Optimal nutrition: fibre and phytochemicals

Ian Rowland
Northern Ireland Centre for Diet and Health, School of Biomedical Sciences, University of Ulster, Coleraine BT52 1SA, UK

There is currently intense research interest in secondary plant metabolites because of their potential preventative effects on the chronic diseases of Western societies, especially cardiovascular disease and cancer. To date most of the research has focused on the identification of plant-derived substances and their potential protective effects against specific chronic diseases. The important issue of determining the optimal intake of those substances, such that the beneficial effects are maximized without manifestation of adverse effects, has yet to be addressed in most cases. Furthermore, there are no specific functional markers that can be used to assess optimal intake, although it may be possible to use biomarkers such as serum cholesterol if the rest of the diet is strictly controlled. The present review discusses a wide range of substances associated with plants, including dietary fibre, resistant starch, oligosaccharides, phyto-oestrogens, phytosterols, flavonoids, terpenes and isothiocyanates, and attempts where possible to indicate optimal intakes and to suggest functional markers.

Phytochemicals: Secondary plant metabolites: Heart disease: Cancer

Plants and plant extracts have been used for centuries throughout the world in traditional cures and herbal remedies and as homeopathic medicines. More recently, there has been a resurgence of interest in secondary plant metabolites because of potential preventative effects on the chronic diseases of Western societies, especially cardiovascular disease and cancer. This interest has arisen because of the strength of the epidemiological evidence indicating a protective effect of vegetable and fruit intake against cardiovascular disease and cancer at most sites (Steinmetz & Potter, 1991). The isolation, identification and quantification of phytochemicals in foods and the evaluation of their potential benefits to human health has now become a major research topic.

Clearly such research has been stimulated by scientific curiosity in the substances and mechanisms involved in the protective effects of fruit and vegetables. In addition, however, such knowledge should enable us to maximize intake of the most bioactive components, for example by selecting and developing new strains of plants (using conventional breeding or genetic manipulation) that contain elevated levels of beneficial constituents. It is clear from *in vitro* and animal data that the actions of some phytochemicals (e.g. terpenes and organosulphides as anti-tumour agents, phytosterols as cholesterol-lowering agents) are likely to be achieved only at doses much higher than those that can be obtained from eating plants. Thus, extraction or synthesis of the active ingredient is essential if they are to be of prophylactic or therapeutic value in human subjects. Finally, knowledge of the biological activity of phytochemicals may allow supplementation of foods that naturally contain low amounts or none of the phytochemicals, but which are more acceptable to consumers, thus circumventing some of the problems of encouraging people to eat more fruit and vegetables. Careful consideration of possible adverse effects of this approach of supplementing the diet with specific phytochemicals is essential; the warning from the intervention studies with β-carotene should be heeded (Omenn *et al*. 1994; Rowe, 1996).

General considerations and problems associated with phytochemical research

Research into bioactive components of plants falls into two stages:

(1) to identify plant-derived substances and their potential protective effects against specific chronic diseases;
(2) to determine the optimal intake of those substances, such that the beneficial effects are maximized without manifestation of adverse effects.

Corresponding author: Professor Ian Rowland, fax +44 (0)1265 323023, email i.rowland@ulst.ac.uk

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For most phytochemicals we are in the first stage of the process. At present, it is difficult to make quantitative recommendations for specific phytochemicals in the same way that recommendations have been made over certain vitamins (Woteki, 1995). This is partly because of analytical complexities that hamper accurate estimates of levels of phytochemicals in foods, and also because of difficulties encountered in experimental studies, often in relation to metabolism. The following are some of these complicating factors:

