kaempferol (R_1=R_2=H) Myricetin (R_1=R_2=OH)	Fruit: apples (34.2 mg/kg), plums (12.5 mg/kg), cranberries (170 mg/kg), strawberries (39 mg/kg), grapes (31.7 mg/kg) Vegetables: kale (35–321 mg/kg), onions (0.2–1096 mg/kg), broccoli (36–231 mg/kg); celery stalks (ND); tomatoes (3–191 mg/kg) Beverages: red wine (13.4 mg/l), green tea (39 mg/l), black tea (30.4 mg/l), grape juice (4.2 mg/kg)
Flavones 	Apigenin (R_1 = R_2 = H) Luteolin (R_1=OH, R_2=H)	Vegetables: celery (130 mg/kg), green olives (142.3 mg/kg), sweet peppers (11 mg/kg)
Flavan-3-ols 	Catechin (R_1=H, R_2=OH) Epigallocatechin (R_1=OH, R_2=OH) Epigallocatechingallate (R_1=OH, R_2=-OC-Ph(OH)$_3$)	Fruit: apples (84.3 mg/kg), plums (23.6 mg/kg), Beverages: green tea, black tea, red wine (110.0 mg/kg), grape juice (5.2 mg/kg)
Flavanones 	Hesperetin (R_1=OMe, R_2=OH) Naringenin (R_1-OH, R_2=H)	Fruit: Citrus fruits: oranges (577 mg/kg), lemons (219 mg/kg) Beverages: grape juice (2 mg/kg)
Anthrocyanidins 	Cyanidin (R_1=OH) Delphinidin (R_2=H)	Fruit: black grapes (92.5 mg/kg) Beverages: red wine (2 mg/l), grape juice (2 mg/l)
Isoflavones 	Genistein (R_1=OH) Daidzein (R_1=H)	Legumes: soyabeans (373–1403 mg/kg) chickpeas (11.5–36 mg/kg) Processed products: soya-based, non-dairy, cream cheese (177 mg/kg); vegetarian chilli (32 mg/kg)

Fig. 2. Classification of some common dietary flavonoids indicating some major food sources. Data from Kuhnau (1976); Hertog *et al.* (1992*b*, 1993*c*); Harborne (1994); Linseisen *et al.* (1997); McDonald *et al.* (1998).

methodology. In addition, their concentrations in foods can vary by many orders of magnitude and are influenced by several factors including species, variety, light, degree of ripeness, processing and storage (Kuhnau, 1976; Hermann, 1988; Robards & Antolovich, 1997; Peterson & Dwyer, 1998). For example, teas contain numerous but differing quantities of polyphenols, particularly catechin and its derivatives (for review see Beecher *et al.* 1999). The types and proportion of catechins in the tea leaf varies with season, the age of the leaf, climate, and horticultural practices. In addition, major changes occur during processing. In the rolling and crushing used to manufacture oolong and black tea, polyphenol oxidase (*EC* 1.10.3.1) is released from the leaf endoplasmic reticulum and catalyses the condensation of the catechins to a range of theaflavins, thearubigens, catechin dimers (bisflavonols) and epitheaflavic acids (Stagg & Millin, 1975). Similarly, although red wine is a rich source of phenolics, the flavonol content can vary 10-fold and there are also marked variations in catechins, anthocyanins, resveratrol and hydroxycinnamates (McDonald *et al.* 1998). Varietal differences in products can also markedly confound the estimation of dietary intakes. For example, quercetin contents of cherry tomatoes are approximately 30 mg/kg compared with about 5 mg/kg for those of 'normal' size and the flavonol content of lettuce ranges from 10 to 900 mg/kg, depending on variety (Crozier *et al.* 1997).

https://doi.org/10.1079/095442200108729016 Published online by Cambridge University Press

Dietary intakes

Interest in polyphenols has generated a need for dietary compositional information to facilitate epidemiological and intervention studies with human subjects. To date, formulation of databases to allow estimates of dietary polyphenol intakes has mainly focused on flavonols and flavones. In the early 1970s flavonoid intake in the USA was estimated at approximately 1 g/d of which flavanones, flavonols and flavones contributed 110 mg/d (Kuhnau, 1976). Subsequent improvements in analytical methodology of food items (Hertog *et al*. 1992*b*) indicate that this estimate may be too high, as more recent analyses of nine fruits, twenty-eight vegetables and a range of commonly consumed beverages in the Netherlands generally provide lower values (Hertog *et al*. 1992*a*, 1993*c*). Calculated dietary flavonol and flavone intake in the Netherlands is 23 mg/d (expressed as aglycones) of which 70 % is quercetin, 17 % kaempferol and 6 % myricetin (Hertog *et al*. 1993*a*). This Dutch compositional data has now been used in numerous studies, often with the addition of estimates of flavonoid content of local food preferences such as berries. Estimated intakes range from 3 mg/d in Finland to 65 mg/d in Japan (Table 1) although a more recent study (Kimira *et al*. 1998) suggests that intakes in Japan are less than previously estimated, possibly owing to the increasing popularity of 'Western' diets. Major food sources of flavonoids in Europe are black tea, wine, onions and apples whereas green tea is the major flavonoid source in Japan. In general, dietary intakes of flavonols and flavones are quantitatively similar to those of the previously categorized antioxidants such as vitamin E and vitamin C.

Antioxidant action

Polyphenols are effective antioxidants in a wide range of chemical oxidation systems, being capable, for example, of scavenging peroxyl radicals, alkyl peroxyl radicals, superoxide, hydroxyl radicals, nitric oxide and peroxynitrite in aqueous and organic environments (for review see Duthie & Crozier, 2000). In a similar manner to vitamin E, this activity is essentially due to the ease with which an H atom from an aromatic hydroxyl (OH) group can be donated to a free radical and the ability of an aromatic compound to support an unpaired electron due to delocalization around the π-electron system. The stoichiometry and kinetics of these reactions are influenced by a number of structural determinants including the number and position of OH

Table 1. Estimated daily intakes (mg/d) of flavonols and flavones in different countries*

Country	(Mean values) Flavonol and flavone intake	Quercetin intake
The Netherlands	23–33	13–16
Finland	3–6	3–6
USA	13	11
Serbia	12	10
Greece	16	15
Italy	27	21
Croatia	49	30
Japan	16–65	8–31
Wales	26	14
Scotland	17	14
Bavaria	12†	10

* Data from several sources (Hertog *et al*. 1993*a*,*b*, 1994, 1995, 1997; Knekt *et al*. 1996, 1997; Rimm *et al*. 1996; Linseisen *et al*. 1997; Kimira *et al*. 1999; Yochum *et al*. 1999).
† Flavonols only.

https://doi.org/10.1079/095442200108729016 Published online by Cambridge University Press

Table 2. Flavonoid-mediated cytoprotection in cultured cells

Model cell type	Protectant	Biomarker	Toxic agent	Reference
Rat hepatocytes	(+)-cyanidol-3 (10^{-4} M)	Cytotoxicity (T blue exclusion) LDH leakage MDA formation	Bromotrichloromethane (2 mM)	Kappus *et al.* (1979)
Rat hepatocyte	Catechin (1 μM)	LDH leakage AST leakage ALT leakage	Erythromycin estolate (1–2×10^{-4} M) Aminotriptyline/nortriptyline (1×10^{-3}, 1×10^{-4} M) TBOOH (1×10^{-3}, 1×10^{-4} M)	Davila *et al.* (1989)
Rat hepatocytes	Catechin (50–150 μM) Quercetin (50–150 μM) Disometin (150–400 μM)	MDA formation LDH leakage	Ferric-nitrilotriacetate (100 μM)	Morel *et al.* (1993)
Rat hepatocyte	Caffeic acid (20–100 μM) Oleuropein Tyrosol Hydroxytyrosol	MDA formation	Ferric-nitrilotriacetate (100 μM)	Chimi *et al.* (1995)
Lymphoid cells	Quercetin (0–100 μM) Rutin (0–100 μM)	Oxidation of LDL Cytotoxicity (T blue exclusion) TBARS formation	Oxidized LDL (u.v. irradiation)	Negre-Salvayre & Salvayre (1992)
Bovine endothelial cells	Rutin (0–100 μM)	Oxidation of LDL TBARS formation Cytotoxicity (MTT)	Oxidized LDL (Cu, u.v. irradiation)	Negre-Salvayre *et al.* (1995)
V79 Chinese hamster cells	Quercetin (0–20 μM) Catechin (0–20 μM) Kaempferol (0–1 mM) Taxifolin (0–240 μM)	Cytotoxicity (colony forming ability)	H_2O_2 (60 μM) HX/XO (50 μM, 0·025 U)	Nakayama *et al.* (1993)
Porcine aortic endothelial cells	Quercetin Catechin Morin (0–500 μM)	Cell necrosis	HX/XO (1 mM, 17 U/l)	Zhang *et al.* (1997)

Continued...

Table 2. *Continued*

Model cell type	Protectant	Biomarker	Toxic agent	Reference
Human fibroblasts, keratinocytes and endothelial cells, chick dorsal root ganglion neurones	Quercetin Dihydroquercetin Rutin (0–100 μM)	Cytotoxicity (MTT)	BSO (500 μM)	Skaper *et al.* (1997)
Calf aortic endothelial cells	Quercetin Myricetin Kaempferol Rutin (0–50 μM)	Cytotoxicity (proliferation)	Daunomycin (5–10 nmol/l)	Melzig *et al.* (1997)
A549 human lung cells	GTP (100 μg/ml)	Lipid peroxidation DNA strand breaks Cytotoxicity (proliferation)	H_2O_2 (200 μM) $FeCl_3$ (10 μM) Cigarette smoke (25 % solution)	Leanderson *et al.* (1997)
U937 cells	Quercetin (0–1 mM)	Cell viability (T blue exclusion) DNA strand breakage	TBOOH (200 μM, 3 mM) H_2O_2 (200 μM)	Sestili *et al.* (1998)
Caco-2 human colonocytes	Quercetin (50 μM) Myricetin (1 mM) Kaempferol (1 mM) Rutin (1 mM)	DNA strand breakage Oxidized DNA bases Cell viability (T blue, proliferation)	H_2O_2 (200 μM)	Duthie & Dobson (1999)

T blue, Trypan blue; LDH, lactate dehydrogenase; MDA, malondialdehyde; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TBARS, thiobarbituric acid reactive substances; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; TBOOH, *tert*-butylhydroperoxide; HX/XO, hypoxanthine/xanthine oxidase; GTP, green tea polyphenols; BSO, buthionine sulfoximine.

groups, the type and position of glycosylation and the degree of steric hindrance at the site of H abstraction. In addition, many polyphenols can bind transition metal ion catalysts such as Cu and Fe to prevent generation of initiating-free radicals through Fenton reactions.

Certain polyphenols may have antioxidant ability in biological systems as they decrease markers of oxidative damage to lipids, proteins and DNA in primary cell cultures as well as in immortalized and transformed cells (Table 2). For example, bromotrichloromethane-induced lipid peroxidation is inhibited by (+)-cyanidol-3 (Kappus *et al.* 1979) and catechin, quercetin and myricetin reduce lipid peroxidation and enzyme leakage associated with Fe overloading in rat hepatocyte cultures (Morel *et al.* 1993). Similarly, several phenolics from olive oil inhibit Fe-generated malondialdehyde formation, an index of lipid peroxidation, in rat hepatocytes (Chimi *et al.* 1995). These compounds can scavenge both the hydroxyl radical and lipid radicals, suggesting that they act to reduce toxicity by interfering with the lipid peroxidation cascade (Negre-Salvayre & Salvayre, 1992; Negre-Salvayre *et al.* 1995).

Polyphenols can also act as free radical scavengers in human cells *ex vivo*. The dietary flavonoids kaempferol, naringin, naringenin and apigenin all decrease oxidant-induced lipid peroxidation and K permeability due to disrupted membrane function in isolated human erythrocytes (Maridonneau *et al.* 1986). The ability of these compounds to chelate metal ions, thereby preventing the formation of reactive O species such as the hydroxyl radical, is suggested as one possible mechanism of cytoprotection. Similarly, rutin, at concentrations as low as 3·3 μM, prevents the H_2O_2-mediated oxidation of erythrocyte haemoglobin following treatment with the pro-oxidant antimalarial drug primaquine (Grinberg *et al.* 1994). Primaquine- and H_2O_2-induced lipid peroxidation and erythrocyte haemolysis are considerably reduced (80 %) in the presence of tea polyphenols (10 μg/ml). These dietary compounds decrease hydroxyl radical efflux in the Fe/ascorbate free-radical generating system, implying that protection may be caused by polyphenol-mediated Fe chelation and the subsequent formation of a redox inactive complex (Grinberg *et al.* 1997). Quercetin also protects against phenylhydrazine- and acrolein-induced lipid peroxidation in mouse erythrocytes probably via its ability to chelate Fe (Ferrali *et al.* 1997).

