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INVITED REVIEW

Proposed Criteria for Assessing the Efficacy of Cancer Reduction by Plant Foods Enriched in Carotenoids, Glucosinolates, Polyphenols and Selenocompounds

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• *Background and Aims* The cancer-protective properties of vegetable consumption are most likely mediated through 'bioactive compounds' that induce a variety of physiologic functions including acting as direct or indirect antioxidants, regulating enzymes and controlling apoptosis and the cell cycle. The 'functional food' industry has produced and marketed foods enriched with bioactive compounds, but there are no universally accepted criteria for judging efficacy of the compounds or enriched foods.
• *Scope* Carotenoids, glucosinolates, polyphenols and selenocompounds are families of bioactive compounds common to vegetables. Although numerous studies have investigated the agricultural and human health implications of enriching foods with one or more of these compounds, inadequate chemical identification of compounds, lack of relevant endpoints and inconsistencies in mechanistic hypotheses and experimental methodologies leave many critical gaps in our understanding of the benefits of such compounds. This review proposes a decision-making process for determining whether there is reasonable evidence of efficacy for the both the compound and the enriched food. These criteria have been used to judge the evidence of efficacy for cancer prevention by carotenoids, polyphenols, glucosinolates and selenocompounds.
• *Conclusions* The evidence of efficacy is weak for carotenoids and polyphenols; the evidence is stronger for glucosinolates and lycopene, but production of enriched foods still is premature. Additionally there is unacceptable variability in the amount and chemical form of these compounds in plants. The evidence of efficacy for selenocompounds is strong, but the clinical study that is potentially the most convincing is still in progress; also the variability in amount and chemical form of Se in plants is a problem. These gaps in understanding bioactive compounds and their health benefits should not serve to reduce research interest but should, instead, encourage plant and nutritional scientists to work together to develop strategies for improvement of health through food.

Key words: Human health, cancer, vegetable, carotenoids, glucosinolates, polyphenol, selenium, bioactive compound, functional food.

INTRODUCTION

Recognition of diet as a primary causative factor for cancer risk has directed much research attention toward the chemoprotective (i.e. reduction of cancer risk by specific chemical compounds) role of certain compounds in foods. Technological progress in manipulating plant metabolism and metabolites, combined with the explosive growth of the 'functional food' industry (for the purposes of this review, functional foods are defined as suggested by the International Life Sciences Institute, i.e. 'foods that, by virtue of physiologically-active components, provide a health benefit beyond basic nutrition') (International Life Sciences Institute, 1999) has led to many attempts to enhance the concentrations of these health-promoting compounds in specific foods (while animal-based foods also may contain health-promoting compounds, this review will be limited to phytonutrients in plant-based foods). To protect the health of the consumer, as well as to ensure the viability of the functional food industry, there must be stringent criteria to judge whether a compound actually provides a health benefit. Likewise, if the market strategy for a food is based on a specific health-promoting compound, stringent criteria must be set to determine the safety and efficacy of the food product. The following review proposes such criteria, and uses the criteria to assess data regarding the cancer-preventive benefits of plant sources of carotenoids, glucosinolates, polyphenols and selenocompounds.

A comprehensive report by the American Institute for Cancer Research and the World Cancer Research Fund (World Cancer Research Fund and American Institute for Cancer Research, 1997) emphasized the importance of a plant-based diet for cancer prevention. Although mechanistic research in this area is often confusing and contradictory, a general theory is emerging that 'bioactive components' in plants induce metabolic effects such as functioning as antioxidants and switching on genes that eliminate carcinogens. Bioactive components are generally defined as compounds in foods that deliver a health benefit beyond basic nutrition (International Life Sciences Institute, 1999). Classical genetic, as well as transgenic, approaches are being used to increase the content of specific bioactive components of plants, but the ability to manipulate plant metabolism

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often is far ahead of our understanding of whether or how such bioactive components work. There is an increasing awareness that multiple genetic and environmental factors affect production and accumulation of bioactive compounds, but these factors are seldom taken into consideration when a 'functional food' is marketed.

The assumption underlying 'functional foods' is that the bioactive components (in the food) are efficacious for the improvement of health; the available evidence should be rigorously scrutinized to ascertain this. Rigorous and unbiased evaluation of the scientific evidence requires a defined set of criteria that may be applied for the evaluation process. For the purpose of allowing health claims on food products, the Food and Drug Administration (FDA) has developed an extensive set of such criteria that are used to decide whether there is 'significant scientific agreement' or 'emerging evidence' regarding biological functionality of food components (US Food and Drug Administration Center for Food Safety and Applied Nutrition, 1999). Unqualified FDA health claims are allowed for only a very few compounds for which there is overwhelming evidence of efficacy. However, there are many other compounds that will not meet FDA criteria but may potentially provide an important health benefit to the consumer and be financially beneficial to the food industry as well. The FDA model cannot be directly applied to such compounds and a new set of criteria need to be developed; the following proposes such criteria.

A proposed decision process for determining efficacy of a compound and of a functional food is given in Fig. 1. Similar to the FDA model, several criteria must be met to allow initial review of pertinent data. Primarily, the compound of interest must be chemically identifiable, and the proposed health benefit must have discrete and measurable endpoints. Reports of unidentified 'factors' or loosely defined categories of substances do not lend themselves to controlled experimentation and/or characterization. For example, there are numerous reports of an 'insulin potentiating' or 'glucose tolerance' factors; however, no definitive compound has been identified and possible candidates range from chromium (Amoikon *et al*., 1995) to inositol derivatives (Larner, 2002). With the current increase in diabetes, such a factor could be greatly beneficial to health, but controlled experimentation is not possible until compounds are isolated and positively identified, and such reports should be treated as preliminary evidence to be monitored for further development. Likewise, the health endpoints must be discrete and measurable and related to a specific disease/physiologic condition. Claims such as 'improved antioxidant status' are not directly related to a specific condition and, consequently, are not meaningful measures of health.

The relevant data concerning a compound that meets entry criteria must be further reviewed to determine whether the compound is bioactive as measured by the ability to alter a specific metabolic/disease endpoint. Initial indications of efficacy often are from epidemiologic or observational studies that are useful for detecting moderate to large effects (Hennekens and Buring, 1994). However, epidemiologic data suffer from many potential biases including ill-defined choice and categorization of exposure variables, inadequate attention to confounding variables, inadequate sample size (Pocock *et al*., 2004), inadequate means of dietary assessment instruments (Dennis *et al*., 2004), recall bias (especially by diseased individuals in case-control studies) and inter-correlations with other dietary components (Freudenheim, 1999). The reader should be familiar with common sources of error and/or bias when using epidemiologic data to determine potential efficacy of a compound. Also, all epidemiology is not equally strong; case-control studies compare the dietary habits of subjects with cancer with parallel healthy controls, and dietary information may be recalled retrospectively following development of cancer. Alternatively cohort studies follow a cohort of subjects until they develop cancer, and prospective cohort studies compare the dietary habits of cancer patients assessed before development of cancer with the habits of subjects who did not develop cancer; more weight is generally given to prospective cohort studies than to retrospective case-control studies (Stephenson and Babiker, 2000).

Epidemiologic evidence is more convincing when it is supported by properly designed and executed animal and cell culture studies that are done within the context of a plausible and consistent mechanistic hypothesis. The FDA approval process will not consider animal and *in vitro* data alone, categorizing it instead as emerging evidence (US Food and Drug Administration Center for Food Safety and Applied Nutrition, 1999). The strongest evidence of efficacy is from well-designed and controlled direct intervention studies, with the 'gold standard' being the randomized, placebo-controlled trial (Stephenson and Babiker, 2000). Certainly strong data from such trials would be persuasive evidence of efficacy and be a solid basis for manipulation of plant compounds and/or development of a functional food [an important consideration in all human studies and, to an extent, also in animal trials is whether the compound is studied in the context of the food because studies with an isolated compound (Marwick, 1996) may give very different results from studies of the compound within a food matrix]. However, it may not be practical to conduct clinical trials on all candidate bioactive compounds, nor may it be financially wise to wait until a compound is proven efficacious before beginning work on a product. Consequently, a measure of discretion is advised and, as shown in Fig. 1, strong, consistent epidemiologic evidence supported by equally strong and consistent animal/*in vitro* data may sufficient evidence to develop a candidate food without the backing of clinical trial data. The investigator is strongly cautioned, however, that what seems to be consistent and supportive data are often proven wrong (or interpreted wrongly) by clinical trials; the example of β-carotene, presented later in this review is illustrative of this (Marwick, 1996).