(1) many phytochemicals (e.g. flavonoids, isoflavonoids and lignans) are present in plants as conjugated forms (usually as glycosides). After ingestion, the conjugates may be hydrolysed to aglycones. This process complicates not only the analysis of the compounds, but also the evaluation of their physiological effects. For example, there is currently controversy over whether glycosides can be absorbed intact, and the relative biological activities of glycosides and aglycones of many phytochemicals have not been evaluated;
(2) human metabolism of phytochemicals is complex; often both mammalian and gut microbial pathways are involved. For many compounds (e.g. isoflavonoids and lignans) there is enormous inter-individual variation in metabolism, which could impinge on biological activity (Bowey *et al*. 1998);
(3) analysis is complicated by the fact that there is a wide variety of phytochemicals even within the same overall group, and that metabolism may occur during crushing or processing of plants (e.g. for Allium and brassica compounds), thus increasing the complexity of the mixture. For many phytochemicals analysis requires mass spectroscopy and therefore is time-consuming and expensive. Furthermore, some compounds appear to be bound to macromolecules, making quantitative extraction difficult;
(4) many of the phytochemicals have a range of different biochemical and physiological effects, e.g. isoflavonoids have antioxidant and anti-oestrogenic activities. These activities may require different plasma or tissue concentrations for optimum effects. In addition, plants contain mixtures of phytochemicals (Table 1), so there is considerable opportunity for interaction;
(5) For most plant compounds, studies have been conducted with single, relatively high-exposure levels to establish whether or not beneficial effects occur. Few *in vivo* dose – response studies, which are vital for determination of optimal exposure levels, have been performed.

Activities of phytochemicals and potential functional markers

For a detailed description of the activities of the various phytochemicals, the reader is referred to recent reviews (for example, see Watzl & Leitzmann, 1995; American Institute for Cancer Research, 1996). A summary of the main groups of bioactive chemicals in plants, their sources and their biological activities is presented in Table 2.

An attempt has also been made to suggest possible functional markers that could be used to assess optimal intake values for phytochemicals (Table 3). At present, for most phytochemicals, there are no specific functional markers that can be used to assess optimal intake, unlike, for example, blood homocysteine for folic acid. Consequently, it is necessary to rely on non-specific biomarkers, usually related to their biological activity, e.g. lowering of serum cholesterol. Such biomarkers are often strongly influenced by other dietary factors, so that careful dietary control is essential when performing intervention studies. Further discussion of possible functional markers for some phytochemicals is given later (pp. 417–418), although it should be noted that the suggestions are speculative in many cases.

Non-startch polysaccharides

NSP have a wide range of effects, including changes in gut physiology, lipid metabolism and glucose uptake. NSP are usually divided into soluble and non-soluble forms, since these forms have distinct sites of action. The insoluble forms of fibre tend to influence events in the lower gut (e.g. faecal weight and transit time), whereas soluble fibres such as guar and pectins have their effects in the upper gut, influencing nutrient absorption. It is possible, therefore, to use changes in faecal weight as a crude measure of response to insoluble fibre sources such as wheat bran. It should be remembered, however, that the effects on events such as colo-rectal carcinogenesis might not be directly related to faecal bulking. Reduction in serum cholesterol may be used to assess optimal intakes of soluble fibres, under controlled dietary conditions to minimize confounding factors.

Table 1. Phytochemical content of some edible plants

Plants	Flavonoids	Isoflavonoids	Lignans	Organo-sulphides	Glucos-inolates	Phenolic acids	Oligo-saccharides	Terpenes	NSP
Soyabeans	✓	✓				✓	✓		✓
Cereal grains	✓		✓			✓			✓
Garlic and onions				✓		✓	✓	✓	
Cruciferae	✓			✓	✓	✓		✓	✓
Solanacae	✓					✓		✓	
Umbelliferae	✓					✓		✓	✓
Citrus fruits	✓					✓		✓	✓
Flaxseed	✓		✓			✓			✓

✓, Present.

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Table 2. Non-nutrient phytochemicals, their sources and biological activities