Supporting the possibility that certain polyphenols may have antioxidant function *in vivo* are several rodent feeding studies indicating that compounds such as rutin and phenolic-rich extracts of red wine, tea and fruit juice lower oxidative products such as protein carbonyls, DNA base damage and malonaldehyde in blood and a range of tissues (Vertommen *et al.* 1994; Yoshina *et al.* 1994; Martin-Aragon *et al.* 1997; Fremont *et al.* 1998; Miyake *et al.* 1998; Casalini *et al.* 1999; Freese *et al*, 1999; Funabiki *et al.* 1999; Roig *et al.* 1999). However, it must be stressed that the amounts of polyphenols consumed in such studies are likely to be an order of magnitude higher than in a 'normal' diet.

Studies attempting to show antioxidant effects in human subjects tend to assess the ability of a compound to moderate indices of oxidative damage to DNA, protein and lipids in blood and urine. One problem with this approach is that many such indices are non-specific and subject to interference from compounds of non-peroxidative origin. In addition, such indices may only be significantly elevated in individuals with overt clinical conditions or nutrient deficiencies. Thus they may not respond in healthy individuals to intervention with polyphenolic antioxidants (Duthie, 1999). This may partly explain why many studies have given contradictory results. For example, whereas there appears to be no effect of consumption of catechin-rich green or black tea on the oxidation of LDL of smokers *ex vivo* (Princen *et al.* 1998; Cherubini *et al.* 1999), a process potentially associated with the development of the atheromatous plaque, other trials indicate that tea consumption by smokers and non-smokers is associated with a decrease in markers of oxidative DNA damage as estimated by 8-hydrox-

deoxyguanosine in leucocytes and urine (Klaunig *et al*. 1999). Urinary and plasma malonaldehyde concentrations, which are crude measures of lipid peroxidation, were also decreased in smokers (Klaunig *et al*. 1999) and healthy females (Freese *et al*. 1999) following consumption of catechin-rich beverages or extracts.

In addition to assays for estimating indices of oxidative damage, various methods have been devised to measure the overall or 'global' antioxidant activity of plasma or serum subsequent to intervention with nutritional antioxidants. Most measure the inhibition of an artificially generated oxidative process in the plasma. Although they differ in choice of oxidation source, target and the type of measurement used to detect the oxidized product (Woodford & Whitehead, 1998), in general such methods have detected transient increases in plasma antioxidant capacity following consumption of polyphenol-rich preparations of green tea (Serafini *et al*. 1996; Benzie *et al*. 1999; Nakagawa *et al*. 1999), red wine (Fuhrman *et al*. 1995; Whitehead *et al*. 1995; Duthie *et al*. 1998), alcohol-free red wine (Serafini *et al*. 1998), whisky (Duthie *et al*. 1998), grape seeds (Koga *et al*. 1999) and onions (McAnlis *et al*. 1999). However, any changes in plasma antioxidant capacity after consumption of polyphenols does not necessarily imply with certainty that analogous changes in redox status occur within relevant cells and tissue.

Polyphenols and cancer

There are many *in vitro* and animal model studies suggesting that polyphenols could inhibit the development of cancer although as a caveat it must be noted that concentrations used in some cell culture studies may substantially exceed those that may be achieved in tissue by dietary means.

Cancer cells in vitro

Numerous studies have reported flavonoid-mediated antiproliferative effects against both human and rodent ovarian, leukaemic, intestinal, lung, breast and bladder cancer cells. For example, quercetin (10 μM) strongly suppresses transformed OVCA 433 human ovarian cancer cell growth. Moreover, quercetin inhibits normal proliferation in cultured primary ovarian adenocarcinoma tumour cells (Scambia *et al*. 1994*a*,*b*). At low micromolar concentrations, quercetin inhibits DNA synthesis (IC_{50} 10 μM) and growth (IC_{50} 7·7 μM) in HL60 human promyelocytic leukaemia cells (Uddin & Choudhry, 1995; Kang & Liang, 1997). The citrus flavonoid tangeretin suppresses HL60 cell proliferation (measured as tritiated thymidine incorporation into DNA) even more strongly, with an IC_{50} of 0·17 μM (Hirano *et al*. 1995), while genistein is inhibitory at concentrations similar to conventional anticancer agents such as doxorubicin and methotrexate (Hirano *et al*. 1994). Genistein, kaempferol and quercetin inhibit the proliferation of the human colon cancer cells Caco-2 and HT29 (Agullo *et al*. 1994; Kuo, 1996) while naringenin and catechin do not (Kuo *et al*. 1997). Curcumin is cytostatic in several hormone-dependent (MCF-7 and T-47D) and -independent (SK-BR3, BT-20 and MDA231) breast-tumour cell lines (Mehta *et al*. 1997), while genistein and quercetin, in addition to their antiproliferative action, appear to alter the metastatic potential of rat breast adenocarcinoma cells, measured as a reduced ability to migrate within a collagen matrix (Lu *et al*. 1996). Quercetin inhibits tritiated thymidine uptake and proliferation in several non-small-cell lung

carcinoma cell lines and reduces bromodeoxyuridine incorporation in primary lung tumour slices (Caltagirone *et al*. 1997). Quercetin also inhibits ML-3 murine hepatoma cell growth (Chi *et al*. 1997).

Very few studies have investigated the cytostatic ability of flavonoids both in malignant cells and in their untransformed counterparts although several polyphenols, most notably genistein, while showing considerable growth inhibition in HL60 cells had little or no effect on mitogen-induced blastogenesis in normal human peripheral blood lymphocytes (Hirano *et al*. 1994). Similarly, tritiated thymidine uptake is inhibited in HL60 cells following exposure to tangeretin, but is unchanged in normal lymphocytes (Hirano *et al*. 1995). The polyhydroxylated flavonoids quercetin and taxifolin and the polymethoxylated flavonoids nobiletin and tangeretin inhibit HTB 43 squamous cell carcinoma cell and 9L gliosarcoma cell growth, but are less effective in untransformed human CCl human embryonic fibroblast-like cells (Kandaswami *et al*. 1992). While these studies appear to suggest that the flavonoids display a tumour-specific action, it should be noted that comparisons were not made on cells derived from the same tissue. In an elegant study by Chen *et al*. (1998), epigallocatechin gallate (EGCG), the major polyphenol in green tea, inhibited colorectal cancer and breast cancer cell growth more than in their respective normal counterparts. Similarly, EGCG reduced W138 human lung fibroblast cell growth only weakly compared with their virally transformed (VA) counterparts. The IC_{50} value of EGCG was 120 μM in W138 cells compared with only 10 μM for W138VA cells. However, while SV40-transfection immortalizes cells they may not be analogous to cancer cells. Conversely, the flavonoids quercetin and genistein are equally as toxic towards colonic cancer cells and non-transformed intestinal crypt cells (Kuo, 1996).

Animal models

In addition to cell culture studies, the capacity of certain dietary polyphenols to protect against both chemically induced and spontaneous formation of tumours in animals is well established. For example, quercetin administered to rats in combination with dimethylbenz[*a*]anthracene (DMBA) or *N*-nitrosomethylurea (NMU) reduces the incidence and multiplicity of mammary tumours by 30 and 50 % respectively (Verma *et al*. 1988). Quercetin and luteolin (10 g/kg diet) decrease fibrosarcoma incidence (52 % and 60 % respectively) and tumour size in male Swiss albino mice following treatment with the model chemical carcinogen 20-methylcholanthrene (Elangovan *et al*. 1994). Quercetin (20 g/kg) also increases the survival and reduces the tumour burden of mice (Balb/c) transplanted intrasplenically with ML-3 hepatoma cells (Chi *et al*. 1997). The citrus flavonoid naringin inhibits the *in vivo* development of DMBA-induced mammary tumours in Sprague–Dawley rats (So *et al*. 1996).

Several studies have described a protective effect for tea polyphenols against carcinogenesis. Rats fed on a diet containing 10 g green tea catechins/kg have a considerably reduced mortality (7 % reduced mortality) from mammary tumours following DMBA treatment compared with rats given the carcinogen alone (66 %) (Hirose *et al*. 1994). Similarly, hamsters fed on green tea polyphenols display fewer hyperplastic pancreatic duct lesions after treatment with *N*-nitrosobis(2-oxopropyl)amine (Majima *et al*. 1998). In a comprehensive study, Yang *et al*. (1998) describes the ability of both green and black tea infusions to inhibit *N*-nitrosodiethylamine-induced lung carcinogenesis in A/J mice. Green tea (12·5 g/kg) decreases tumour incidence and multiplicity by 39 % and 56 % respectively when fed before the carcinogen. A similar pattern of protection is observed when the infusion is administered afterwards, indi-

cating an effect both on carcinogenic initiation and promotion. Decaffeinated tea preparations were equally effective in reducing the incidence of lung cancer induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. Tumorigenesis is inhibited by 65 % when fed pre-initiation and 85 % when administered post-initiation. Tea extract also significantly reduces the progression of chemical-induced, non-malignant adenomas to malignant adenocarcinomas (27 % in rats fed on the infusion compared with 80 % in rats given the carcinogen alone). Finally, the spontaneous formation of lung tumours and rhabdosarcomas is inhibited by 50 % in rats fed on either black or green tea infusions.

Anticancer mechanisms

There are several suggested mechanisms by which polyphenols exert anticarcinogenic effects:

Antioxidant effects. Carcinogenesis is a multi-stage process of genetic change affecting proto-oncogenes or tumour suppressor genes in a single cell or clone of cells. Such genetic alteration may be initiated by increased and persistent damage to DNA causing permanent alterations in the genetic message when the cell replicates its DNA and divides. Reactive O and N species are potential carcinogens as they can directly and indirectly induce structural alterations in DNA by oxidation, methylation, depurination and deamination reactions. The ability of certain polyphenols to inhibit oxidative DNA damage is well documented. For example, luteolin, kaempferol, quercetin and myricetin at relatively low concentrations (50–100 μM) significantly reduce DNA strand breakage and oxidized pyrimidine levels in H_2O_2-stressed lymphocytes (Duthie *et al*. 1997*a*,*b*; Noroozi *et al*. 1998). Similarly, tea polyphenols decrease the incidence of hydroxyl radical-generated chromatid breaks in lymphocytes exposed to fluorescent light irradiation (Parshad *et al*. 1998). The number and positioning of the hydroxyl groups in the flavonoid structure appear important to the antioxidant and cytoprotective potential of the compounds. There are also many studies with Caco-2 cells, which are generally accepted as a good model for normal human colonocytes, which indicate a cytoprotective ability of flavonoids against oxidative DNA damage (Raeissi *et al*. 1997; Ricchi *et al*. 1997; Venturi *et al*. 1997; Duthie & Dobson, 1999).

Ex vivo studies also suggest that the antioxidant potential of polyphenols may be anticarcinogenic. For example, the ability of plasma to inhibit O free radical-induced DNA damage to lymphocytes was increased by 20 % 1 hour after consumption of 300 ml wine (Fenech *et al*. 1997). Moreover, indices of oxidized DNA in bladder mucosal cells of smokers inversely correlate with the level of phenolics measured in their urine (Malaveille *et al*. 1998).

Modulation of enzyme activities associated with carcinogen activation and detoxification. One of the mechanisms by which polyphenols may exert their anticarcinogenic effect is by modulating the enzyme systems that metabolize carcinogens or pro-carcinogens to genotoxins. In this way, the activation of the carcinogen may be inhibited, or it may be converted to a less reactive compound before it reacts with DNA and initiates carcinogenesis. The cytochrome P450 superfamily of enzymes metabolizes a large number of procarcinogens to reactive intermediates, which bind covalently to DNA and can induce malignant transformation. The activity of some P450s are either induced or inhibited by flavonoids. For example, naringenin and tangeretin are potent inhibitors of microsomal 7-ethoxyresorufin-O-deethylase (EROD) activity, which is a marker substrate for P450 1A (Obermeier *et al*. 1995). Similarly, quercetin inhibits EROD activity ($IC_{50} < 1\ \mu$M) in microsomes from human hepatoma HepG2 cells (Musonda *et al*. 1997). Pentoxyresorufin-O-dealkylase (PROD) activity is also decreased,

indicating ability of the flavonoids to inhibit P450 2B activity. Tangeretin inhibits nifedepine oxidase, (P450 3A) in human liver microsomes (Obermeier *et al*. 1995). Flavone and several hydroxylated derivatives (3-OH-, 5-OH-, 7-OH- and 3,7-dihydroxyflavone) are potent inhibitors of cDNA-expressed human P450s 1A1 and 1A2 ($IC_{50} < 1\ \mu M$), while galangin is a selective inhibitor of P450 1A2 (Zhai *et al*. 1998). The ability of flavonoids to inhibit P450 1A is directly related to their antimutagenic properties. Several flavones, including apigenin and luteolin, and flavonols such as kaempferol, quercetin and myricetin, reduce the mutagenicity of the food-derived heterocyclic amine 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2) in the Ames test (*Salmonella typhimurium* TA98). Trp-P-2 is metabolized by P450 1A to the ultimate mutagen *N*-hydroxy-Trp-P-2 that binds to the DNA molecule and initiates carcinogenesis (Kanazawa *et al*. 1998).