If a plant/food that is enhanced in a bioactive compound is developed, then as depicted in Fig. 1, further criteria need to be examined to ascertain that the compound has bioactivity when consumed *through the food*. A further degree of discretion is advised as it is neither necessary nor financially possible to conduct clinical trials with all food products. Instead, it is proposed that sufficient information be available to (*a*) prove the compound is in the food in the amount and form claimed and (*b*) that the compound is bioavailable. It is further proposed (*c*) that the food/plant should be tested to

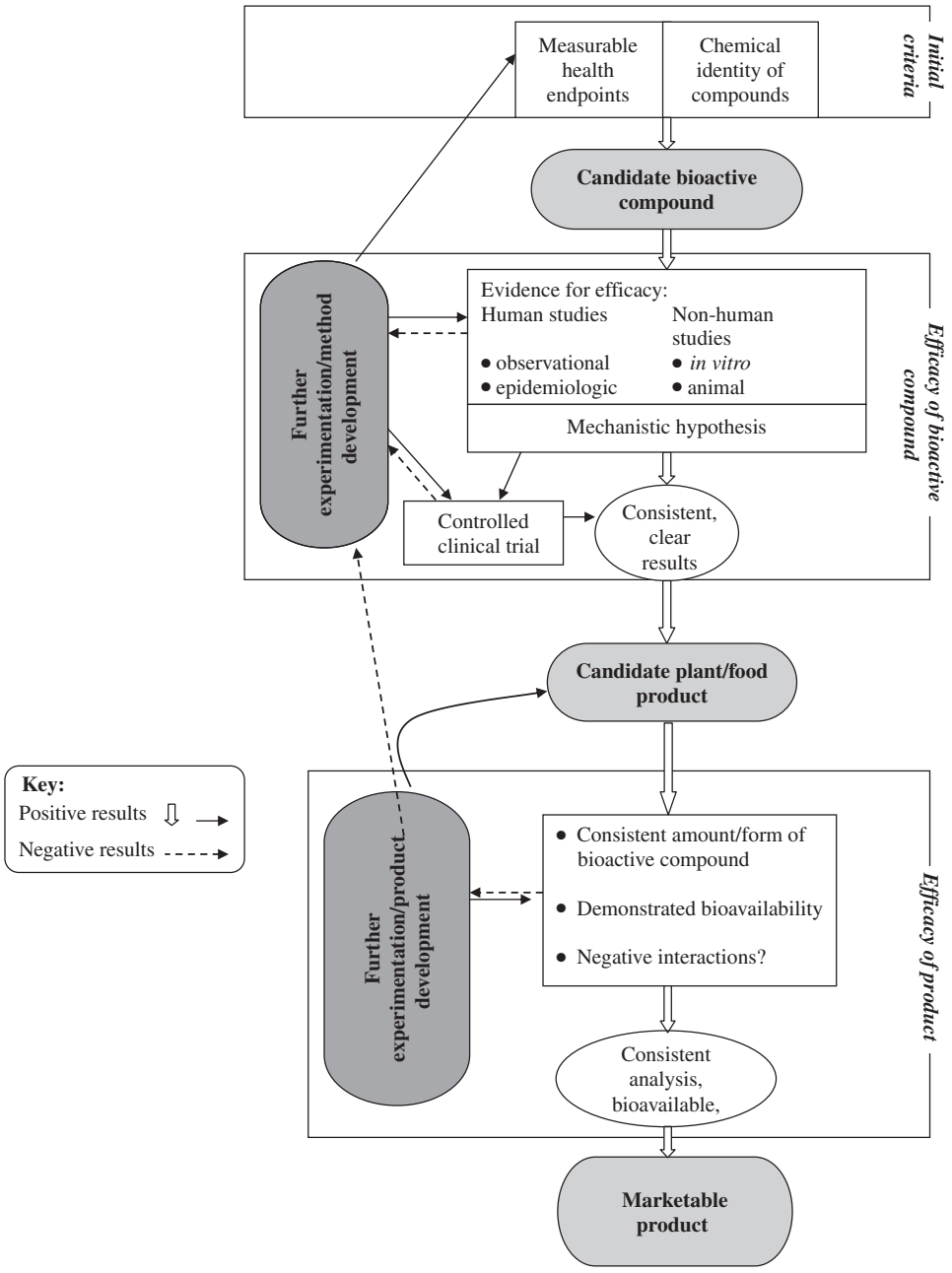


FIG. 1. Proposed process for determining the efficacy of consumption of a bioactive component or a food enriched in a bioactive component for the reduction of cancer risk.

determine whether enhancement of one compound causes negative interactions with other compounds. These criteria are similar to those of the US Federal Trade Commission (FTC) rules to ensure fairness in advertising (Federal Trade Commission, 2004), which state that product marketing must be based on truthful, non-deceptive, accurate and complete (i.e. must not leave out contradictory or negative) information that is available before a product is marketed.

THE CANCER PROCESS

Cancer is a family of diseases of multifactorial origin and progression; the process of cancer and how diet impacts that process is complex and the reader is directed to several excellent reviews of the subject (Szarka *et al*., 1994; World Cancer Research Fund and American Institute for Cancer Research, 1997; Kelloff *et al*., 2000; Willett, 2000). The following is intended as an abbreviated overview of the subject and is not exhaustive.

Cancer is generally divided into the stages of initiation, promotion and progression. Because cancer is the unrestricted division and proliferation of cells, initiation must be a genetic or epigenetic event, i.e. something must cause a misreading of the genetic code, or normal control of gene expression must be lost; both events ultimately result in abnormal cell division. Diet may contribute chemicals

that initiate the cancer process (Cejas *et al*., 2004; Wertz *et al*., 2004), but this is considered a minor effect. Of far greater importance, diet may be a source of bioactive compounds (Doll and Peto, 1981) that suppress cancer by mechanisms such as regulation of the cell cycle, induction of apoptosis in compromised cells, and/or regulation of detoxification enzyme systems. Ironically, up-regulation of Phase I enzymes in the first stage of detoxification may result in the activation of compounds that are initially un-reactive and non-carcinogenic.

Promotion occurs after the initial cellular insult, when a chemical signal or event stimulates the expansion of the insulted cell into a clone of cancer cells. Multiple dietary compounds may exert their effects in this stage by regulating processes such as the cell cycle/apoptosis, angiogenesis (inhibition) and the immune system. Progression is the terminal stage of cancer when the clonal group of cells expands into an uncontrolled tumour or multiple tumours; important physiologic events that may be regulated at this stage include angiogenesis and the immune response.

Diet and cancer

Cancer was the second leading cause of death in the US in 2001, accounting for 22·9 % of all deaths (Anderson and Smith, 2003). A landmark review by Doll and Peto in 1981 (Doll and Peto, 1981) summarized the available evidence for causes of cancer and suggested that diet is the primary causative factor in 35 % of all cancer deaths. Although diet may be a source of carcinogens, the authors concluded the most important role was as a source of cancer-inhibiting bioactive compounds, and diets that do not provide enough bioactive compounds may increase the risk of specific cancers.

Fruit and vegetable consumption and cancer risk

Meta-analyses of epidemiologic studies have generally concluded that vegetable and fruit consumption is inversely associated with cancer incidence and mortality; however, the data are not unequivocal. Although case-control studies have suggested that fruit and vegetable consumption reduce the risk of breast cancer, a recent summary of all cohort studies concluded there was no protective benefit (van Gils *et al*., 2005). Riboli and Norat (2003) reviewed 29 case-control and 17 cohort studies that examined the association between vegetable/fruit consumption and the risk of mortality from oesophageal, laryngeal, stomach, colo-rectal, breast, lung and bladder cancers. They concluded that case-control studies provided evidence that vegetable consumption decreased oesophageal, breast, lung, stomach and colorectal cancers, whereas cohort studies did not give convincing evidence for associations with any of the cancers. Steinmetz and Potter (1991) reviewed 13 ecologic, nine cohort and 115 case-control studies examining the same relationships. They concluded there is consistent, but not universal, evidence for an inverse association between fruit and vegetable consumption and epithelial, but not hormone-related, cancer. They also concluded that there is some evidence that raw foods are more efficacious than cooked. Trock *et al*. (1990) concluded that case-control and observational studies provide evidence for a protective effect of vegetable consumption on colon cancer, but Steinmaus *et al*. (2000) concluded that there was only a minor effect of vegetable intake on bladder cancer (although the relationship between fruit intake and bladder cancer risk was quite strong).