Group	Examples	Main food sources	Activity and functional marker
Fibre and related compounds	NSP		
	Soluble (e.g. pectins, gums)	Fruit (apples, citrus), oats, soyabean, algae	Lowers serum cholesterol
	Insoluble (celluloses)	Cereals (wheat, rye), vegetables	Prevention of colon and breast cancer, diverticular disease. Alleviates constipation
	Resistant starch, retrograded starch	High-amylose starches, processed starches, whole grains and seeds	Increased butyrate in faeces. Prevention of colon cancer
	Phytate	Cereals, grains, soyabeans	Binds minerals. Colon cancer prevention?
	Oligosaccharides	Chicory, soyabeans, artichokes, onion	Modify gut flora, modulate lipid metabolism? Cancer prevention?
Flavonoids	Flavonols: quercetin, kaempferol	Vegetables (onion, lettuce, tomatoes, peppers) wine, tea	Antioxidants, modulate phase 1 enzymes, inhibit protein kinase C. Cancer prevention? CVD protection? Immune modulation?
	Flavanones: tangeritin, naringenin, hesperitin	Citrus fruits	
	Flavanols: catechins, epicatechins	Green tea	
Tea polyphenols	Catechins, epicatechins	Green tea	Antioxidants. CHD prevention?
Derived tannins	Theaflavins, thearubigens	Black tea, red wine, roasted coffe	
Isoflavonoids	Daidzein, genistein	Soyabean products	Anti-oestrogenic effects, effects on serum lipids, prevention of breast and prostate cancers
Lignans	Secoisolariciresinol, matairesinol	Rye bran, flaxseed, berries, nuts	Antioxidant and anti-oestrogenic effects. Prevention of colon an prostate cancer?
Glucosinolates	Glucobrassicin, indole-3-carbinol	Cruciferous vegetables (broccoli, cabbage, Brussel sprouts, water-cress, mustard)	Induction of phase 2 enzymes. Cancer prevention?
Isothiocyanates	Allylisothiocyanates, indoles, sulforaphane		
Simple phenols	*p*-Cresol, ethyl phenol, hydroquinone	Raspberry, cocoa beans	
Phenolic acids, condensed phenols	Gallic acid, tannins, ellagic acid	Green tea, black tea, strawberries	Antioxidants
Monoterpenes	D-Limonene, D-carvone, perillyl alcohol	Citrus fruits, cherries, herbs	Induce phase 1 and phase 2 enzymes. Anti-tumour activity
Hydroxycinnamic acids	Caffeic, ferulic, chlorogenic acids, curcumin	Apples, pears, coffee, mustard, curry	Inhibit nitrosation by trapping nitrite, nucleophiles, antioxidants
Phytosterols	β-Sitosterol, campesterol, stigmasterol	Vegetable oils (soyabean, rape seed, maize, sunflower)	Lower serum cholesterol
Organosulphides (allium compounds)	Diallyl sulphide, allyl methyl sulphide, S-allylcysteine	Garlic, onions, leeks	Phase 2 enzyme induction, effects on serum lipids and platelet aggregation. Cancer prevention

CVD, cardiovascular disease.

Table 3. Potential functional markers for phytochemicals

Phytochemical	Functional marker
NSP: Insoluble	Faecal bulking
Soluble; phytosterols	Cholesterol lowering
Resistant starch	SCFA profile (*n*-butyrate)
Non-digestible oligo-saccharides	Faecal bifidobacteria
Phyto-oestrogens	Plasma SHBG, LDL oxidation
Terpene	Ras processing, phase 2 enzymes
Allium compounds and isothiocyanates	Phase 2 enzymes in blood and tissues

SCFA, short-chain fatty acids; SHBG, sex hormone-binding proteins.

Resistant starch

There is currently considerable interest in resistant starches (i.e. those that are poorly digested by amylase (*EC* 3.2.1.1) in the small intestine, and so reach the colon where they are fermented by gut bacteria). Possible protective effects against colon cancer are currently under investigation. A possible functional marker for studying optimal resistant starch intake is the proportion of *n*-butyrate in faeces. Starches are well fermented by the gut microflora and tend to favour production of butyrate, in contrast to many NSP. The *in vitro* activities of *n*-butyrate (e.g. inhibition of growth and stimulation of differentiation of cancer cell lines) suggest that it may play a role in colon cancer prevention.

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Non-digestible oligosaccharides

Non-digestible oligosaccharides are low-molecular-weight carbohydrates (usually with a degree of polymerization between two and ten) that are poorly absorbed from the small intestine but fermented by the colonic microflora. There is preliminary evidence that they may have beneficial effects on colon cancer, blood lipids and Ca absorption. A common feature of oligosaccharides is that they increase the proportion of bifidobacteria in the faeces as a consequence of their fermentation in the colon (Gibson *et al*. 1995). This feature could be used as a functional marker for non-digestible oligosaccharides, particularly as it appears to be dose-dependent, with no effects being seen for a fructo-oligosaccharide at intakes of 5 g/d, but increases in bifidobacteria being apparent at 10 g/d and above (Gibson *et al*. 1995). The current developments in molecular methods for analysis of gut microflora could also simplify the determination of bifidobacteria in faeces.