Specific flavonoids also induce P450 activities. P450s 1A and 2B proteins are increased in rats *in vivo* fed on flavone and flavanone in the diet (10 mg/kg DM) over 32 d. EROD, methoxyresorufino-D-methylase and PROD activities are maximally induced after 4–8 d. Tangeretin, a citrus flavonoid, induces a similar pattern of activities, but with a lesser magnitude and after a longer delay (Siess *et al*. 1996).

In addition to modulating metabolic activation, flavonoids affect the activities of phase II enzymes. The glutathione transferases (GST (*EC* 2.5.1.18)), together with the tripeptide glutathione, conjugate the highly reactive and potentially carcinogenic metabolites of phase I activation. Conjugation renders the metabolites more polar, thereby facilitating excretion. Moreover, GSH itself is a reductant, with oxidized glutathione (GSSG) recycled back to GSH by the enzyme glutathione reductase (*EC* 1.6.4.2). Quercetin (1–9 μg/ml), given for 8 weeks to Swiss NMRI mice in drinking water, increases hepatic and pulmonary GST (30–40 % respectively). GSH levels double following treatment with the highest dose of quercetin (Gandhi & Khanduja, 1993). Flavone, flavanone and tangeretin in the diet similarly increase hepatic GST activity in rats (Siess *et al*. 1996). GST induction is generally considered to reflect an increase in cellular protection, ensuring that potential toxins are conjugated and excreted more rapidly. However, increased GST protein or activity might also reflect a cellular response to toxic insult by the flavonoid itself. This remains to be established. Conversely, quercetin does not induce GST protein or activity in human hepatoma cells (Musonda *et al*. 1997), while it acts as an inhibitor of GST activity in a purely chemical system (Ito *et al*. 1993). Fisetin and myricetin are also inhibitors of GST (IC_{50} 14 μM and 24 μM respectively) and glutathione reductase (Ito *et al*. 1993). Similarly, narigenin, quercetin, luteolin and kaempferol all inhibit glutathione reductase activity (Zhang *et al*. 1997).

DT-diaphorase, or quinone reductase (*EC* 1.6.99.2), limits cellular toxicity by catalysing the reduction of numerous chemicals and quinones and diverting their metabolism away from redox cycling and the generation of reactive O species. The ability of dietary agents to induce quinone reductase activity is considered a marker of anticarcinogenic potential. Galangin, kaempferol and quercetin are all potent inducers of quinone reductase activity in mouse hepatoma cells, increasing enzyme activity 2-fold at flavonol concentrations between 6 μM and 13 μM (Uda *et al*. 1997). Flavanols and flavans are not effective inducers (Uda *et al*. 1997). Certain glycosides of quercetin, which are highly prevalent in the diet, can also increase quinone reductase activity *in vitro* (Williamson *et al*. 1996). However, quercetin (50 μM) has been found to inhibit quinone reductase activity in mouse brain (Tamura *et al*. 1994).

Therefore, the effect of flavonoids on xenobiotic metabolizing enzymes is complex and highly dependent on a number of factors including the chemical structure of the flavonoid, the species under investigation and the model system being employed.

Flavonoid-mediated modulation of gene expression, apoptosis and malignant transformation. By modification of gene expression, certain polyphenols may prevent or reverse carcinogenesis by inducing apoptosis, thus eliminating damaged cells, or by inhibiting neoplastic transformation. The inhibition of proliferation by polyphenols can be associated with an increase in apoptosis. For example, tangeretin induces apoptosis, measured as an increase in DNA fragmentation, in HL-60 human leukaemia cells following arrest in the G2-M stage of the cell cycle (Hirano *et al*. 1995). Similarly, quercetin (15–120 μM) increases apoptosis in HL60 cells following inhibition of growth (Dong *et al*. 1997). EGCG induces apoptosis in human tumour cells isolated from various sites in the body following cell cycle arrest in G0–G1. More than 70 % of cancer cells were apoptotic following exposure to EGCG (Ahmad *et al*. 1997).

Flavonoid-enhanced apoptosis appears to be regulated via alteration in gene expression. The tumour suppressor gene p53 regulates cell cycle arrest and apoptosis. Apigenin, luteolin and quercetin increase apoptosis in C3H10T1/2CL8 cells as a direct result of p53 upregulation and accumulation in the nucleus. Cell cycle arrest and apoptosis did not occur following exposure to the flavonoids in p53 knockout fibroblasts (Plaumann *et al*. 1996). Treatment of human mammary epithelial cells with the chemical carcinogen, B[*a*]P, induces uncontrolled proliferation. EGCG (2·2 μM) or genistein (2·5 μM), given together with the carcinogen, inhibit cell growth and increase cell death. The induction of apoptosis follows upregulation and expression of p53 (Katdare *et al*. 1998). The flavonoids may also work to downregulate mutated forms of the p53 gene, which are associated with malignant transformation and the development of various forms of breast cancer. Quercetin (75 μg/ml for 3 d) causes accumulation of human breast cancer cells (MDA-MB468) in G2-M while downregulating the expression of a mutated form of the p53 protein. Inhibition is at the level of transcription and is specific for the tumour suppressor protein.

Flavonoids can also downregulate proto-oncogenes, which may be over-expressed in tumour cells. Quercetin (55 μM) reduces the levels of the proto-oncogene Ki-*ras*, which is over-expressed in the human leukaemia cell line, K562. Downregulation of the proto-oncogene is associated with inhibition of proliferation, an increase in apoptosis and induction of cellular differentiation (Csokay *et al*. 1997).

In addition, certain polyphenols may alter gene expression by interacting directly with the DNA molecule. Alternatively, they may block signal transduction pathways by inhibiting second messengers such as protein tyrosine kinase (*EC* 2.7.1.112) and inositol-1,4,5-triphosphate (Csokay *et al*. 1997; Lin *et al*. 1997).

Several studies report a selective effect of flavonoids in inducing apoptosis in cancer cells. EGCG induces apoptosis in virally transformed human fibroblasts but not in their normal counterparts (Chen *et al*. 1998). Similarly, green tea polyphenols and EGCG increase DNA fragmentation in several human and rodent carcinoma cells but not in normal human epidermal keratinocytes (Ahmad *et al*. 1997). However, induction of apoptosis by flavonoids is not entirely restricted to cancer cells. Both quercetin and genistein cause apoptosis in non-transformed rat intestinal crypt cells in addition to their malignant counterparts (Kuo *et al*. 1996), while EGCG and genistein induce apoptosis in normal human mammary epithelial cells derived from explants (Katdare *et al*. 1998).

Flavonoids may also act by downregulating genes associated with malignant transformation and tumorigenesis. The AP-1 complex, containing c-*jun* and c-*fos* oncogene products, is a transcription factor for several genes, which, amongst other functions, can induce matrix proteases crucial for angiogenesis and malignant transformation. Using a c-*fos*-transfected rat liver epithelial cell line, Lagarrigue *et al*. (1995) showed that upregulation in AP-1 resulted in spontaneous transformation of the cell line, expressed as an ability to grow in soft agar.

Quercetin (10 μM) inhibited malignant transformation by inhibiting AP-1 transcription. Similarly, apigenin, kaempferol and genistein (25 μM) all cause reversion, in v-H-*ras* transformed NIH3T3 cells, back to a non-transformed phenotype (Lin *et al*. 1997). Exactly how flavonoids act to prevent or revert neoplastic transformation is unknown. However, the polyphenols may induce the expression of Fra-1 and Fra-2 proteins which bind to *jun* to form an AP-1 heterodimer which does not possess transactivating ability but which competes with the functional AP-1 complex and thereby reduces subsequent gene activation. Fra-1 and Fra-2 expression is induced by flavonoids activating transcription factors which bind to antioxidant-responsive elements in the *Fra* gene promoter regions (McCarty, 1998).

Upregulation of intracellular gap junctional communication and inhibition of neoplastic transformation. Cell-to-cell communication, mediated via transmembranal gap junctions, is crucial in regulating normal cellular homoeostasis, cell proliferation and differentiation. Gap junctions, composed of transmembrane connexin proteins, allow the transfer of growth-controlling signals or molecules between neighbouring cells and in doing so may prevent malignant transformation. Detrimental changes in gap junctional intracellular communication are now considered to be instrumental in the early development of cancer. Cell-to-cell communication is presented experimentally as transfer of the fluorescent dye Lucifer Yellow following microinjection. Malignant transformation is routinely demonstrated in cultured animal cells as an increase in uncontrolled cell growth resulting in distinct areas of multilayered foci. Franke *et al*. (1998), investigating the ability of nineteen dietary flavonoids to inhibit the 3-MC-induced neoplastic transformation of C3H10T1/2 murine fibroblasts, found that most of the phenolics were at least equal to, or in many cases superior to, other dietary protectant agents such as β-carotene or vitamin E. The citrus flavonoids hesperetin and hesperedin were amongst the most potent, inhibiting malignant transformation almost completely (98 %) at very low concentrations (1 μM). Quercetin and rutin at the same dose inhibited carcinogenesis by 50 % and 30 % respectively. Most agents tested showed concentration-dependent cytoprotection. Moreover, the levels of isoflavonoids which were effective in decreasing neoplastic transformation *in vitro* were within the range of concentrations detected in human biofluids (urine, plasma and breast milk) following consumption of an isoflavonoid-rich meal (20 g roasted soyabeans). Tangeretin increases gap junctional intracellular communication in rat liver epithelial cells and inhibits the transformation of V79 lung fibroblasts (Chaumontet *et al*. 1996). Protection is probably afforded by upregulating the production of connexin43. Apigenin and tangeretin (10 μM or 25 μM) increase gap junctional intracellular communication between rat liver epithelial cells in a time-and concentration-dependent manner while similarly inducing connexin43 expression (Chaumontet *et al*. 1994). However, tangeretin (1 g/kg) fed to rats for 3 months actually inhibits gap junctional intracellular communication by 50 % (measured as transfer of Lucifer Yellow in isolated tissue slices) indicating that the relatively high concentrations of this flavonoid may be acting as a tumour-promoter *in vivo* (Chaumontet *et al*. 1996).

P-glycoprotein activation. P-glycoprotein is a membrane protein which may contribute to cellular defences against naturally occurring xenobiotics by facilitating their speedy and efficient removal before they can be activated into potential carcinogens. Quercetin, galangin and kaempferol (100 μM) all markedly inhibit the accumulation of the xenobiotic adriamycin presumably by upregulation of P-glycoprotein activity (Critchfield *et al*. 1994). Moreover, adriamycin efflux in the target HCT-15 colon cells was increased 2-fold by the polyphenols. This could be ascribed to their altering the phosphorylation state of the membrane pump or directly modulating P-glycoprotein expression, thereby enhancing the ability of the pump to efflux xenobiotics. However, in direct contrast, quercetin and 3′,4′,7-trimethyloxyquercetin, at lower concentrations (1–10 μM), potentiate the growth-inhibitory action of adriamycin on

MCF-7, ADR-resistant breast cancer cells *in vitro* by inhibiting P-glycoprotein efflux activity and downregulating expression of the membrane pump (Scambia *et al*. 1994*b*). The reason for the marked discrepancy is unclear but may indicate that the flavonoids act through a very sensitive tissue-specific and concentration-dependent mechanism.

Studies with human subjects

Despite the considerable experimental evidence that certain polyphenols have potent anti-carcinogenic activity, epidemiological support is contradictory. For example, some ecological, cohort and case–control studies suggest that tea consumption lowers the risk of developing cancer whereas other investigations have failed to find such associations or have even indicated procarcinogenic effects (Blot *et al*. 1996). In addition, no correlation was observed between estimated flavonoid intake (determined in 1985) and cancer incidence ($P = 0{\cdot}54$) and mortality ($P = 0{\cdot}51$) at all sites after a 5-year period in 738 elderly Dutch men (65–84 years; Hertog *et al*. 1994). Similarly, in a retrospective cross-cultural study involving sixteen cohorts in seven countries, total flavonoid intake, estimated by dietary analysis of food composites taken in 1960, was not associated with mortality from all causes of cancer 25 years later. Current fruit and vegetable consumptions were used to estimate the amount of flavonoid the subjects had consumed at the beginning of the study. However, intake was strongly and positively related to mortality from stomach cancer. Moreover, this was confounded by vitamin C intake and indicates the complex relationship and potential synergistic action between flavonoids and other dietary components.