Consumption of cruciferous vegetables may be more protective than consumption of vegetables in general. Verhoeven *et al*. (1996) reviewed the evidence for *Brassica* consumption and cancer risk, and reported that 67 % of all studies showed an inverse association between consumption of total *Brassica* vegetable intake and risk of cancer at various sites; cohort studies found the greatest inverse associations between the consumption of broccoli and risk of several cancers including lung and stomach. Cohen *et al*. (2000) provided evidence that cruciferous vegetable intake was strongly (and inversely) associated with prostate cancer risk.

CAROTENOIDS AND CANCER

Carotenoid chemistry and biochemistry is well defined and is reviewed elsewhere (Fraser and Bramley, 2004). Carotenoids include compounds as diverse as α- and β-carotene, lycopene, lutein and xanthophylls, and carotenoids are found in almost all coloured vegetables (Fig. 2).

β-*Carotene*

Much research has been conducted on the relationship between β-carotene and cancer. Because β-carotene has a defined chemical structure, and because cancer has measurable endpoints, β-carotene is a candidate compound for determining efficacy for cancer prevention. β-Carotene is the primary carotenoid found in many vegetables, and the cancer-inhibitory functions of β-carotene are likely to be distinct from its nutritionally essential role as a precursor to vitamin A (Nagao, 2004). Prior to 1995, substantial epidemiologic evidence was seen as supportive of the hypothesis that β-carotene was the primary bioactive component in fruit that reduced cancer risk (Wald, 1987; Willett, 1990; Lippman *et al*., 1993; Hennekens, 1994), and specifically reduced lung-cancer risk (Willett, 1990). Moreover, limited studies from animals (Schwartz and Shklar, 1988; Lambert *et al*., 1990; Steinel and Baker, 1990; Appel *et al*., 1991; Moreno *et al*., 1991; Sherenesheva and Fin'ko, 1992; Chen *et al*., 1993) and cultured cell models (Hazuka *et al*., 1990; Nyandieka *et al*., 1990; Schwartz *et al*., 1990; Bertram *et al*., 1991; Zhang *et al*., 1991; Das *et al*., 1992; Moon *et al*., 1992; Cooney *et al*., 1993) supported this hypothesis (although very few studies were conducted in models of lung cancer) (Castonguay *et al*., 1991). A mechanistic hypothesis was developed that explained β-carotene's function as an *in vivo* antioxidant that protected against oxidation-induced cellular damage (Di Mascio *et al*., 1990; Dorgan and Schatzkin, 1991; Malone, 1991; Borek, 1993). All of this evidence seemed to provide the consistent

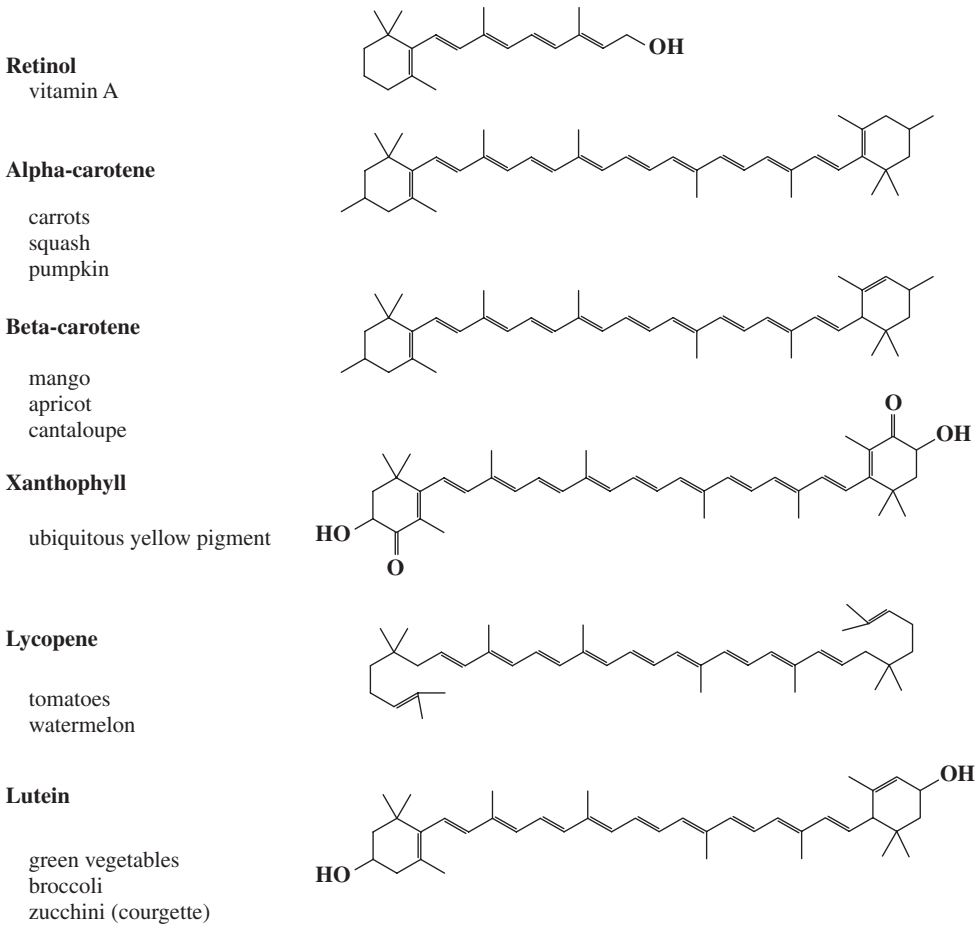


FIG. 2. Structures of common carotenoids and the foods in which they are abundant.

results needed to conduct a randomized and blinded clinical trial that supplemented Finnish male smokers (n = 29 133, 50–69 years of age) with α-tocopherol (50 mg d^{-1}), β-carotene (20 mg d^{-1}) or a placebo (the ATBC study) (The Alpha-Tocopherol BCCPSG, 1994). Unexpectedly β-carotene supplementation increased lung cancer incidence (474 *vs*. 402 cases for supplemented and un-supplemented subjects, respectively), resulting in an incidence of 56·3 and 46·5 cases per 100 000 people. (It should be noted that the increase was relatively small and only detectable because of the large sample size). The results of a second intervention trial (the CARET study) conducted in the United States were very similar to the results of the ATBC trial (Omenn *et al*., 1996). The relationship between β-carotene and cancer was further obscured by a finding in the ATBC trial that there was a significant inverse relationship between dietary intake of β-carotene and lung cancer at baseline (The Alpha-Tocopherol BCC PSG, 1994).

Assessment of evidence of efficacy for β*-carotene.* When the evidence for chemoprevention by β-carotene is considered in the context of the proposed decision criteria (Fig. 1), multiple problems are encountered. β-Carotene is chemically identifiable, and there are clear endpoints for epidemiologic studies. However, although epidemiology found a strong relationship between β-carotene intake and cancer reduction, the evidence primarily was for β-carotene as a component of fruit and vegetable intake and not as β-carotene *per se*. There were supporting data from animal and *in vitro* studies, but data from these studies may be questioned for being insufficient in total number and for relatively few using models of lung cancer. The data also may be faulted for having a relatively weak mechanistic hypothesis. Figure 1 would suggest that such data were insufficient to proceed to intervention trials, and required further experimentation in animal and cell culture models, as well as the development of a more physiology-based hypothesis. The greatest problem, however, may be that data from β-carotene intake from foods was used to justify trials with purified β-carotene (this is the opposite situation for most functional foods; many times the pure compound is proven to be effective, and from that it is extrapolated that the compound in the food is effective).

Therefore, the criteria proposed in Fig. 1 would suggest further experimentation is needed to more completely define the physiologic role of β-carotene before beginning development of β-carotene-enhanced food products. A review in the *Journal of Nutrition* summarized problems with studies of the health benefits of carotenoids and

concluded: 'Authoritative scientific evaluations by leading thinkers have not been able to ascribe a disease prevention *function* to carotenoids because of the absence of definitive evidence. These leaders recommend that future research . . . deal with the complexities of diet, genetics and environment . . . ' (Cooper, 2004).