Phyto-oestrogens

Isoflavonoids found in soyabean products and lignans present in a wide variety of cereal brans, berries and in flaxseed (Thompson *et al*. 1991; Reinli & Block, 1996) have a range of biological activities including antioxidant and anti-oestrogenic effects (Markiewicz *et al*. 1993; Zava & Duwe, 1997; Arora *et al*. 1998; Milligan *et al*. 1998). *In vitro*, animal and epidemiological studies suggest that they have beneficial effects on cancer (particularly hormone-dependent cancers; Adlercreutz, 1995; Sathyamoorthy & Wang, 1997). It has been proposed that isoflavonoids exert these effects by stimulating sex hormone-binding protein, since sex hormone-binding protein lowers the level of circulating free hormones in the blood (Adlercreutz, 1995). Intervention studies with soyabean products, however, provide little support for this theory (Bingham *et al*. 1998). Thus, sex hormone-binding protein level, although a potentially-useful functional marker for phyto-oestrogen intake, needs further evaluation in relation to other dietary factors that could interfere. We have recently demonstrated that isoflavonoids, at realistic daily intakes, can affect LDL oxidation in human volunteers (Reilly *et al*. 1999), so it may also be feasible to use such a measurement as a non-specific functional marker for optimal intake of isoflavonoids.

Monoterpenes

Monoterpenes are simple C_{10} isoprenoid compounds found in the essential oils of citrus fruits, cherries and herbs such as dill (*Anethum graveolens*), mint (*Mentha* spp.) and caraway (*Carum carvi*). The major terpenes found are D-limonene, carvone and perrillyl alcohol, and they exhibit chemotherapeutic activity against tumours of the pancreas, prostate and breast (Cromwell *et al*. 1996). *In vitro* studies (Hohl, 1996) indicate that one of the mechanisms of action of the monoterpenes is inhibition of post-translational farnesylation of the Ras oncoprotein, a process that is crucial to its functioning. Although speculative at present, the development of methods for assessing protein farnesylation *in vivo* would provide a valuable functional marker for assessing optimal intakes of these potential powerful anti-tumourigenic compounds.

Isothiocyanates

Isothiocyanates occur as glucosinolate conjugates in cruciferous vegetables. The glucosinolates are hydrolyzed by the action of myrosinase (*EC* 3.2.3.1), which is released when the plants are cut or processed. Isothiocyanates prevent tumours at numerous sites in rodent studies (Hecht, 1995). The mechanism appears to be via modulation of enzymes involved in activation and detoxification of carcinogens, in particular inhibition of cytochrome P450 enzymes and induction of GSH transferases. Measurement of changes in the activity of these phase 1 and phase 2 pathways of carcinogen metabolism is feasible in human intervention studies and could be used as a means of assessing optimal intakes of isothiocyanates, and indeed other phytochemicals with similar modulating activities. As with other functional markers, careful control is needed in the human studies because of the non-specific nature of the end point, and there is a further complication in the form of extensive genetic polymorphisms in these enzyme systems.

Phytosterols

Vegetable oils contain a wide range of plant sterols such as β-sitosterol, camposterol and stigmasterol with structural similarities to cholesterol. They have been shown to reduce plasma total cholesterol and LDL-cholesterol in human dietary intervention studies (Miettinen *et al*. 1995). The amounts required appear to be at least 2 g/d, i.e. about ten times the normal intake in the UK from vegetable oils. Measurement of plasma cholesterol-lowering effect, within a suitable controlled diet, would provide a suitable method for evaluating optimal intake of the phytosterols and for comparing the various esterified and free forms of the compounds currently being used, or proposed for use, in phytosterol-supplemented margarines (Miettinen *et al*. 1995).

Conclusions

Development of simple functional biomarkers for assessing optimal levels of intake of phytochemicals is of crucial importance because of the structural diversity and complexity of the phytochemical mixtures found in plants. There are, however, a number of potential methods, which have been suggested in the present review. In many cases the feasibility of their use in human intervention studies and their validation will be an important area of research for the future development of phytoprotectants.

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References

Adlercreutz H (1995) Phytoestrogens: Epidemiology and possible role in cancer protection. *Environmental Health Perspectives* **103**, Suppl. 7, 103–112.

American Institute for Cancer Research (editors) (1998) *Dietary Phytochemicals in Cancer Prevention and Treatment. Advances in Experimental Biology and Medicine*, vol. 401. New York: Plenum Press.