The inconclusive nature of the epidemiological studies may reflect a lack of information on the duration and amount of polyphenol intake, inadequate control of confounding and potential biases in recall and reporting of intake patterns. In addition, the majority of studies estimating flavonoid intake rely on data gathered from self-reported dietary questionnaires together with a relatively short follow-up period from which to determine cancer incidence. In contrast, a recent study (Knekt *et al*. 1997) using a 20-year follow-up of 10 000 men and women (aged 15–99 years) observed an inverse correlation between the intake of flavonoids and the relative risk of all cancers (relative risk 0·8, 95 % CI 0·67, 0·96). This association was the result of significant protection against lung cancer (relative risk 0·54, 95 % CI 0·34, 0·87), especially in younger subjects, and was unaffected by smoking and the intake of other dietary antioxidants such as vitamin C.

Polyphenols and heart disease

Low-density lipoprotein oxidation

Considerable evidence *in vitro* implicates the oxidation of LDL in atherogenesis (Steinberg, 1997). In brief, LDL is a heterogeneous structure containing phospholipids, free and esterified cholesterol, triacylglycerols, and amino acids, which form apolipoprotein B. The proteins and the polyunsaturated fatty acid components of the LDL are susceptible to free radical-mediated oxidation, particularly if the antioxidant content of the LDL is low. When LDL is oxidized *in vitro* there is a loss of polyunsaturated fatty acids to yield a range of fragments of 3–9 C lengths including hydroperoxides, aldehydes and ketones, which conjugate with other LDL-bound

lipids and the apolipoprotein B. In cell cultures, this 'minimally modified' LDL has a number of properties that could increase its atherogenicity. It is recognized by at least three types of scavenger receptors in macrophages, which rapidly internalize the oxidized LDL. The macrophages are transformed into 'foam-like cells' which *in vivo* are regarded as precursors to the development of the occlusive plaque (Westhuyzen, 1997). In addition, oxidized LDL stimulates the release of macrophage colony stimulating factor and monocyte chemoattractant protein 1 from cells (Diaz *et al*. 1997).

Such observations have led to the proposal that, *in vivo*, LDL in arterial endothelial cells may be oxidized by cellular enzymes such as NADPH oxidase (*EC* 1.11.1.2), myeloperoxidase (*EC* 1.11.1.7) or lipoxygenase (*EC* 1.13.11.12), or by the leakage of free radicals from the mitochondrial electron transport chain. The presence of minimally-modified LDL induces the surrounding vascular cells to produce the chemoattractants and stimulating factors that cause monocytic accumulation and their subsequent differentiation to macrophages. On transformation of monocytes to macrophages, the oxidized LDL limits further macrophage mobility and decreases their ability to migrate away from the arterial wall. The enhanced rate of uptake of oxidized LDL by the macrophages, via the scavenger receptor pathways, may then convert them into foam cells. In addition, since the macrophage can oxidatively modify native LDL via the respiratory burst, autocatalytic progression may lead to their continuous growth to form the plaque that begins to occlude the artery. These proposed events have not, as yet, been demonstrated *in vivo*. However, LDL extracted from human atherosclerotic lesions but not from normal arteries contains products of lipid peroxidation such as F_2-isoprostanes and malonaldehyde (Pratico *et al*. 1997). Moreover, antibodies raised against oxidized LDL react with such lesions (Holvoet & Collen, 1998) and elevated amounts of oxidized LDL are present in blood of patients with atherosclerotic disease (Holvoet *et al*. 1995). Therefore it is plausible that oxidized LDL is involved in the atherosclerotic process.

Polyphenols and low-density lipoprotein oxidation

In studies *in vitro*, the oxidation of LDL by endothelial cells, macrophages and Cu^{2+} can be inhibited by a wide range of polyphenols and polyphenol-rich extracts (Frankel *et al*. 1993; Laranjinha *et al*. 1994; Miura *et al*. 1994, 1995; Viana *et al*. 1996; Bourne & RiceEvans, 1997; Yokozawa & Dong, 1997; Aviram & Fuhram, 1998; Brown & RiceEvans, 1998; Hodgson *et al*. 1999; Kerry & Abbey, 1999; Rifici *et al*. 1999). Such effects may be due to direct scavenging by the polyphenols of the oxidizing species or may result from the regeneration by the polyphenol of vitamin E in the LDL molecule (Zhu *et al*. 1999) and/or its ability to bind LDL protein (Wang & Goodman, 1999).

In addition to studies *in vitro*, several animal models and studies with human subjects indicate that ingestion of polyphenols or polyphenol-rich extracts increases the resistance of LDL to oxidation *ex vivo* (e.g. Fuhrman *et al*. 1995; Ishikawa *et al*. 1997; Carbonneau *et al*. 1998; Nigdikar *et al*. 1998). However, other studies have failed to detect changes in the oxidizability of LDL *ex vivo* following consumption of such preparations (van het Hof *et al*. 1997, 1999; Princen *et al*. 1998). The reasons for the disparity between studies are unclear but may reflect the type of polyphenol used, variation in absorption kinetics and the antioxidant content of the LDL before consumption of the polyphenol.

Changes in LDL oxidation induced by polyphenols do not necessarily imply a causal relationship with the progression of vascular disease. However, dietary polyphenols have

reduced the lesions in arteries of animals which either are genetically susceptible to vascular disease or have been fed on atherosclerosis-promoting diets (Kirk *et al*. 1998). For example, consumption of red wine, quercetin and catechin decreased atherosclerotic lesion areas by 31–52 % in apolipoprotein E-deficient mice (Hayek *et al*. 1997) and green tea consumption decreases aortic lesion formation in hypercholesterolaemic rabbits by 31 % (Tijburg *et al*. 1997).

Other mechanisms

Consumption of polyphenols may also have beneficial effects in the prevention of heart disease by mechanisms that do not necessarily implicate their antioxidant properties (Kritz & Sinzinger, 1997). For example, tea extracts may prevent platelet adhesion and aggregation by inhibiting the cyclooxygenase (*EC* 1.14.99.1) pathway and reducing the cyclic 3′,5′-adenosine monophosphate response of platelets to prostaglandin I_2. Moreover, vasodilatory effects of tea extracts and polyphenols may be due to their affecting enhanced NO generation, cyclic guanosine 3′5′-monophosphate accumulation and other endothelium-dependent relaxation factors (Bravo, 1998; Di Carlo *et al*. 1999).

Epidemiology

In contrast to the lack of clear associations between intakes of polyphenols and cancer, several epidemiological studies have reported inverse associations between intakes of flavonols and flavones and CHD, with relative risk ranging from 0·3 to 1·6. However, other studies have failed to detect a significant statistical association (Hertog *et al*. 1993*a*, 1995, 1997; Knekt *et al*. 1996; Rimm *et al*. 1996; Yochum *et al*. 1999). In general, analyses of dietary flavonoid intakes include only five flavonols and flavones expressed as aglycones (quercetin, kaempferol, myricetin, luteolin and apigenin) and there is little information available for the relationship between the consumption of the myriad of other phenolics in the diet and mortality from CHD. However, supporting a role for polyphenols in the prevention of heart disease are epidemiological studies focusing on the consumption of polyphenol-rich beverages. For example, in a prospective study of 3454 men and women aged 55 years and older, there was a significant inverse association between the intake of catechin-rich tea and radiographically quantified aortic atherosclerosis (Geleijnse *et al*. 1999). Similarly, inverse associations between the consumption of red wine and CHD mortality (the French paradox) are well known (e.g. St. Leger *et al*. 1979; Renaud & Longeril, 1992) and may reflect, in part, the antioxidant ability of the wine phenolics to inhibit the oxidation of LDL to an atherogenic form (Frankel *et al*. 1993). The relationship between red wine consumption and CHD has been recently reviewed (Waterhouse *et al*. 1998).

Bioavailability

Polyphenols have to be absorbed from the gut if they are to exert a protective effect against heart disease and cancer. Bioavailability refers to the fraction of an ingested nutrient that is

available to the body for use in normal physiological functions or storage (Jackson, 1997). As yet, little is known about the bioavailability of polyphenols although it will probably be affected by numerous factors including molecular structure, the amount consumed, the food matrix, degree of bioconversion in the gut and tissues, the nutrient status of the host and genetic factors. Clarification of the absorption, bioavailability and metabolism of the plethora of polyphenols in our diet will be an important research area in the future. Ultimately, the many potentially anticarcinogenic and anti-atherogenic effects observed in cell cultures will not be of nutritional relevance unless polyphenols or their active metabolites gain access to the appropriate sites within the tissues of the body.

Animal studies

Animal studies aimed at elucidating the degree and mechanisms of absorption of polyphenols have given contradictory results, possibly reflecting complex catabolic interactions of polyphenols with intestinal bacteria. Rodent models in which gut bacterial activity was suppressed (Nakagawa *et al*. 1965; Das, 1969) appeared to indicate that flavonoids, for example, were absorbed only to a limited degree because gut micro-organisms preferentially destroy the heterocyclic rings of the compounds before any absorption takes place in the small intestine. Moreover, Kuhnau (1976) concluded that glycoside forms may not be absorbed from the intestine without extensive and time-consuming hydrolysis to the aglycone. Moreover, any flavonoids subsequently crossing the intestinal wall were rapidly bound in the liver and excreted into the bile (Barrow *et al*. 1971). In contrast, over 40 years ago the urine from rabbits fed on quercetin and rutin was shown to contain phenolic aromatic acids (Murray *et al*. 1954), which suggests the initial absorption of both aglycones and sugar conjugates.

Subsequent improvements in analytical methodology have allowed the detection of various polyphenols and their conjugated derivatives in the plasma and urine of rats. Compounds detected include catechin derivatives and metabolites (Chen *et al*. 1997; Okushio *et al*. 1999), cyanidin glucosides (Miyazawa *et al*. 1999; Tsuda *et al*. 1999), hydroxycinnamic acids (Choudhury *et al*. 1999), luteolin glucosides (Shimoi *et al*. 1998) and metabolites of quercetin and rutin (Manach *et al*. 1995, 1996). Thus the bioavailability of some polyphenols may be greater than was previously assumed. This view is supported by indications of significant transport of some polyphenols, particularly as glucuronides, across isolated jejunal and ileal gut preparations (Spencer *et al*. 1999) and also across endothelial cells (Schramm *et al*. 1999). This may indicate that specific transport mechanisms exist to facilitate the uptake of selected polyphenols into blood and tissues (Noteborn *et al*. 1997). Moreover, increased concentrations of quercetin and its methylated derivative isorhamnetin have been detected in liver, kidney, heart and testes of rats consuming diets supplemented with quercetin (Morrice *et al*. 2000) and [^{3}H]catechin derivatives were widely distributed in brain, liver, pancreas and bladder of predosed rats (Suganuma *et al*. 1998). However, studies that detect significant changes in concentrations of phenolics in blood and tissues usually involve the use of doses of the compounds that markedly exceed what may be achievable from diet alone. The nutritional relevance of these studies therefore remains uncertain.

Studies with human subjects

An increasing number of studies have now enabled detection of selected polyphenols and their metabolites in plasma and urine of human subjects following the consumption of pure com-

pounds and polyphenol-rich extracts and beverages. For example, flavanols, flavonols and anthocyanins and their metabolites increase in plasma and urine following the consumption of wine, tea, parsley, onions, red fruits and *Ginkgo biloba* tablets (van het Hof *et al*. 1997; Bourne & RiceEvans, 1999; Cao & Prior, 1999; Donovan *et al*. 1999; Miyazawa *et al*. 1999; Nielsen *et al*. 1999; Watson & Oliveira, 1999; Young *et al*. 1999). Particular attention has been paid to quercetin, the major representative of the flavonol subclass, and it is suggested that conjugation with glucose may enhance its absorption from the small intestine (Hollman *et al*. 1995, 1997*a*,*b*; Hollman & Katan, 1997, 1998). However, wide individual variation in absorptive response exists. For example, absorption of quercetin appears to range from 0 to $> 50\,\%$ of the dose (Graefe *et al*. 1999). This may partly reflect the type of polyphenol, its conjugation and lack of highly specific and sensitive assay methodology.

Toxicity

Although several polyphenols may have potent anticarcinogenic effects in cell culture, it should be noted that they can also be toxic as many function in plants to discourage attack by fungal parasites, herbivorous grazers and pathogens. Not surprisingly, therefore, many are also toxic and mutagenic in cell culture systems and consumption to excess by mammals could cause adverse metabolic reactions (Brusick *et al*. 1993). For example, quercetin can bind with DNA *in vitro* (calf thymus, plasmid or phage DNA) and induce damage (Alvi *et al*. 1986; Rahman *et al*. 1992; Ahmed *et al*. 1994*a*) by intercalating directly with nucleotide bases (Alvi *et al*. 1986; Ahmed *et al*. 1994*b*).