Lycopene

The chemistry and biochemistry of lycopene, a carotenoid that is the subject of much ongoing research, is well characterized (Minorsky, 2002; Muller *et al*., 2003). Epidemiologic studies of the relationship between lycopene and cancer (particularly prostate cancer) are suggestive of a protective effect, but are not consistent. The Health Professionals Follow-up Study followed a cohort of 47 894 men and did extensive assessment of dietary intakes. Over the course of the study 812 new cases of prostate cancer were diagnosed; only lycopene intake, and not intakes of β-carotene, alpha-carotene, lutein and beta-cryptoxanthin, was significantly related to lower cancer risk (Giovannucci *et al*., 1995). Subsequent studies have not been consistent, with some finding significant associations, others finding marginal associations, and many finding no association (for complete reviews, see Barber and Barber, 2002; Giovannucci, 2002; Everson and McQueen, 2004; Tapiero *et al*., 2004). However, these epidemiologic data are complicated by studies reporting correlations of cancer risk with multiple variables including lycopene concentrations in the blood, lycopene intakes, tomato intake or intake of tomato products. Overall conclusions are also made difficult by studies using different numbers of subjects and subjects with widely varying baseline lycopene intakes and/or plasma levels. Epidemiologic evidence has resulted in several intervention studies that have used lycopene; however, results of these studies should be viewed with caution as many have utilized purified lycopene administered to patients with diagnosed prostatic cancer and, thus, are not directly applicable to the study of dietary lycopene and cancer prevention (Kucuk *et al*., 2001, 2002; Ansari and Gupta, 2004).

Chemoprevention of cancer by lycopene has also been studied in animal and cell-culture models, and lycopene has been demonstrated to have multiple cellular effects including functioning as an antioxidant (Di Mascio *et al*., 1989; Bohm *et al*., 1995), inhibition of cell cycle progression and inhibition of signalling pathways (Karas *et al*., 2000). Additionally, lycopene has been demonstrated to accumulate in human prostate tissue (Kaplan *et al*., 1990; Stahl *et al*., 1992). However, in a review Cohen (2002) concluded that there are relatively few reports of cancer chemoprevention by lycopene in animals, and while most were positive, there were also negative reports. A recent report in the *Journal of the National Cancer Institute* (Boileau *et al*., 2003) may provide insight into the apparent discrepancies between studies. Prostate cancer was chemically induced in male rats fed control diets or diets containing lycopene or tomato powder; cancer was not significantly different between controls and lycopene-fed animals, but animals fed tomato powder had a significantly lower death rate. The authors concluded that lycopene alone does not inhibit prostate cancer, but rather bioactivity is a function of the complex mix of multiple phytonutrients present in tomatoes.

Thus a review of the available literature shows the data for the efficacy of prostate cancer reduction by lycopene to be equivocal. Despite these limitations of the data, lycopene is already being incorporated into and used to promote some foods, especially tomato-based products. Therefore further criteria should be used to evaluate the chemoprotective effectiveness of lycopene from tomato-based foods.

The primary obstacle to producing lycopene-enhanced plant foods is the variability in the amount and chemical form that accumulates. Studies with tomatoes have demonstrated multiple genetic and environmental factors that may affect lycopene metabolism at virtually every step of tomato production and processing (Fig. 3). Species of tomatoes are not absolutely distinct (for a complete review, see Davies and Hobson, 1981), but despite this inter-relatedness, red varieties of tomatoes may contain as much as 30-fold more lycopene than yellow varieties (Hart and Scott, 1995). In addition, lighter coloured varieties of tomatoes may accumulate the 7,9,7′,9′-tetra-*cis*-lycopene isomer, whereas deep red varieties accumulate almost all *trans*-lycopene (Giuliano *et al*., 2002). The lycopene biosynthetic pathway changes as the fruit ripens and mRNA for proteins that convert lycopene to other carotenoids disappear (Ronen *et al*., 1999), therefore fruit picked at a more ripe stage have more lycopene than unripe fruit (Liu and Luh, 1977). Additionally, vine-ripened fruit contains higher concentrations of lycopene than fruit picked green and ripened in storage, and tomatoes produced in a greenhouse have lower lycopene concentrations than tomatoes produced outside in the summer (Gould, 2004).

Processing has the greatest effect on lycopene bioavailability. Human serum lycopene concentrations are greater when heat-processed tomatoes are consumed, as compared with unprocessed tomatoes (Giovannucci *et al*., 1995). This is in part because cooking and grinding disrupts lycopene complexes and breaks down cell walls (Hussein and el Tohamy, 1990). Additionally; unprocessed tomatoes contain primarily the *trans* isomer of lycopene, but heat processing converts a substantial amount to the *cis* isomer which may be better absorbed (Schierle *et al*., 1996). Bioavailability is especially enhanced when tomatoes are processed in the presence of 1 % corn oil, perhaps because more is incorporated into micelles and absorption is increased (Stahl and Sies, 1992). Because of all of these influences, stability of the lycopene content of a specific tomato-based food would depend on rigorous control of the entire production and processing system.

Assessment of evidence of efficacy for lycopene-enriched plant foods. Based of criteria from Fig. 1, the absence of clear and consistent evidence from human studies, and the absence of clear, consistent mechanistic studies done within the context of an over-arching hypothesis suggests that, at present, lycopene is not a compound for which enhanced foods should be considered. Certainly there is

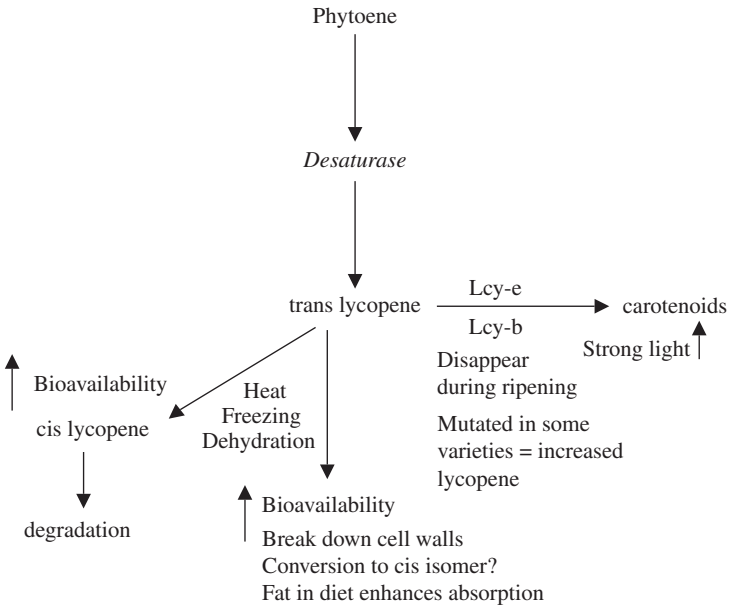


FIG. 3. Lycopene biosynthesis and factors that affect its accumulation and bioavailability; for complete review see Bramley (2002).

evidence supportive of reduction of cancer (primarily prostate) risk by lycopene, but there also is much equivocal evidence and a substantial amount of negative evidence. Further, the supportive evidence is complicated by inconsistencies between experimental models, conditions and methodologies as well as failure to agree on a primary mechanism of action. An important question to be answered is whether lycopene *per se* is bioactive for cancer reduction or, as suggested by some researchers, the combination of multiple phytochemicals in tomatoes is the actual bioactive component. Failure to provide a definitive answer to this question poses a risk similar to that for β-carotene supplementation studies, i.e. the isolated compound is at best ineffective and, under the worst circumstances, perhaps even harmful. Evidence of cancer chemoprevention by lycopene would be greatly enhanced by well-designed, controlled intervention studies that use food-based sources of lycopene and examine reduction of cancer risk (not improvement of an existing cancer condition). The criteria presented in Fig. 1 would suggest that further experimentation is needed before candidate lycopene-enhanced foods can be developed.

The question of efficacy aside, the decision process proposed in Fig. 1 also can be used to judge the potential benefits of lycopene-enhanced foods. Consistency in the amount and chemical form of a bioactive compound in a food product are primary criteria to be evaluated, and product inconsistency is the greatest problem with lycopene-containing plant foods. Not only is the content of lycopene in plants affected by virtually every step of the production process, lycopene content and bioavailability are also greatly affected by processing conditions. It will be essential to demonstrate product standardization and quality control for any plant-based product. According to the proposed decision process, this will require further product development and experimentation.

CRUCIFEROUS VEGETABLES AND GLUCOSINOLATES

Consumption of cruciferous vegetables is more strongly associated with cancer protection than vegetable consumption in general. The plant family Cruciferae (also called the mustard family or Brassicaceae) includes broccoli, parsnip, Brussels sprouts, Chinese cabbage, radish, horseradish, wasabi, white mustard, watercress and cauliflower. Crucifers contain many bioactive components including flavonoids such as quercetin (Williamson *et al*., 1996), minerals such as selenium (Se) (Finley *et al*., 2000) and vitamins such as vitamin C (Proteggente *et al*., 2002). Among the most-studied bioactive compounds in crucifers associated with cancer protection are glucosinolates (GS) (Fenwick *et al*., 1983). More than 120 GS have been characterized; although their function in the plant is unclear, their potent odour and taste suggests a role in herbivore and microbial defense (Fenwick *et al*., 1983).