Arora A, Nair MG & Strasburg GM (1998) Antioxidant activities of isoflavones and their biological metabolites in a liposomal system. *Archives of Biochemistry and Biophysics* **356**, 133–141.

Bingham SA, Atkinson C, Liggins J, Bluck L & Coward A (1998) Phyto-oestrogens: where are we now? *British Journal of Nutrition* **79**, 393–406.

Bowey EA, Rowland IR, Adlercreutz H, Sanders TAB & Wiseman H (1998) Interindividual variation in soya isoflavone metabolism: the role of habitual diet. *Proceedings of the Nutrition Society* **58**, 000A.

Cromwell PL, Siar Ayoubi A & Burke YD (199) Antitumorigenic effects of limonene and perillyl alcohol against pancreatic and breast cancer. In *Dietary Phytochemicals in Cancer Prevention and Treatment. Advances in Experimental Biology and Medicine*, vol. 401, pp. 131–136 [American Institute for Cancer Research, editors]. New York: Plenum Press.

Gibson GR, Beatty ER, Wang X & Cummings JH (1995) Selective stimulation of Bifidobacteria in the human colon by oligofructose and inulin. *Gastroenterology* **108**, 975–982.

Hecht SS (1995) Chemoprevention by isothiocyanates. *Journal of Cellular Biochemistry* **22**, Suppl., 195–209.

Hohl RJ (1996) Monoterpenes as regulators of malignant cell proliferation. In *Dietary Phytochemicals in Cancer Prevention and Treatment. Advances in Experimental Biology and Medicine*, vol. 401, pp. 137–146 [American Institute for Cancer Research, editors]. New York: Plenum Press.

Markiewicz L, Garey J, Aldercreutz H & Gurpide E (1993) In vitro bioassays of non-steroidal phytoestrogens. *Journal of Steroid Biochemistry* **45**, 399–405.

Miettinen TA, Puska P, Gylling H, Vanhanen H & Vartiainen E (1995) Reduction in serum cholesterol with sitostanol-ester margarine in a mildly hypercholesterolemic population. *New England Journal of Medicine* **16**, 1308–1312.

Milligan SR, Balasubramanian AV & Kalita JC (1998) Relative potency of xenobiotic estrogens in an acute *in vivo* mammalian assay. *Environmental Health Perspective* **106**, 23–26.

Omenn GS, Goodman G, Thornquist M, Grizzle J, Rosenstock L, Barnhart S, Balmes J, Cherniack MG, Cullen MR, Glass A, Keogh J, Meyskens F, Valanis B & Williams J (1994) The beta carotene and retinol efficacy trial (CARET) for chemoprevention of lung cancer in high risk populations: smokers and asbestos-exposed workers. *Cancer Research* **54**, 2038s–2043s.

Reilly J, Dean TS, Rowland I, Sanders TAB & Wiseman H (1999) The influence of dietary isoflavones on markers of lipid peroxidation in healthy male and female volunteers. *Proceedings of the Nutrition Society* **58** (In the Press).

Reinli K & Block G (1996) Phytoestrogen content of foods – a compendium of literature values. *Nutrition and Cancer* **26**, 123–148.

Rowe PM (1996) Beta carotene takes a collective beating. *Lancet* **347**, 249.

Sathyamoorthy N & Wang TTY (1997) Differential effects of dietary phyto-oestrogens daidzein and equol on human breast cancer MCF-7 cells. *European Journal of Cancer* **33**, 2384–2389.

Steinmetz KA & Potter JD (1991) Vegetables, fruit and cancer. *Epidemiology Cancer Causes and Control* **2**, 325–357.

Thompson LU, Robb P, Serraino M & Cheung F (1991) Mammalian lignan production from various foods. *Nutrition and Cancer* **16**, 43–52.

Watzl B & Leitzman C (1995) *Bioaktive Substanzen in Lebenmitteln (Bioactive Substances in Foods)*. Stuttgart, Germany: Hippokrates Verlag.

Woteki CE (1995) Consumption, intake patterns, and exposure. *Critical Reviews in Food Science and Nutrition* **35**, 143–147.

Zava DT & Duwe G (1998) Estrogenic and antiproliferative properties of genistein and other flavonoids in human breast cancer cells *in vitro*. *Nutrition and Cancer* **27**, 31–40.

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