In addition, mutagenic, genotoxic and clastogenic responses to polyphenols by cells may reflect pro-oxidant rather than antioxidant activity. O free radicals may be generated following degradation or auto-oxidation of flavonoids (Miura *et al*. 1998), leading to DNA single or double strand breakage (Rahman *et al*. 1992) and to enhanced oxidation of LDL. Quercetin, myricetin and kaempferol ($100\,\mu$M) all increase lipid peroxidation and DNA strand breakage in isolated rat liver nuclei (Sahu & Washington, 1991*a*; Sahu & Gray, 1993, 1994). Genotoxicity occurs under aerobic conditions, is enhanced in the presence of Fe or Cu ions and is decreased by preincubation with the hydroxyl radical scavenger mannitol (Sahu & Washington, 1991*a*,*b*). Thus, quercetin auto-oxidation, close to or within the DNA molecule catalysed by metal ions, may act as a pro-oxidant and induce the generation of reactive O species, which are themselves genotoxic. Quercetin-mediated chromosome damage (chromatid breaks, sister chromatid exchanges, dicentrics) is highly pH-dependent, supporting the hypothesis that flavonoid degradation increases the formation of highly reactive and destructive radicals (Gaspar *et al*. 1994). In addition to inducing DNA strand breakage, quercetin is cytotoxic and cytostatic in several human cell types (Duthie *et al*. 1997*b*). In these experiments, GSH levels were depleted before membrane damage and cell death. This may indicate conversion of GSH to its oxidized form GSSG as a result of oxidative stress (Duthie *et al*. 1997*a*).

While certain flavonoids appear to be mutagenic and genotoxic *in vitro*, there is very little evidence to date that they are carcinogenic *in vivo*. Many studies have found the incidence of various tumours in rodents fed on quercetin (or its glycoside rutin) throughout their life span, not to be statistically different from control groups (Saito *et al*. 1980; Hirono *et al*. 1981; Morino *et al*. 1982). Similarly, quercetin does not appear to induce DNA damage to any significant degree *in vivo*. For example, neither unscheduled DNA synthesis nor induction of bone

marrow or peripheral blood micronuclei is increased in mice and rats fed on quercetin (Ngomuo & Jones, 1996).

Conclusion

Despite the apparent lack of toxicity of polyphenols *in vivo* and their undoubted anti-carcinogenic and anti-atherogenic effects in cell cultures, little is known about their uptake and metabolism *in vivo*. Consequently, it may be premature to regard them as micronutrients with important health benefits. In addition, it is unwise, at present, to recommend that intakes should be increased by supplementation or food fortification. This is particularly apposite in view of the unexpected adverse effects that were apparent in recent intervention trials with supplements of some of the well-recognized antioxidant nutrients (Omenn *et al*. 1996; Rapola *et al*. 1997). Increasing consumption of polyphenol-rich foods such as fresh fruits and vegetables is possibly a more appropriate strategy to increase intake of polyphenols than is supplementation. Until we know more about the activity and metabolic fate of polyphenols in the body, it would be better to be very cautious about the consumption of supra-nutritional amounts of such bio-active compounds.

Acknowledgements

We are grateful to the Scottish Executive for Rural Affairs Department (SERAD), the Ministry of Agriculture, Fisheries and Food (MAFF), the EU, and the World Cancer Research Fund (WCRF) for financial support of work in our laboratories.

References

Agullo G, Gamet L, Besson C, Demigne C & Remesy C (1994) Quercetin exerts a preferential cytotoxic effect on active dividing colon carcinoma HT29 and Caco-2 cells. *Cancer Letters* **87**, 55–63.

Ahmad N, Feyes DK, Nieminen AL, Agarwal R & Mukhtar H (1997) Green tea constituent epigallocatechin-3-gallate and induction of apoptosis and cell cycle arrest in human carcinoma cells. *Journal of The National Cancer Institute* **89**, 1881–1886.

Ahmed MS, Ainley K, Parish JH & Hadi SM (1994*a*) Free radical-induced fragmentation of proteins by quercetin. *Carcinogenesis* **15**, 1627–1630.

Ahmed MS, Ramesh V, Nagaraja V, Parish JH & Hadi SM (1994*b*) Mode of binding of quercetin to DNA. *Mutagenesis* **9**, 193–197.

Alvi NK, Rizvi RY & Hadi SM (1986) Interaction of quercetin with DNA. *Bioscience Reports* **6**, 861–868.

Aviram M & Fuhram B (1998) Polyphenolic flavonoids inhibit macrophage-mediated oxidation of LDL and attenuate atherogenesis. *Atherosclerosis* **137**, S45–S50.

Barrow A & Griffiths LA (1971) The biliary excretion of hydroxyethylrutosides and other flavonoids in the rat. *Biochemical Journal* **125**, 24P–25P.

Beecher GR, Warden BA & Merken H (1999) Analysis of tea polyphenols. *Proceedings of the Society for Experimental Biology and Medicine* **220**, 267–270.

Benzie IF, Szeto YT, Strain JJ & Tomlinson B (1999) Consumption of green tea causes rapid increase in plasma antioxidant power in humans. *Nutrition & Cancer* **34**, 83–87.

Blot WJ, Chow WH & McLaughlin N (1996) Tea and cancer: a review of the epidemiological evidence. *European Journal of Cancer Prevention* **5**, 425–438.

Bourne LC & RiceEvans CA (1997) The effect of the phenolic antioxidant ferulic acid on the oxidation of low density lipoprotein depends on the pro-oxidant used. *Free Radical Research* **27**, 337–344.

Bourne LC & RiceEvans CA (1999) Detecting and measuring bioavailability of phenolics and flavonoids in human: Pharmacokinetics of urinary excretion of dietary ferulic acid. *Methods in Enzymology* **299**, 91–106.
Bravo L (1998) Polyphenols: Chemistry, dietary sources, metabolism and nutritional significance. *Nutrition Reviews* **56**, 317–333.
Brown JE & RiceEvans CA (1998) Luteolin-rich artichoke extract protects low density lipoprotein from oxidation *in vitro*. *Free Radical Research* **29**, 247–255.
Brusick D (1993) Genotoxicity of phenolic antioxidants. *Toxicology and Industrial Health* **9**, 223–230.
Cao GH & Prior RL (1999) Anthocyanins are detected in human plasma after oral administration of an elderberry extract. *Clinical Chemistry* **45**, 574–576.
Caltagirone S, Ranelletti FO, Rinelli A, Maggiano N, Colasante A, Musiani P, Aiello FB & Piantelli M (1997) Interaction with type II estrogen binding sites and antiproliferative activity of tamoxifen and quercetin in human non-small-cell lung cancer. *American Journal of Respiratory Cell & Molecular Biology* **17**, 51–59.
Carbonneau MA, Leger CL, Descomps B, Michel F & Monnier L (1998) Improvement in the antioxidant status of plasma and low density lipoprotein in subjects receiving a red wine phenolics mixture. *Journal of the American Oil Chemists Society* **75**, 235–240.
Casalini C, Lodovici M, Briani C, Paganelli G, Remy S, Cheynier V & Dolara P (1999) Effect of complex polyphenols and tannins from red wine (WCPT) on chemically induced oxidative DNA damage in the rat. *European Journal of Nutrition* **38**, 190–195.
Chaumontet C, Bex V, GaillardSanchez I, Seillanheberden C, Suschetet M & Martel P (1994) Apigenin and tangeretin enhance gap junctional intercellular communication in rat-liver epithelial-cells. *Carcinogenesis* **15**, 2325–2330.
Chaumontet C, Suschetet M, HonikmanLeban E, Krutovskikh VA, Berges R, LeBon AM, Heberden C, Shahin MM, Yamasaki H & Martel P (1996) Lack of tumor-promoting effects of flavonoids: Studies on rat liver preneoplastic foci and on in vivo and in vitro gap junctional intercellular communication. *Nutrition and Cancer* **26**, 251–263.
Chen L, Lee MJ, Li H & Yang CS (1997) Absorption, distribution, elimination of tea polyphenols in rats. *Drug Metabolism & Disposition* **25**, 1045–1050.
Chen ZP, Schell JB, Ho CT & Chen KY (1998) Green tea epigallocatechin gallate shows a pronounced growth inhibitory effect on cancerous cells but not on their normal counterparts. *Cancer Letters* **129**, 173–179.
Cherubini A, Beal MF & Frei B (1999) Black tea increases the resistance of human plasma to lipid peroxidation *in vitro* but not *ex vivo*. *Free Radical Biology & Medicine* **27**, 381–387.
Chesson A, Russell WR & Provan GJ (1997) Metabolites of the phenylpropanoid pathway—common origin, common properties. In *Polyphenols in Foods*, pp. 17–23. Luxembourg: Office for Official Publications of the European Communities.
Chi CW, Chang YF, Ou YR, Hsieh CC, Lui YW, Peng FK & Liu TY (1997) Effect of quercetin on the in vitro and in vivo growth of mouse hepatoma cells. *Oncology Reports* **4**, 1021–1024.
Chimi H, Morel I, Lescoat G, Pasdeloup N, Cillard P & Cillard J (1995) Inhibition of iron toxicity in rat hepatocyte culture by natural phenolic-compounds. *Toxicology In Vitro* **9**, 695–702.
Choudhury R, Srai SK, Debnam E & RiceEvans CA (1999) Urinary excretion of hydroxycinnamates and flavonoids after oral intravenous administration. *Free Radical Biology & Medicine* **27**, 278–286.
Combs GF (1992) *The Vitamins. Fundamental Aspects in Nutrition and Health*. London: Academic Press Inc.
Critchfield JW, Welsh CJ, Phang JM & Yeh GC (1994) Modulation of adriamycin(r) accumulation and efflux by flavonoids in hct-15 colon cells—activation of p-glycoprotein as a putative mechanism. *Biochemical Pharmacology* **48**, 1437–1445.
Croft KG (1998) The chemistry and biological effects of flavonoids and phenolic acids. *Annals of the New York Academy of Sciences* **20**, 435–442.
Crozier A, Lean MEJ, McDonald MS & Black C (1997) Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce and celery. *Journal of Agricultural and Food Chemistry* **45**, 490–495.
Csokay B, Prajda N, Weber G & Olah E (1997) Molecular mechanisms in the antiproliferative action of quercetin. *Life Sciences* **60**, 2157–2163.
Das NP (1969) Studies on flavonoid metabolism. Degradation of (+)-catechin by rat intestinal contents. *Biochimica et Biophysica Acta* **177**, 668–670.
Davila JC, Lenherr A & Acosta D (1989) Protective effect of flavonoids on drug-induced hepatotoxicity in vitro. *Toxicology* **57**, 267–286.
Dewick PM (1997) *Medicinal Natural Products: A Biosynthetic Approach*. Chichester, England: John Wiley & Sons Ltd.
Diaz MN, Frei B, Vita JA & Keaney JF (1997) Antioxidants and atherosclerotic heart disease. *New England Journal of Medicine* **337**, 408–416.
Di Carlo G, Mascolo N, Izzo AA & Capasso F (1999) Flavonoids: Old and new aspects of a class of natural therapeutic drugs. *Life Sciences* **65**, 337–353.
Diplock AT, Charleux JL, Crozier-Willi G, Kok FJ, RiceEvans CA, Roberfroid M, Stahl W & Vina-Ribes J (1998) Functional food science and defence against reactive oxidative species. *British Journal of Nutrition* **80**, S77–S112.
Dong X, Shou-Peng Z & Zhen-Lun G (1997) Quercetin induced apoptosis in human leukemia HL-60 cells. *Acta Pharmacologica Sinica* **18**, 280–283.
Donovan JL, Bell JR, Kasim-Karakas S, German JB, Walzem RL, Hansen RJ & Waterhouse AL (1999) Catechin is present as metabolites in human plasma after consumption of red wine. *Journal of Nutrition* **129**, 1662–1668.