Glucosinolates are chemically defined compounds; all characterized GS share a similar basic structure consisting of a β-D-thioglucose group, a sulfonated oxime group and a side chain derived from methionine, phenylalanine, tryptophane or branched-chain amino acids (Fig. 4). The sulfate group of a GS molecule is strongly acidic and plants accumulate GS by sequestering them as potassium salts in plant vacuoles (Keck and Finley, 2004). Glucosinolates are not bioactive in the animal that consumes them until they have been enzymatically hydrolysed to an associated isothiocyanate (Rouzaud *et al*., 2003) by the endogenous myrosinase enzyme that is released by disruption of the plant cell through harvesting, processing, or mastication. The hydrolysis products of common GS are shown in Fig. 4; glucoraphanin is converted to sulforaphane (Sf) and Sf nitrile, sinigrin to allyl isothiocyanate, gluconasturtin to phenethyl isothiocyanate and glucobrassicin to indole-3-carbinol (Keck and Finley, 2004).

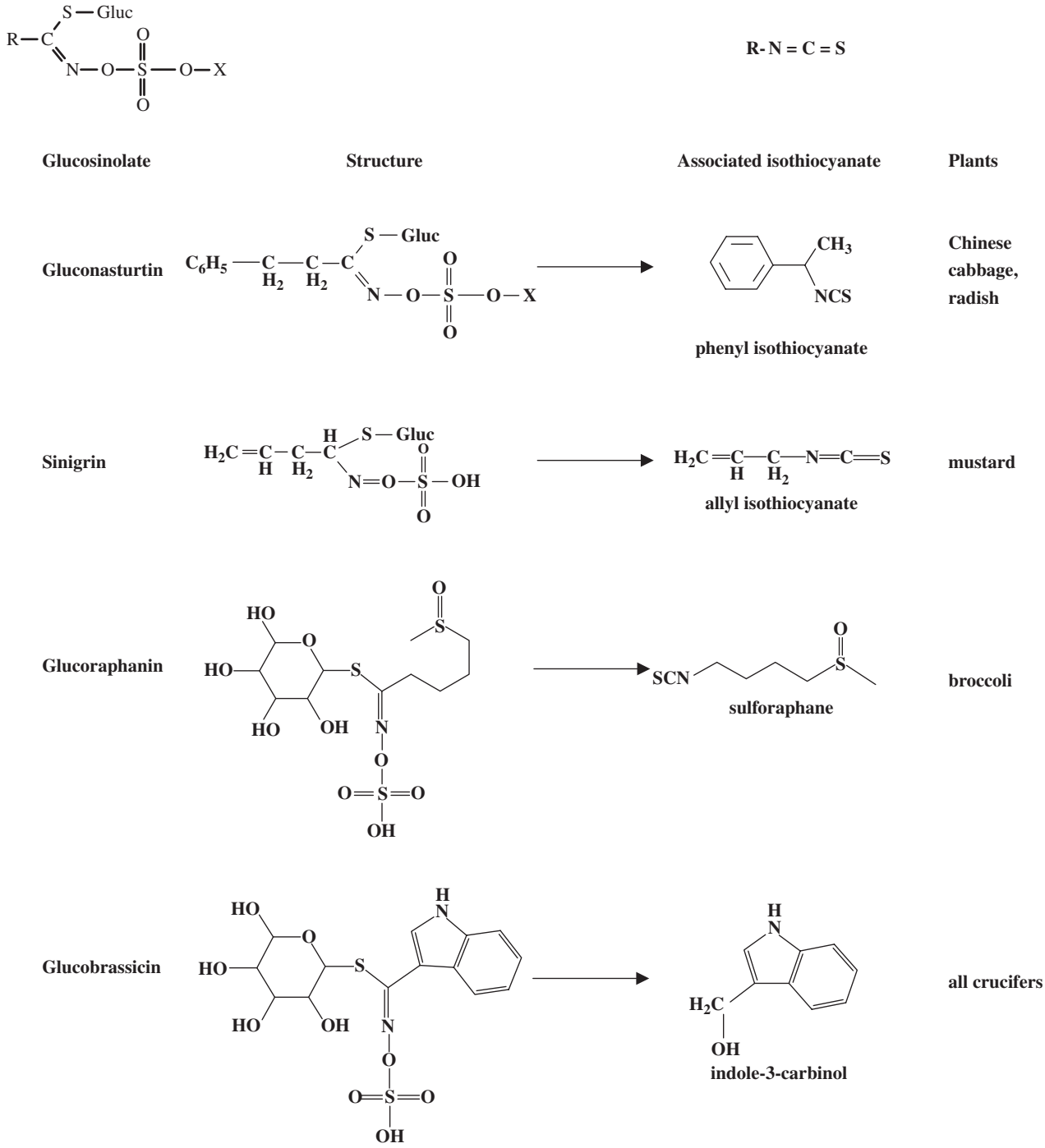


FIG. 4. Common glucosinolates and their hydrolysis products.

In vitro and *in vivo* studies have reported that isothiocyanates affect many steps of cancer development including modulation of phase I and II detoxification enzymes (Rabot *et al*., 1993; Bogaards *et al*., 1994; Jiao *et al*., 1996; Talalay and Fahey, 2001), functioning as a direct antioxidant (Zhu *et al*., 2000; Zhu and Loft, 2001, 2003) or as an indirect antioxidant by phase II enzyme induction (Hayes and McLellan, 1999; Talalay and Fahey, 2001; McWalter *et al*., 2004), modulating cell signalling (Xu and Thornalley, 2001), induction of apoptosis (Yu *et al*., 1998; Chiao *et al*., 2002; Yang *et al*., 2002), control of the cell cycle (Yu *et al*., 1998; Zhang *et al*., 2003*b*; Wang *et al*., 2004) and reduction of *helicobacter* infections (Fahey *et al*., 2002). The most characterized GS compounds are Sf, phenethyl isothiocyanate, allyl isothiocyanate and indole-3-carbinol (Hecht, 1999), but many other isothiocyanates that are present in lower quantities also may contribute to the anti-carcinogenic properties of crucifers.

Apoptosis and modulation of phase I and phase II detoxification pathways have been the most studied mechanisms by which GS/isothiocyanates inhibit carcinogenesis. There are numerous reports of GS/isothiocyanate activation of cellular control and apoptosis-inducing genes, including the caspases (Rose *et al*., 2003; Pham *et al*., 2004), p53 (Fimognari *et al*., 2004*a*, *b*), cyclin-dependent kinases (Srivastava *et al*., 2003; Fimognari *et al*., 2004*b*; Singh *et al*., 2004; Xiao *et al*., 2004), bax (Fimognari *et al*., 2004*b*) and nuclear factor signalling pathways (Jeong *et al*., 2004; Srivastava and Singh, 2004). Others have also proposed that apoptosis may be mediated by disruption of tubulin polymerization (Jackson and Singletary, 2004), or increased oxidative stress caused by superoxide radical bursts (Rose *et al*., 2003) or decreased intracellular antioxidant concentrations (Pham *et al*., 2004).

Other researchers have concentrated on GS/isothiocyanate-mediated changes in detoxification enzyme systems; such changes are hypothesized to reduce cancer risk by decreasing activation of pro-carcinogens and/or increasing excretion of carcinogens (Talalay and Fahey, 2001). Additionally, some have suggested that activation of these enzymes also provides *in vivo* catalytic antioxidant protection and decreases oxidative stress (Talalay and Fahey, 2001). Recent research on regulation of antioxidant genes has suggested that a promoter sequence found in multiple phase II enzymes (Bonnesen *et al*., 2001) called the antioxidant response element (ARE) (for a review, see Finley, 2003*b*) may respond to various dietary constituents and simultaneously activate multiple enzyme systems. Sulforaphane is the dietary constituent that is the most powerful inducer of the ARE (Morimitsu *et al*., 2002).

There are reports of interventional studies with GS in humans, although they are limited in number and scope, and most have examined GS bioavailability and excretion (Shapiro *et al*., 1998; Conaway *et al*., 2000; Ye *et al*., 2002). A few have examined functional changes: Bogaards *et al*. (1994) and Verhagen *et al*. (1997) reported two studies that showed decreased markers of oxidative damage with consumption of Brussels sprouts. Cashman *et al*. (1999) reported that flavin monooxygenase-3 activity was reduced by dietary consumption of Brussels sprouts, and Bogaards *et al*. (1994) and Nijhoff *et al*. (1995) reported increased GST activity with consumption of Brussels sprouts.

Some have concluded that the evidence for health benefits by GS is strong enough to warrant product development, and broccoli sprouts with a uniformly high concentration of Sf are a patented, commercially available product (Brassica Protection Products LLC, 1999; Fahey *et al*., 1997). However, the GS content of most crucifers consumed for food is highly variable, and the effect of this variability on estimating the protective benefits of crucifer consumption was elegantly demonstrated by Dekker and Verkerk (2003). A modelling procedure was utilized to introduce estimated variation in the glucosinolate content of crucifers reported in cancer studies, and the effect of GS intake on relative risk of cancer was recalculated. If glucosinolate intake was assumed to be a constant function of crucifer intake, then increasing crucifer consumption cuts the relative risk of cancer by as much as half. However, cultivation, processing and domestic cooking all affect glucosinolate content; when variability from these factors was introduced into the model, GS consumption did not significantly reduce cancer risk.

Epidemiologic studies often consider crucifers as a group, but the chemical form and total amount of GS differ more than 10-fold within and between crucifer species. Glucobrassicin and glucoraphanin are generally found in high concentrations in broccoli (0·1–2·8 and 0·8–21·7 mmol g^{-1} d. wt, respectively) and constitute as much as 95 % of the total amount of GS (Kushad *et al*., 1999). Brussels sprouts, cabbage and cauliflower contain little or no glucoraphanin, and crucifers other than broccoli generally contain high concentrations of sinigrin. Gluconasturtiin is abundant in Chinese cabbage, radishes and watercress (Fenwick *et al*., 1983). Kushad *et al*. (1999) reported that, although the average total GS content of Brussels sprouts was twice that of broccoli, the average glucoraphanin content (the parent compound of Sf) of broccoli was 7-fold that of Brussels sprouts. The same study also reported remarkable variation between different varieties of the same species; e.g. the glucoraphanin content of broccoli varied from 0·8 to 21·7 μmol g^{-1} d. wt, and total GS content was not necessarily predictive of concentrations. Moreover, environmental variables such as location (Shelp *et al*., 1993) and harvest date (Kushad *et al*., 1999) affect GS concentrations and profile as much as or more than variety.

Assessment of evidence of efficacy for glucosinolates. Glucosinolates are chemically defined and there is limited supportive epidemiologic evidence for efficacy of cancer reduction (at least for crucifer consumption); however, studies with β-carotene have certainly demonstrated the danger of using epidemiologic data from foods to predict efficacy of isolated chemical compounds. Basic animal and cell culture studies have demonstrated plausible mechanisms of action, but there is no agreement as to which mechanism is of primary importance. Human interventional studies with GS in humans have been conducted, but they are limited in number and scope. By criteria proposed in Fig. 1, there are no overwhelming, clear and consistent data showing a cancer-reductive benefit of glucosinolates.

According to criteria proposed in Fig. 1, the available data would suggest that further experimentation, especially randomized and controlled human intervention trials, is needed before candidate glucosinolate-enhanced foods are proposed. However, as with lycopene, this point is irrelevant as such foods are already being produced and marketed, and the decision process should then be used to determine whether such enhanced foods are effective for the hypothesized health benefits. The first of the criteria (Fig. 1) for evaluating a food is that the bioactive compound must be found in consistent amounts and chemical forms in the food product. Also similar to lycopene, extensive evidence shows GS content and chemical forms vary dramatically under common agricultural production conditions, and at this point it may be very difficult to provide a consistent product. Consequently, further product development,

experimentation and, perhaps, even development of new methodology is required.

POLYPHENOLS: THE MOST ABUNDANT DIETARY ANTIOXIDANTS?

Polyphenols are an enormous general class of chemicals with over 8000 described compounds (Ross and Kasum, 2002); general structures of the main classes are shown in Fig. 5. Although the hydrophobic phenolic group is common to all, glycosylation by sugars such as glucose, rhamnose, galactose and arabinose makes them water soluble (Yang *et al*., 2001*b*). The chemistry and nutritional properties of phenolic compounds have been extensively reviewed (Yang *et al*., 2001*b*; Robbins, 2003; Manach *et al*., 2004). Although ten different general classes of phenols are recognized, the majority of plant polyphenols are simple phenols and flavonoids (Kris-Etherton *et al*., 2002).

Epidemiologic evidence for the reduction of cancer risk by dietary sources of polyphenols is emerging but not convincing. Unbiased studies are difficult because of the vast number of potential compounds and because the phenolic content of most foods is not well established. The best evidence comes from studies of polyphenols in tea drinkers which was reviewed by Yang *et al*. (2001*a*). Four of seven case control studies reported a significant inverse relationship and two reported a numerical, but statistically insignificant relationship between green tea consumption and cancer risk (Yang *et al*., 2001*a*), whereas a cohort study did not find a protective effect (Tsubono *et al*., 2001). Studies of black tea consumption are equivocal; a cohort study in the Netherlands did not find any benefit (Goldbohm *et al*., 1996), whereas a cohort study in the US found a protective effect on colon cancer (Su and Arab, 2002). Epidemiologic studies of consumption of other flavonoid-rich foods and cancer include a report of a significant inverse relationship between apple consumption and lung cancer in Finnish men (Knekt *et al*., 1997), and a protective effect of onions, grapefruit and apples, primary sources of quercitin, on lung cancer (Le Marchand *et al*., 2000). There are multiple reports of isoflavones and lignans protecting against breast cancer (Messina *et al*., 1994; Wu, 1999), but such effects are probably a result of phytoestrogenic activity (Kurzer and Xu, 1997) and are distinct from the chemoprotective mechanisms of other polyphenols.

Epidemiologic evidence is accompanied by a large volume of basic *in vitro* and animal studies (approx. 1000 articles dealing with polyphenols in plant foods published since 2000 according to PubMed database) (National Library of Medicine, 2004). The primary problems with these studies, however, are that the endpoints measured may not be physiologically relevant and/or they lack a consistent and plausible mechanistic hypothesis.

The problem with the endpoints utilized in studies of polyphenols is that most reports have focused on the *in vitro* 'antioxidant activity' of polyphenols or phenolic-rich foods, i.e. the ability of a food extract to reduce a test compound, but the tests used may not generate data that have any relationship to *in vivo* amelioration of oxidative stress. Many studies reported in the plant-science literature have come to conclusions such as *in vitro* antioxidant activity is well correlated to phenolic content in *Vaccinium* berries (wild blueberry-like berries) (Taruscio *et al*., 2004), processed tomatoes (Gahler *et al*., 2003), nectarines, peaches and plums (Gil *et al*., 2002), grapefruit juice (Gorinstein *et al*., 2004), apple extracts (Chinnici *et al*., 2004) and yucca extracts (Piacente *et al*., 2004). In fact, a search of the PubMed database for original research articles published from the year 2000 to the present found more than 700 reports of the antioxidant potential of phenolics in plants (excluding the reports of antioxidants associated with oils or oilseeds). Most used only *in vitro* assessments, and less than 100 reports used an animal model.

A close scrutiny of the antioxidant tests used shows many may have little or no relevance to human health. Common tests include the Trolox equivalence (TEAC) assay (Bohm *et al*., 2002), the diphenyl-1-picrylhydrazyly (DPPH) assay (Polasek *et al*., 2004) and the 2,2′anziobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay. All of these tests measure the ability of a test substance or extract to scavenge a spontaneously formed radical cation chromophore (Rice-Evans and Miller, 1997) (Bonina *et al*., 2000). At least 200 studies published since 2000 used one of these methods to relate the antioxidant ability of a plant extract to its polyphenol content, and the popularity of these tests is most likely their simplicity, not their *in vivo* significance (Antolovich *et al*., 2002). Because these tests measure scavenging capacity of a radical formed spontaneously, they do not use an oxidant initiator, but an oxidant initiator is considered an essential part of a valid test (Rice-Evans and Miller, 1997; Antolovich *et al*., 2002).

Other assays in common use are the ferric-reducing antioxidant power (FRAP) (Pulido *et al*., 2000) and the oxygen radical absorbance capacity (ORAC) assays. The ORAC assay follows the disappearance of oxidized β-phycoerythrin (DeLange and Glazer, 1989) or fluorescein (3′,6′-dihydroxyspiro[isobenzofuran-1[3H],9′[9H]-xanthen]-3-one), while the FRAP assay measures reduction of Fe^{3+} tripyridyltriazine complex to Fe^{2+} tripyridyltriazine; 43 PubMed-listed studies used these assays. Antolovitch *et al*. (2002) faults the FRAP assay because it measures total antioxidant concentrations and not antioxidant activity. Results of the ORAC assay are significantly correlated with HPLC data for some phenolic acids, whereas the correlations were meaningless for others, especially flavonoid glycosides (Antolovich *et al*., 2002).