Duthie GG (1999) Determination of activity of antioxidants in human subjects. *Proceedings of the Nutrition Society* **58**, 1–10.
Duthie GG & Crozier A (2000) Plant-derived phenolic antioxidants. *Current Opinions in Lipidology* **11**, 43–47.
Duthie GG, Pedersen MW, Gardner PT, Morrice PC, Jenkinson A McE, McPhail DB & Steele GM (1998) The effect of whisky and wine consumption on total phenol content and antioxidant capacity of plasma from healthy volunteers. *European Journal of Clinical Nutrition* **52**, 733–736.
Duthie SJ, Collins AR, Duthie GG & Dobson VL (1997*a*) Quercetin and myricetin protect against hydrogen peroxide-induced DNA damage (strand breaks and oxidized pyrimidines) in human lymphocytes. *Mutation Research Genetic Toxicology EM* **393**, 223–231.
Duthie SJ & Dobson VL (1999) Dietary flavonoids protect human colonocyte DNA from oxidative attack in vitro. *European Journal of Nutrition* **38**, 28–34.
Duthie SJ, Johnson W & Dobson VL (1997*b*) The effect of dietary flavonoids on DNA damage (strand breaks and oxidized pyrimidines) and growth in human cells. *Mutation Research Genetic Toxicology EM* **390**, 141–151.
Elangovan V, Sekar N & Govindasamy S (1994) Chemopreventive potential of dietary biflavonoids against 20-methylcholanthrene-induced tumorigenesis. *Cancer Letters* **87**, 107–113.
Fenech M, Stockly C & Aitken C (1997) Moderate wine consumption protects against hydrogen peroxide-induced damage. *Mutagenesis* **12**, 289–296.
Ferrali M, Signorini C, Caciotti B, Sugherini L, Ciccoli L, Giachetti D & Comporti M (1997) Protection against oxidative damage of erythrocyte membrane by the flavonoid quercetin and its relation to iron chelating activity. *FEBS Letters* **416**, 123–129.
Fieschi M, Codignola A & Luppi Mosa AM (1989) Mutagenic flavonol aglycones in infusions and in fresh and pickled vegetables. *Food Science* **54**, 1492–1495.
Formica JV & Regelson AW (1995) Review of the biology of quercetin and related bioflavanoids. *Food and Chemical Toxicology* **33**, 1061–1080.
Franke AA, Cooney RV, Custer LJ, Mordan LJ & Tanka Y (1998) Inhibition of neoplastic transformation and bioavailability of dietary flavonoid agents. *Advances in Experimental Medicine and Biology* **439**, 237–248.
Frankel EN, Kanner J, German JB, Parks E & Kinsella JE (1993) Inhibition of oxidation of human low density lipoprotein by phenolic substances in red wine. *Lancet* **341**, 454–457.
Freese R, Basu S, Hietanen E, Nair J, Nakachi K, Bartsch H & Mutanen M (1999) Green tea extract decreases plasma malonaldehyde concentration but does not affect other indicators of oxidative stress, nitric oxide production, or hemostatic factors during a high-linoleic acid diet in healthy volunteers. *European Journal of Nutrition* **38**, 149–157.
Fremont L, Gozzelino MT, Franchi MP & Linard A (1998) Dietary flavonoids reduce lipid peroxidation in rats fed polyunsaturated or monounsaturated fat diets. *Journal of Nutrition* **128**, 1495–1502.
Fuhrman B, Lavy A & Aviram M (1995) Consumption of red wine with meals reduces the susceptibility of human plasma and low-density lipoprotein to lipid peroxidation. *American Journal of Clinical Nutrition* **61**, 549–554.
Funabiki R, Takeshita K, Miura Y, Shibasto M & Nagasawa T (1999) Dietary supplement of G-rutin reduces oxidative damage in the rodent model. *Journal of Agricultural and Food Chemistry* **47**, 1078–1082.
Gandhi RK & Khanduja KL (1993) Impact of quercetin consumption on phase-i and phase-ii drug metabolizing enzymes in mice. *Journal of Clinical Biochemistry and Nutrition* **14**, 107–112.
Gaspar J, Rodrigues A, Laires A, Silva F, Costa S, Monteiro MJ & Rueff J (1994) On the mechanisms of genotoxicity and metabolism of quercetin. *Mutagenesis* **9**, 445–449.
Geleijnse JM, Launer LJ, Hofman A, Pols HAP & Witteman JCM (1999) Tea flavonoids may protect against atherosclerosis—The Rotterdam Study. *Archives of Internal Medicine* **159**, 2170–2174.
Gradelet S, Astorg P, Leclerc J, Chevalier J, Vernevaut MF & Siess MH (1996) Effects of canthaxanthin, astaxanthin, lycopene and lutein on liver xenobiotic-metabolizing enzymes in the rat. *Xenobiotica* **26**, 49–63.
Graefe EU, Derendorf H & Veit M (1999) Pharmacokinetics and bioavailability of the flavonol quercetin in humans. *International Journal of Clinical Pharmacology & Therapeutics* **37**, 219–233.
Grinberg LN, Newmark H, Kitrossky N, Rahamim E, Chevion M & Rachmilewitz EA (1997) Protective effects of tea polyphenols against oxidative damage to red blood cells. *Biochemical Pharmacology* **54**, 973–978.
Grinberg LN, Rachmilewitz EA & Newmark H (1994) Protective effects of rutin against hemoglobin oxidation. *Biochemical Pharmacology* **48**, 643–649.
Halliwell B (1987) Oxidants and human disease: some new concepts. *FASEB Journal* **1**, 358–364.
Harborne JB (1994) *The Flavonoids: Advances in Research Since 1986*. London: Chapman & Hall.
Hayek T, Fuhrman B, Vaya J, Rosenblat M, Belinky P, Coleman R, Elis A & Aviram M (1997) Reduced progression of atherosclerosis in 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. *Arteriosclerosis Thrombosis & Vascular Biology* **17**, 2744–2752.
Hermann K (1988) On the occurrence of flavone glycosides in vegetables. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung* **186**, 1–5.
Hertog MGL, Hollman PCH & Katan MB (1992*a*) Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *Journal of Agricultural and Food Chemistry* **40**, 2379–2383.
Hertog MGL, Hollman PCH & Venema DP (1992*b*) Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. *Journal of Agricultural and Food Chemistry* **40**, 1591–1598.

Hertog MGL, Feskens EJM, Hollman PCH, Katan MB & Kromhout D (1993*a*) Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen elderly study. *Lancet* **342**, 1007–1011.
Hertog MGL, Hollman PCH, Katan MB & Kromhout D (1993*b*) Intake of potentially anticarcinogenic flavonoids and their determinants in adults in the Netherlands. *Nutrition & Cancer* **20**, 21–29.
Hertog MGL, Hollman PCH & van de Putte B (1993*c*) Content of potentially anticarcinogenic flavonoids of tea infusions, wines, and fruit juices. *Journal of Agricultural and Food Chemistry* **41**, 1242–1246.
Hertog MGL, Feskens EJM, Hollman PCH, Katan MB & Kromhout D (1994) Dietary flavonoids and cancer risk in the Zutphen elderly study. *Nutrition & Cancer* **22**, 175–184.
Hertog MGL, Kromhout D, Aravanis C, Blackburn H, Buzina R, Fidanza F, Giampaoli S, Jansen A, Menotti A & Nedeljkovic S (1995) Flavonoid intake and long-term risk of coronary heart disease. *Archives of Internal Medicine* **27**, 381–386.
Hertog MGL, Sweetnam PM, Fehily AM, Elwood PC & Kromhout D (1997) Antioxidant flavonols and ischemic heart disease in a Welsh population of men: the Caerphilly Study. *American Journal of Clinical Nutrition* **65**, 1489–1494.
Hirano T, Abe K, Gotoh M & Oka K (1995) Citrus flavone tangeretin inhibits leukaemic HL-60 cell-growth partially through induction of apoptosis with less cytotoxicity on normal lymphocytes. *British Journal of Cancer* **72**, 1380–1388.
Hirano T, Gotoh M & Oka K (1994) Natural flavonoids and lignans are potent cytostatic agents against human leukemic HL-60 cells. *Life Sciences* **55**, 1061–1069.
Hirono I, Ueno I, Hosaka S, Takanashi H, Matsushima T, Sugimura T & Natori S (1981) Carcinogenicity examination of quercetin and rutin in aci rats. *Cancer Letters* **13**, 15–21.
Hirose M, Hoshiya T, Akagi K, Futakuchi M & Ito N (1994) Inhibition of mammary gland carcinogenesis by green tea catechins and other naturally occurring antioxidants in female Sprague–Dawley rats pretreated with 7,12-dimethylbenz[alpha]anthracene. *Cancer Letters* **83**, 149–156.
Hodgson JM, Proudfoot JM, Croft KD, Puddey IB, Mori TA & Beilin LJ (1999) Comparison of the effects of black and green tea on *in vitro* lipoprotein oxidation in human serum. *Journal of the Science of Food and Agriculture* **79**, 561–566.
Hollman PCH, de Vries JHM, van Leeuwen SD, Mengelers MJB & Katan MB (1995) Absorption of dietary quercetin glycosides and quercetin in healthy ileostomy volunteers. *American Journal of Clinical Nutrition* **62**, 1276–1282.
Hollman PCH & Katan MB (1997) Absorption, metabolism and health effects of dietary flavonoids in man. *Biomedicine and Pharmacotherapeutics* **51**, 305–310.
Hollman PCH & Katan MB (1998) Bioavailability and health effects of dietary flavonols in man. *Archives of Toxicology Supplement* **20**, 237–248.
Hollman PCH & Katan MB (1999) Dietary flavonoids: Intake, health effects and bioavailability. *Food and Chemical Toxicology* **37**, 937–942.
Hollman PCH, van Trijp JMP, Buysman MNCP, Gaag MSvd, Mengelers MJB, de Vries JHM & Katan MB (1997*a*) Relative bioavailability of the antioxidant flavonoid quercetin from various foods in man. *FEBS Letters* **418**, 152–156.
Hollman PCH, van Trijp JMP, Mengelers MJB, de Vries JHM & Katan MB (1997*b*) Bioavailability of the dietary antioxidant flavonol quercetin in man. *Cancer Letters* **114**, 139–140.
Holvoet P & Collen D (1998) Oxidation of low density lipoproteins in the pathogenesis of atherosclerosis. *Atherosclerosis* **137**, S33–S38.
Holvoet P, Perez G & Zhao Z (1995) Malonaldehyde-modified low density lipoproteins in patients with atherosclerotic disease. *Journal of Clinical Investigation* **95**, 2611–2619.
Ishikawa T, Suzukawa M, Ito T, Yoshida H, Ayaori M, Nishiwaki M, Yonemura A, Hara Y & Nakamura H (1997) Effect of tea flavonoid supplementation on the susceptibility of low density lipoprotein to oxidative modification. *American Journal of Clinical Nutrition* **66**, 261–266.
Ito M, Kawaguchi H, Sakota Y, Otonari J & Nitahara H (1993) Effects of polyphenols, including flavonoids, on glutathione-S-transferase and glutathione-reductase. *Bioscience, Biotechnology and Biochemistry* **57**, 1678–1680.
Jackson MJ (1997) The assessment of bioavailability of micronutrients: Introduction. *European Journal of Clinical Nutrition* **51**, 51–52.
Kanazawa K, Yamashita T, Ashida H & Danno G (1998) Antimutagenicity of flavones and flavonols to heterocyclic amines by specific and strong inhibition of the cytochrome p450 1a family. *Bioscience, Biotechnology and Biochemistry* **62**, 970–977.
Kandaswami C, Perkins E, Drzewiecki G, Soloniuk DS & Middleton E Jr (1992) Differential inhibition of proliferation of human squamous cell carcinoma, gliosarcoma and embryonic fibroblast-like lung cells in culture by plant flavonoids. *Anticancer Drugs* **3**, 525–530.
Kang TB & Liang NC (1997) Studies on the inhibitory effects of quercetin on the growth of HL-60 leukemia cells. *Biochemical Pharmacology* **54**, 1013–1018.
Kappus H, Koster-Albrecht D & Remmer H (1979) 2-Hydroxyoestradiol and (+)-cyanidanol-3 prevent lipid peroxidation of isolated rat hepatocytes. *Archives of Toxicology Supplement* **2**, 321–326.
Katdare M, Osborne MP & Telang NT (1998) Inhibition of aberrant proliferation and induction of apoptosis in pre-neoplastic human mammary epithelial cells by natural phytochemicals. *Oncology Reports* **5**, 311–315.
Kerry N & Abbey M (1999) The isoflavone genistein inhibits copper and peroxyl radical mediated low density lipoprotein oxidation *in vitro*. *Atherosclerosis* **140**, 341–347.