Although the above studies, no doubt, contribute to our understanding of the potential beneficial role of phenolics in plants, problems with the assays themselves, as well as the relevance of the tests, mean that applying the results to the human diet must be done with caution. Only a very small percentage of the studies have simultaneously made *in vitro* measurements and correlated them with *in vivo* changes, thus the functional significance of the reported tests is often not clearly established. Furthermore, methodological concerns make their results of limited use, especially when only one test is reported. Additionally, these studies do not take into account bioavailability or delivery to a specific tissue site (so important in cancer prevention)

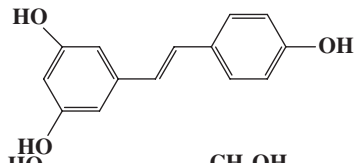
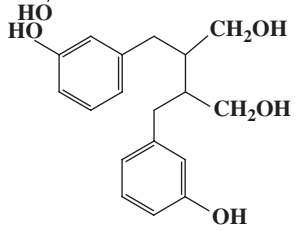
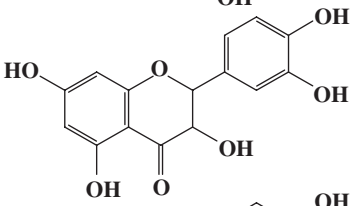
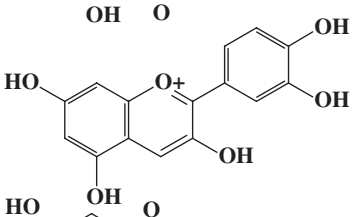
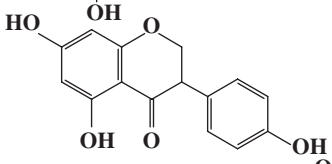
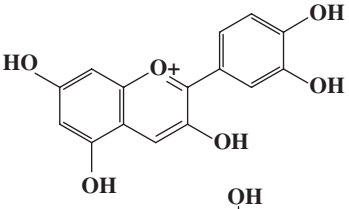
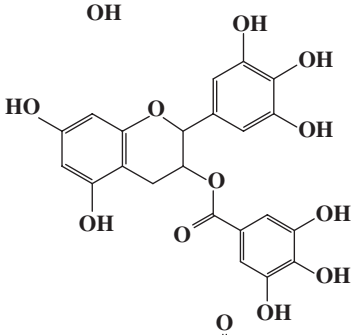
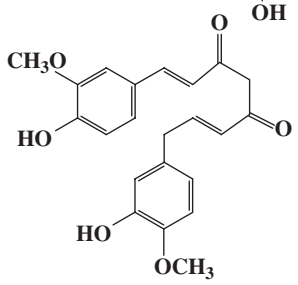
Structure	Name	Source
	Resveratrol	Grapes
	Enetrodiol (lignan)	Flaxseed
	Quercitin (flavonoid)	Broccoli, tea, onions
	Cholorogenic acid (phenolic acid)	Many fruits and vegetables, coffee
	Genestein (isoflavone)	Soy
	Anthocyanidin	Raspberries, strawberries
	Epigallocatechin gallate (EGCG)	Green tea
	Curcumin	Tumeric

FIG. 5. Common polyphenols and foods in which they are found.

into consideration, nor is there consensus on how much of an antioxidant is beneficial. Aruoma (2003), in a review of antioxidant methods, stated '. . . it is clear that not a single method can give a comprehensive prediction of antioxidant efficiency' and suggested that 'the question of bioavailability and fate of metabolites of antioxidant components must be addressed', and concluded that 'we have to agree (to) governance on *in vitro* antioxidant methods based on an understanding of the mechanisms involved'.

The second major problem with polyphenolic research is that while much of the research focus has been on the antioxidant activity of polyphenols, amelioration of oxidative stress may not even be the mechanism by which polyphenols inhibit cancer. A review by Yang *et al*. (2001*b*) stated 'The effects of dietary polyphenols are of great current interest due to their antioxidative and possible anticarcinogenic activities. A popular belief is that dietary polyphenols are anticarcinogens because they are antioxidants, but direct evidence for this supposition is lacking'. Other proposed mechanisms by which polyphenols may inhibit cancer include modulation of molecular events in cancer initiation, promotion and progression.

Some of the best studies of chemoprotection by polyphenols have used green tea and its predominant polyphenol, epigallocatechin gallate (EGCG). However, results of many of these studies are more applicable to mechanisms related to heart disease and stroke rather than cancer. For example, EGCG was reported to attenuate hypoxia-induced oxidative stress (Wei *et al*., 2004), protect against neuronal oxidative damage (Nagai *et al*., 2002; Etus *et al*., 2003; Lee *et al*., 2003), inhibit LDL–cholesterol oxidation (Vinson *et al*., 2002), and ameliorate oxidation-induced hepatotoxicity in mice (Chen *et al*., 2004) by decreasing nitrous oxide-generated mediators of oxidative stress. More applicable to carcinogenesis, green tea and/or components of green tea inhibited the formation of O6-methylguanine and 8-hydroxydeoxyguanosine (8-OH-dGuo) DNA lesions and chemically induced lung tumourogenesis in mice (Xu *et al*., 1992), inhibited DNA methyl-transferase and reactivated methylation-silenced genes important in the cancer process (Fang *et al*., 2003), and scavenged hydrogen peroxide and decreased UV-induced 8-OH-dGuo DNA adducts in calf thymus (Wei *et al*., 1999), while black tea decreased DNA adducts in liver microsomes (Krishnan and Maru, 2004).

Other investigators have suggested that cancer reduction by polyphenolic-rich foods may be mediated by an indirect antioxidant function. Frei and Higdon (2003) reviewed studies regarding the antioxidant activity of green tea and suggested that polyphenols may function indirectly as antioxidants by (*a*) inhibiting redox-sensitive transcription factors such as nuclear factor-kappaB and activator protein-1, (*b*) inhibiting 'pro-oxidant' enzymes such as inducible nitric oxide synthase, lipoxygenases, cyclooxygenases and xanthine oxidase or (*c*) inducing phase II and antioxidant enzymes such as glutathione S-transferases and superoxide dismutases. Such indirect antioxidant activity almost certainly would not be detected by *in vitro* tests such as those described above. Other proposed chemopreventive mechanisms of polyphenolic compounds, particularly EGCG, include induction of apoptosis in smooth muscle cells (Hofmann and Sonenshein, 2003), mouse leukaemia cells (Gao *et al*., 2002), oral carcinoma cells (Hsu *et al*., 2003*a*), and human leukaemia cells (Smith and Dou, 2001; Shiono *et al*., 2002). Induction of apoptosis has become important enough to suggest that *in vitro* apoptotic activity may be used as a screening tool for potential anticancer phenolic phytochemicals (Hsu *et al*., 2003*b*). Cell cycle arrest is induced by green tea polyphenol (Jia *et al*., 2002) and curcumin (Hanif *et al*., 1997). Curcumin also may modulate arachidonic acid metabolism (Rao *et al*., 1995), some pro-oxidant polyphenols (e.g. resveratrol) may be cytotoxic (Hadi *et al*., 2000), some polyphenols may block initiating attacks on DNA (Newmark, 1984), and some may regulate cell signal pathways (Yeh *et al*., 2003).

Assessment of evidence of efficacy for polyphenols. The evidence for the health benefits of polyphenols is intriguing, but they are clearly compounds for which the evidence is emerging at best. Based on Fig. 1, most polyphenols do not meet the criteria for even an initial assessment of chemopreventive efficacy. Although there have been major research advancements in the identification and characterization of specific polyphenols (Robbins, 2003), many remain unidentified. Also the polyphenolic content of most plant foods is uncharacterized, thus making epidemiologic studies very difficult. The other initial assessment criterion proposed in Fig. 1 is there must be measurable endpoints for the intended health benefit. This may be the biggest concern with the data at present as many of the reported analytical methods have focused on *in vitro* antioxidant capability and such studies are not directly applicable to the endpoint of human cancer prevention. Consequently Fig. 1 criteria would suggest that further analytical method development and experimentation are required before polyphenol-enriched candidate foods are proposed.

SELENOCOMPOUNDS AND SELENIUM-ENHANCED FOODS: A MODEL OF PROVEN EFFICACY FOR CANCER REDUCTION?