Kimira M, Arai Y, Shimoi K & Watanabe S (1998) Japanese intake of flavonoids and isoflavonoids from foods. *Journal of Epidemiology* **8**, 168–175.
King A & Young G (1999) Characteristics and occurrence of phenolic phytochemicals. *Journal of the American Dietetic Association* **99**, 213–218.
Kirk EA, Sutherland P, Wang SA, Chait A & LeBoeuf RC (1998) Dietary isoflavones reduce plasma cholesterol and atherosclerosis in C57BL/6 mice but not LDL receptor-deficient mice. *Journal of Nutrition* **128**, 954–959.
Klaunig JE, Xu Y, Han C, Kamendulis LM, Chen JS, Heiser C, Gordon MS & Mohler ER (1999) The effect of tea consumption on oxidative stress in smokers and non-smokers. *Proceedings of the Society of Experimental Biology & Medicine* **220**, 249–254.
Knekt P, Jarvinen R, Seppanen R, Hellovaara M, Teppo L, Pukkala E & Aromaa A (1997) Dietary flavonoids and the risk of lung cancer and other malignant neoplasms. *American Journal of Epidemiology* **146**, 223–230.
Knekt P, Reunanen A & Maatela J (1996) Flavonoid intake and coronary mortality in Finland: a cohort study. *British Medical Journal* **312**, 478–481.
Koga T, Moro K, Nakamori K, Yamakoshi J, Hosoyama H, Kataoka S & Ariga T (1999) Increase in antioxidative potential of rat plasma by oral administration of proanthocyanidin-rich extract from grape seeds. *Journal of Agricultural and Food Chemistry* **47**, 1892–1897.
Krinsky NI (1992) Mechanism of action of biological antioxidants. *Proceedings of the Society of Experimental Biology and Medicine* **200**, 248–254.
Kritz H & Sinzinger H (1997) Tea consumption, lipid metabolism and atherosclerosis. *Weiner Klinische Wochenschrift* **109**, 944–988.
Kuhnau J (1976) The flavonoids. A class of semi-essential food components: Their role in human nutrition. *World Review of Nutrition & Dietetics* **24**, 1117–1191.
Kuo SM (1996) Antiproliferative potency of structurally distinct dietary flavonoids on human colon cancer cells. *Cancer Letters* **110**, 41–48.
Kuo SM, Morehouse HF & Lin CP (1997) Effect of antiproliferative flavonoids on ascorbic acid accumulation in human colon adenocarcinoma cells. *Cancer Letters* **116**, 131–137.
Lagarrigue S, Chaumontet C, Heberden C, Martel P & GaillardSanchez I (1995) Suppression of oncogene-induced transformation by quercetin and retinoic acid in rat liver epithelial cells. *Cellular & Molecular Biology Research* **41**, 551–560.
Lairon D & Amiot MJ (1999) Flavonoids in food and natural antioxidants in wine. *Current Opinions in Lipidology* **10**, 23–28.
Laranjinha JAN, Almeida LM & Madeira VMC (1994) Reactivity of dietary phenolic acids and peroxyl radicals: antioxidant activity upon low density lipoprotein peroxidation. *Biochemical Pharmacology* **48**, 487–494.
Leanderson P, Faresjo AO & Tagesson C (1997) Green tea polyphenols inhibit oxidant-induced DNA strand breakage in cultured lung cells. *Free Radical Biology and Medicine* **23**, 235–242.
Lin JK, Chen YC, Huang YT & Lin-Shiau SY (1997) Suppression of protein kinase C and nuclear oncogene expression as possible molecular mechanisms of cancer chemoprevention by apigenin and curcumin. *Journal of Cellular Biochemistry Supplements* **28/29**, 39–48.
Linseisen J, Radtke J & Wolfram G (1997) Flavonoid intake of adults in a Bavarian subgroup of the national food consumption survey. *Zeitschrift für Ernahrungswissenschaft* **36**, 403–412.
Lu HQ, Niggemann B & Zanker KS (1996) Suppression of the proliferation and migration of oncogenic ras-dependent cell lines, cultured in a three-dimensional collagen matrix, by flavonoid-structured molecules. *Journal of Cancer Research and Clinical Oncology* **122**, 335–342.
McAnlis GT, McEneny J, Pearce J & Young IS (1999) Absorption and antioxidant effects of quercetin from onions, in man. *European Journal of Clinical Nutrition* **53**, 92–96.
McCarty MF (1998) Polyphenol-mediated inhibition of ap-1 transactivating activity may slow cancer growth by impeding angiogenesis and tumor invasiveness. *Medical Hypotheses* **50**, 511–514.
McDonald MS, Hughes M, Burns J, Lean MEJ, Matthews D & Crozier A (1998) Survey of the free and conjugated myricetin and quercetin content of red wines of different geographical origins. *Journal of Agricultural and Food Chemistry* **46**, 368–375.
Majima T, Tsutsumi M, Nishino H, Tsunoda T & Konishi Y (1998) Inhibitory effects of beta-carotene, palm carotene, and green tea polyphenols on pancreatic carcinogenesis initiated by N-nitrosobis(2-oxopropyl)amine in Syrian golden hamsters. *Pancreas* **16**, 13–18.
Malaveille C, Hautefeuille A, Pignatelli B, Talaska G, Vineis P & Bartsch H (1998) Antimutagenic dietary phenolics as antigenotoxic substances in urothelium of smokers. *Mutation Research—Fundamental and Molecular Mechanisms of Mutagenesis* **402**, 219–224.
Manach C, Morand C, Texier O, Favier ML, Agullo G, Demigné C, Régérat F & Rémésy C (1995) Quercetin metabolites in plasma of rats fed diets containing rutin or quercetin. *Journal of Nutrition* **125**, 1911–1922.
Manach C, Texier O, Régérat F, Agullo G, Demigné C & Rémésy C (1996) Dietary quercetin is recovered in rat plasma as conjugated derivatives of isorhamnetin and quercetin. *Nutritional Biochemistry* **7**, 375–380.
Maridonneau I, Braquet P & Garay RP (1986) Heterogeneous effect of flavonoids on K^+ loss and lipid peroxidation induced by oxygen-free radicals in human red cells. *Pharmacological Research Communications* **18**, 61–72.
Martin-Aragon S, Benedi JM & Villar AM (1997) Modifications of antioxidant capacity and liquid peroxidation in mice under fraxetin treatment. *Journal of Pharmacy and Pharmacology* **49**, 49–52.

Mehta K, Pantazis P, McQueen T & Aggarwal BB (1997) Antiproliferative effect of curcumin (diferuloylmethane) against human breast tumor cell lines. *Anticancer Drugs* **8**, 470–481.
Melzig MF, Loose R & Schonherr G (1997) Effect of flavonoids on daunomycin-induced toxicity in cultivated endothelial cells. *Pharmazie* **52**, 793–796.
Miura YH, Tomita I, Watanabe T, Hirayama T & Fukui S (1998) Active oxygen generations by flavonoids. *Biological & Pharmacological Bulletin* **21**, 93–96.
Miura S, Watanabe J, Sano M, Tomita T, Osawa T, Hara T & Tomita I (1995) Effects of various natural antioxidants on the Cu (2 +)-mediated oxidative modification of low density lipoprotein. *Biological & Pharmacological Bulletin* **18**, 1–4.
Miura S, Watanabe J, Tomita T, Sano M & Tomita I (1994) The inhibitory effects of tea polyphenols (flavan-3-ol derivatives) on Cu^{2+} mediated oxidative modification of low density lipoprotein. *Biological & Pharmacological Bulletin* **17**, 1567–1572.
Miyake Y, Yamamoto K, Tsujhara N & Osawa T (1998) Protective effects of lemon flavonoids on oxidative stress in diabetic rats. *Lipids* **33**, 689–695.
Miyazawa T, Nakagawa K, Kudo M, Muraishi K & Someya K (1999) Direct intestinal absorption of red fruit anthocyanins, cyanidin-3-glucoside and cyanidin-3,5-diglucoside, into rats and humans. *Journal of Agricultural & Food Chemistry* **47**, 1083–1092.
Morel I, Lescoat G, Cogrel P, Sergent O, Pasdeloup N, Brissot P, Cillard P & Cillard J (1993) Antioxidant and iron-chelating activities of the flavonoids catechin, quercetin and diosmetin on iron-loaded rat hepatocyte cultures. *Biochemical Pharmacology* **45**, 13–19.
Morino K, Matsukura N, Kawachi T, Ohgaki H, Sugimura T & Hirono I (1982) Carcinogenicity test of quercetin and rutin in golden-hamsters by oral-administration. *Carcinogenesis* **3**, 93–97.
Morrice PC, Wood SG & Duthie GG (2000) High performance liquid chromatographic determination of quercetin and isorhamnetin in rat tissues using beta-glucuronidase and acid hydrolysis. *Journal of Chromatography B* **738**, 413–417.
Murray CW, Booth AN, de Eds F & Jones FT (1954) Absorption and metabolism of rutin and quercetin in the rabbit. *Journal of the American Pharmacological Association* **43**, 361–364.
Musonda CA, Helsby N & Chipman JK (1997) Effects of quercetin on drug metabolizing enzymes and oxidation of 2′,7-dichlorofluorescein in hepg2 cells. *Human & Experimental Toxicology* **16**, 700–708.
Nakagawa K, Ninomiya M, Okubo T, Aoi N, Juneja LR, Kim M, Yamanaka K & Miyazawa T (1999) Tea catechin supplementation increases antioxidant capacity and prevents phospholipid hydroperoxidation in plasma of humans. *Journal of Agricultural and Food Chemistry* **47**, 39967–39973.
Nakagawa Y, Shetlar ME & Wender SH (1965) Urinary products from quercetin in neomycin-treated rats. *Biochimica et Biophysica Acta* **97**, 233–241.
Nakayama T, Yamada M, Osawa T & Kawakishi S (1993) Suppression of active oxygen-induced cytotoxicity by flavonoids. *Biochemical Pharmacology* **45**, 265–267.
Negre Salvayre A, Mabile L, Delchambre J & Salvayre R (1995) α-Tocopherol, ascorbic acid, and rutin inhibit synergistically the copper-promoted LDL oxidation and the cytotoxicity of oxidized LDL to cultured endothelial cells. *Biological Trace Element Research* **47**, 81–91.
Negre Salvayre A & Salvayre R (1992) Quercetin prevents the cytotoxicity of oxidized LDL on lymphoid cell lines. *Free Radical Biology and Medicine* **12**, 101–106.
Ngomuo AJ & Jones RS (1996) Genotoxicity studies of quercetin and shikimate in vivo in the bone marrow of mice and gastric mucosal cells of rats. *Veterinary and Human Toxicology* **38**, 176–180.
Nielsen SE, Young JF, Daneshvar B, Lauridsen ST, Knuthsen P, Sandstrom B & Dragsted LO (1999) Effect of parsley (*Petroselinum crispum*) intake on urinary apigenin excretion, blood antioxidant enzymes and biomarkers for oxidative stress in human subjects. *British Journal of Nutrition* **81**, 447–455.
Nigdikar SV, Williams NR, Griffin BA & Howard AN (1998) Consumption of red wine polyphenols reduces the susceptibility of low density lipoproteins to oxidation *in vivo*. *American Journal of Clinical Nutrition* **68**, 258–265.
Noroozi M, Angerson WJ & Lean MEJ (1998) Effects of flavonoids and vitamin C on oxidative DNA damage to human lymphocytes. *American Journal of Clinical Nutrition* **67**, 1210–1218.
Noteborn HPJM, Jansen E, Benito S & Mengelers MJB (1997) Oral absorption and metabolism of quercetin and sugar-conjugated derivatives in specific transport systems. *Cancer Letters* **114**, 175–177.
Obermeier MT, White RE & Yang CS (1995) Effects of bioflavonoids on hepatic P450 activities. *Xenobiotica* **25**, 575–584.
Okushio K, Suzuki M, Matsumoto N, Nanjo F & Hara Y (1999) Identification of (−)-epicatechin metabolites and their metabolic fate in the rat. *Drug Metabolism & Disposition* **27**, 309–316.
Omenn GS, Goodman GE & Thornquist MD (1996) Effects of a combination of beta-carotene and vitamin A on lung cancer and cardiovascular disease. *New England Journal of Medicine* **334**, 1150–1155.
Parshad R, Sanford KK, Price FM, Steele VE, Tarone RE, Kelloff GJ & Boone CW (1998) Protective action of plant polyphenols on radiation-induced chromatid breaks in cultured human cells. *Anticancer Research* **18**, 3263–3266.
Peterson J & Dwyer J (1998) Flavonoids: dietary occurrence and biochemical activity. *Nutrition Research* **18**, 1995–2018.
Plaumann B, Fritsche M, Rimpler H, Brandner G & Hess RD (1996) Flavonoids activate wild-type p53. *Oncogene* **13**, 1605–1614.

Pratico D, Iuliano L & Mauriello A (1997) Localization of distinct F2-isoprostanes in human atherosclerotic lesions. *Journal of Clinical Investigation* **100**, 2028–2034.
Princen HMG, van Duyvenvoorde W, Buytenhek R, Blonk C, Tijburg LBM, Langius JAE, Meinders E & Pijl H (1998) No effect of consumption of green and black tea on plasma lipid and antioxidant levels and on LDL oxidation in smokers. *Arteriosclerosis, Thrombosis & Vascular Biology* **18**, 833–841.
Puddey IB & Croft KD (1999) Alcohol, stroke and coronary heart disease. Are there anti-oxidants and pro-oxidants in alcoholic beverages that might influence the development of atherosclerotic cardiovascular disease. *Neuroepidemiology* **18**, 292–302.
Raeissi SD, Guo ZY, Dobson GL, Artursson P & Hidalgo IJ (1997) Comparison of cyp3a activities in a subclone of caco-2 cells (tc7) and human intestine. *Pharmaceutical Research* **14**, 1019–1025.
Rahman A, Fazal F, Greensill J, Ainley K, Parish JH & Hadi SM (1992) Strand scission in DNA induced by dietary flavonoids: role of Cu(I) and oxygen free radicals and biological consequences of scission. *Molecular and Cellular Biochemistry* **111**, 3–9.
Rapola J, Virtamo J & Ripatti S (1997) Randomized trial of alpha-tocopherol and beta-carotene supplements on incidence of major coronary events in men with previous myocardial infarction. *Lancet* **349**, 1715–1720.
Renaud S & de Longeril M (1992) Wine alcohol, platelets and the French paradox for coronary heart disease. *Lancet* **339**, 1523–1526.
Ricchi P, Pignata S, DiPopolo A, Memoli A, Apicella A, Zarrilli R & Acquaviva AM (1997) Effect of aspirin on cell proliferation and differentiation of colon adenocarcinoma caco2 cells. *International Journal of Cancer* **73**, 880–884.
RiceEvans CA, Miller NJ & Paganga G (1997) Antioxidant properties of phenolic compounds. *Trends in Plant Science* **2**, 152–159.
Rifici VA, Stephen EM, Schneider SH & Khachadurian AK (1999) Red wine inhibits the cell-mediated oxidation of LDL and HDL. *Journal of the American College of Nutrition* **18**, 137–143.
Rimm EB, Katan MB, Ascherio A, Stampfer MJ & Willett WC (1996) Relation between intake of flavonoids and risk for coronary heart disease in male health professionals. *Annals of Internal Medicine* **125**, 384–389.
Robards K & Antolovich M (1997) Analytical chemistry of fruit bioflavonoids. *Analyst* **122**, R11–R34.
Roig P, Cascon E, Arola L, Blade C & Salvado MJ (1999) Moderate red wine consumption protects the rat against oxidation *in vivo*. *Life Sciences* **6**, 1517–1524.
Sahu SC & Gray GC (1993) Interactions of flavonoids, trace metals and oxygen: nuclear DNA damage and lipid peroxidation induced by myricetin. *Cancer Letters* **70**, 73–79.
Sahu SC & Gray GC (1994) Kaempferol-induced nuclear DNA damage and lipid peroxidation. *Cancer Letters* **85**, 159–164.
Sahu SC & Washington MC (1991*a*) Effects of antioxidants on quercetin-induced nuclear DNA damage and lipid peroxidation. *Cancer Letters* **60**, 259–264.
Sahu SC & Washington MC (1991*b*) Quercetin-induced lipid peroxidation and DNA damage in isolated rat-liver nuclei. *Cancer Letters* **58**, 75–79.
Saito D, Shirai A, Matsushima T, Sugimura T & Hirono I (1980) Test of carcinogenicity of quercetin, a widely distributed mutagen in food. *Teratogenesis, Carcinogenesis and Mutagenesis* **1**, 213–221.
Scambia G, Panici PB, Ranelletti FO, Ferrandina G, Devincenzo R, Piantelli M, Masciullo V, Bonanno G, Isola G & Mancuso S (1994*a*) Quercetin enhances transforming growth-factor beta(1) secretion by human ovarian-cancer cells. *International Journal of Cancer* **57**, 211–215.
Scambia G, Ranelletti FO, Panici PB, Devincenzo R, Bonanno G, Ferrandina G, Piantelli M, Bussa S, Rumi C, Cianfriglia M & Mancuso S (1994*b*) Quercetin potentiates the effect of adriamycin in a multidrug-resistant mcf-7 human breast-cancer cell-line—p-glycoprotein as a possible target. *Cancer Chemotherapy and Pharmacology* **34**, 459–464.
Schramm DD, Collins HE & German JB (1999) Flavonoid transport by mammalian endothelial cells. *Journal of Nutritional Biochemistry* **10**, 193–197.
Serafini M, Ghiselli A & Ferro-Luzzi A (1996) *In vivo* antioxidant effect of green and black tea in man. *European Journal of Clinical Nutrition* **50**, 28–32.
Serafini M, Maiani G & Ferro-Luzzi A (1998) Alcohol-free red wine enhances plasma antioxidant capacity in humans. *Journal of Nutrition* **128**, 1003–1007.
Sestili P, Guidarelli A, Dachá M, & Cantoni O (1998) Quercetin prevents DNA single strand breakage and cytotoxicity caused by *tert*-butylhydroperoxide: free radical scvenging versus iron chelating mechanism. *Free Radical Biology Medicine* **25**, 196–200.
Shimoi K, Okada H, Furugori M, Goda T, Suzuki M, Hara Y, Yamamoto H & Kinae N (1998) Intestinal absorption of luteolin and luteolin 7-O-beta-glucoside in rats and humans. *FEBS Letters* **438**, 220–224.
Siess MH, Mas JP, Canivenc-Lavier MC & Suschetet M (1996) Time course of induction of rat hepatic drug-metabolizing enzyme activities following dietary administration of flavonoids. *Journal of Toxicology and Environmental Health* **49**, 481–496.
Skaper SD, Fabris M, Ferrari V, Carbonare MD & Leon A (1997) Quercetin protects cutaneous tissue-associated cell types including sensory neurons from oxidative stress induced by glutathione depletion: Cooperative effects of ascorbic acid. *Free Radical Biology and Medicine* **22**, 669–678.
Slater TF (1984) Free-radical mechanisms in tissue injury. *Biochemical Journal* **222**, 1–15.

So FV, Guthrie N, Chambers AF, Moussa M & Carroll KK (1996) Inhibition of human breast cancer cell proliferation and delay of mammary tumorigenesis by flavonoids and citrus juices. *Nutrition and Cancer* **26**, 167–181.
Spencer JPE, Chowrimoto G, Choudry R, Debham ES, Srai SK & RiceEvans CA (1999) The small intestine can both absorb and glucuronidate luminal flavonoids. *FEBS Letters* **458**, 224–230.
Stagg GV & Millin DJ (1975) The nutritional and therapeutic value of tea—a review. *Journal of the Science of Food and Agriculture* **26**, 1439–1459.
Steinberg D (1997) Low density lipoprotein and its pathological significance. *Journal of Biological Chemistry* **272**, 20963–20966.
St Leger AS, Cochrane AL & Moore F (1979) Factors associated with cardiac mortality in developed countries with particular reference to the consumption of wine. *Lancet* **12**, 1017–1020.
Suganuma M, Okabe S, Oniyama M, Tada Y, Ito H & Fujika H (1998) Wide distribution of [^{3}H](−)-epigallocatechin gallate, a cancer preventative tea polyphenol in mouse tissue. *Carcinogenesis* **19**, 1771–1776.
Tamura M, Kagawa S, Tsuruo Y, Ishimura K & Morita K (1994) Effects of flavonoid compounds on the activity of NADPH diaphorase prepared from the mouse-brain. *Japanese Journal of Pharmacology* **65**, 372–373.
Tijburg LBM, Wiseman SA, Meijer GW & Weststrate JA (1997) Effects of green tea, black tea and dietary lipophilic antioxidants on LDL oxidizability and atherosclerosis in hypercholesterolaemic rabbits. *Atherosclerosis* **135**, 37–47.
Tsuda T, Horio F & Osawa T (1999) Absorption and metabolism of cyan-O-beta-D-glucoside in rats. *FEBS Letters* **449**, 179–182.
Uda Y, Price KR, Williamson J & Rhodes MJC (1997) Induction of the anticarcinogenic marker enzyme quinone reductase in murine hepatoma cells in vitro by flavonoids. *Cancer Letters* **120**, 213–216.
Uddin S & Choudhry MA (1995) Quercetin, a bioflavonoid, inhibits the DNA synthesis of human leukemia cells. *Biochemistry amd Molecular Biology International* **36**, 545–550.
Ursini F, Tubaro F, Rong J & Sevanian A (1999) Optimization of nutrition: Polyphenols and vascular protection. *Nutrition Reviews* **57**, 241–249.
van het Hof KH, de Boer HS, Wiseman SA, Lien N, Weststrate JA & Tijburg LB (1997) Consumption of green or black tea does not increase resistance of low-density lipoprotein to oxidation in humans. *American Journal of Clinical Nutrition* **66**, 1125–1132.
van het Hof KH, Wiseman SA, Yang CS & Tijburg LBM (1999) Plasma and lipoprotein levels of tea catechins following repeated tea consumption. *Proceedings of the Society for Experimental Biology and Medicine* **220**, 203–209.
Venturi M, Hambly RJ, Glinghammar B, Rafter JJ & Rowland IR (1997) Genotoxic activity in human faecal water and the role of bile acids: a study using the alkaline comet assay. *Carcinogenesis* **18**, 2353–2359.
Verma AK, Johnson JA, Gould MN & Tanner MA (1988) Inhibition of 7,12-dimethylbenz(a)anthracene-and N-nitrosomethylurea-induced rat mammary cancer by dietary flavonol quercetin. *Cancer Research* **48**, 5754–5758.
Vertommen J, Vandenenden M, Simoens L & Deleeuw I (1994) Flavonoid treatment reduces glycation and lipid peroxidation in experimental diabetic rats. *Phytotherapy Research* **8**, 430–432.
Viana M, Barbas C, Bonet B, Bonet MV, Castro M, Fraile MV & Herrera E (1996) *In vitro* effects of a flavonoid-rich extract on LDL oxidation. *Atherosclerosis* **123**, 83–91.
Wang WQ & Goodman MT (1999) Antioxidant property of dietary phenolic agents in a human LDL-oxidation *ex vivo* model. *Nutrition Research* **19**, 191–202.
Waterhouse AL, German JB, Walzem RL, Hansen RJ & Kasim-Karakas SE (1998) Is it time for a wine trial? *American Journal of Clinical Nutrition* **68**, 220–221.
Watson DG & Oliveira EJ (1999) Solid-phase extraction and gas chromatography mass spectrometry determination of kaempferol and quercetin in human urine after consumption of *Ginkgo biloba* tablets. *Journal of Chromatography B* **723**, 203–210.
Westhuyzen J (1997) The oxidation hypothesis of atherosclerosis: an update. *Annals of Clinical & Laboratory Science* **27**, 1–10.
Whitehead TP, Robinson D, Allaway S, Syms J & Hale A (1995) Effect of red wine ingestion on the antioxidant capacity of serum. *Clinical Chemistry* **41**, 32–35.
Williamson G, Plumb GW, Uda Y, Price KR & Rhodes MJC (1996) Dietary quercetin glycosides: antioxidant activity and induction of the anticarcinogenic phase II marker enzyme quinone reductase in Hepalclc7 cells. *Carcinogenesis* **17**, 2385–2387.
Woodford FP & Whitehead TP (1998) Is measuring serum antioxidant capacity clinically useful? *Annals of Clinical Biochemistry* **35**, 48–56.
Yang CS, Yang GY, Landau JM, Kim S & Liao J (1998) Tea and tea polyphenols inhibit cell hyperproliferation, lung tumorigenesis, and tumor progression. *Experimental Lung Research* **24**, 629–639.
Yochum L, Kushi LH, Meyer K & Folsom AR (1999) Dietary flavonoid intake and risk of cardiovascular disease in postmenopausal women. *American Journal of Epidemiology* **149**, 943–949.
Yokozawa T & Dong E (1997) Influence of green tea and its three major components upon low-density lipoprotein oxidation. *Experimental Toxicology and Pathology* **49**, 329–335.
Yoshina K, Tomita I, Sano M, Oguni I, Hara Y & Nakano M (1994) Effects of long term dietary supplementation of tea polyphenols on lipid peroxide levels in rats. *Age* **17**, 79–85.
Young JF, Nielsen SE, Haraldsdottir J, Daneshvar B, Lauridsen ST, Knuthsen P, Crozier A, Sandstrøm B & Dragsted LO (1999) Effect of fruit juice intake on urinary excretion and biomarkers of antioxidative status. *American Journal of Clinical Nutrition* **69**, 87–94.

Zhai S, Dai RK, Friedman FK & Vestal RE (1998) Comparative inhibition of human cytochromes P450 1a1 and 1a2 by flavonoids. *Drug Metabolism and Disposition* **26**, 989–992.
Zhang K, Yang EB, Tang WY, Wong KP & Mack P (1997) Inhibition of glutathione reductase by plant polyphenols. *Biochemical Pharmacology* **54**, 1047–1053.
Zhu QY, Huang Y, Tsang D & Chen ZY (1999) Regeneration of alpha-tocopherol in human low-density lipoprotein by green tea catechin. *Journal of Agricultural and Food Chemistry* **47**, 2020–2025.