Selenium (Se) is a nutritionally essential element and Se deficiency results in disease conditions in humans and domestic livestock (Levander, 1987). Most of the recent interest in Se nutrition, however, is not directed towards restoring adequacy in deficient individuals. Rather, it is directed toward over-supplementation in amounts of 3–6-fold beyond the Recommended Dietary Allowance (RDA; 55 μg d^{-1}) (National Academy of Science, 2001), because there is evidence that such intakes are protective against cancer (Combs *et al*., 2001).

Isolated selenocompounds are chemically characterized and much research has been directed towards determining the various forms in food. Epidemiologic data are supportive of an association between Se intakes and cancer risk, and these data are supported by animal and cell culture studies conducted within the framework of a mechanistic hypothesis. Finally, there have been multiple clinical intervention trials with Se, and trials directed toward confirming

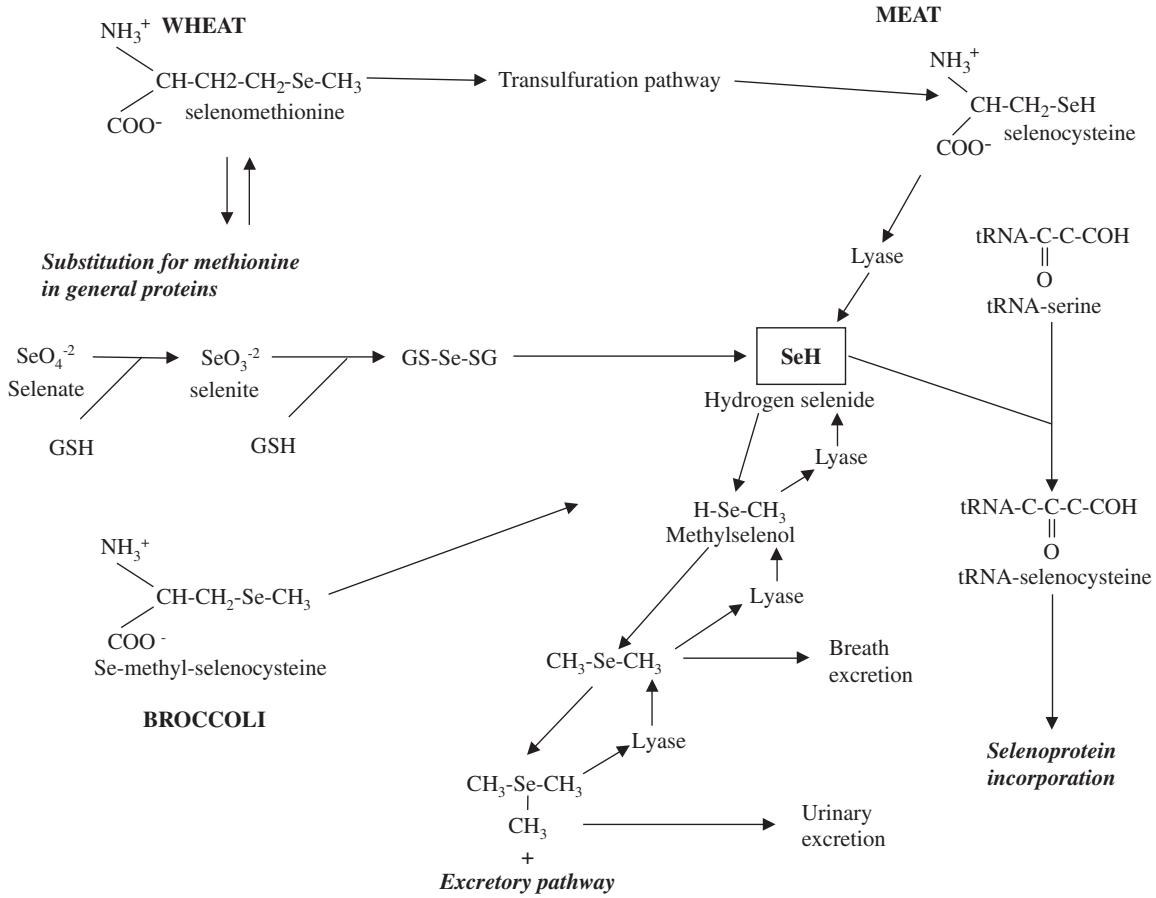


FIG. 6. Important metabolites of selenium and selenium metabolism in animals.

previous results are now under way. There is also interest in Se-enhanced foods because studies have shown that Se can be reliably and repeatedly enhanced in selected foods. Bioavailability studies have been conducted in animals and humans, and other studies have begun to characterize interactions between Se and other food constituents.

Chemical forms of Se

Selenium is covalently bound into multiple compounds in plants, and the amount and chemical forms of these compounds are determined by the environment and plant genetics (Davis and Finley, 2003; Ellis and Salt, 2003); the structures and metabolism of these compounds are reviewed in detail elsewhere (Ganther and Lawrence, 1997). The physiologic effect of Se consumption depends in part on the chemical form of the element. Some forms of Se are preferentially incorporated into selenoproteins (proteins that require Se for catalytic activity), others are non-specifically incorporated into proteins in general, whereas still others are preferentially excreted; Fig. 6 is a simplified picture of this metabolism. Predominate forms of Se found in nature include salts such as sodium selenate and selenite and the amino acid selenocysteine; these forms are readily used by Se-deficient animals for production of selenoproteins. The amino acid selenomethionine (SeMet) may randomly substitute for methionine and thus may accumulate in general methionine-requiring proteins. Methylated amino acids such as Se-methyl selenocysteine (SeMSC) are metabolized primarily in the excretory pathway, and limited data suggests that methyl selenol generated in this pathway is the metabolite most responsible for preventing cancer (Ip and Ganther, 1990).

The biosynthetic pathway for selenocompounds in plants has been delineated (Ellis and Salt, 2003); Se follows the sulfate assimilation pathway and ultimately incorporates into SeCys and SeMet. A specific transferase may add a methyl group to SeCys forming SeMSC (Neuhierl and Bock, 2000), and transfection of that gene into a plant will convert it into a plant that hyperaccumulates Se (Wang *et al*., 1999) in methylated forms such as SeMSC (Pickering *et al*., 2003). Broccoli will hyperaccumulate Se (Finley, 1998) in methylated forms (Cai *et al*., 1995; Roberge *et al*., 2003). Wheat will only accumulate modest amounts of Se (Olson *et al*., 1970; Finley, 1999*a*), primarily as SeMet (Djujic *et al*., 2000; Wolf and Goldschmidt, 2004). Other plants that may accumulate Se include garlic (Ip *et al*., 1992; Ip and Lisk, 1993, 1994*a*), ramps (Whanger *et al*., 2000), various species of mushroom (Stijve, 1977; Spolar *et al*., 1999; Werner and Beelman, 2002), various algae (Saiki

et al., 1993), multiple *Brassica* species (Hamilton, 1975; Nyberg, 1991; Keck and Finley, 2004) and Brazil nuts (Chang *et al*., 1995).

Epidemiologic and investigative evidence for cancer reduction

Clark *et al*. (1991) reported significant associations between the concentration of Se in animal forages and human lung, rectal, bladder, oesophageal, cervical, breast and corpus uteri cancer mortality rates in 200 US counties. More recent epidemiologic evidence is strongly supportive of the hypothesis that Se is protective against prostate cancer (Yoshizawa *et al*., 1998; Nomura *et al*., 2000).

The epidemiologic evidence for Se-mediated chemoprevention of cancer is supported by many studies in animals and cells in culture that have developed distinct hypotheses of the mechanism of action of Se; these studies are extensively reviewed elsewhere (Combs *et al*., 2001; Ganther, 2001; Kim and Milner, 2001; Davis and Finley, 2003). Combs (1999) has proposed a multistage hypothesis for the biological action of Se in cancer prevention. During Se deficiency, addition of small amounts of Se to the diet increases the activity of selenoproteins, improves the function of the immune system and may regulate Phase I and Phase II detoxification enzymes. However, when Se is consumed in amounts beyond the dietary requirement (so-called 'supranutritional intakes') it probably exerts its effects through completely different mechanisms such as control of the cell cycle, apoptosis and angiogenesis. Summarization of the literature (Davis and Finley, 2003) suggests several primary anti-carcinogenic mechanisms of Se that include irreversible apoptosis with DNA strand breaks (Spallholz, 1994; Davis *et al*., 1998; Spallholz *et al*., 2001), cell cycle arrest and/or apoptosis independent of DNA strand breaks (Wilson *et al*., 1992; Lu *et al*., 1995), changes in the mitogen-activated cell signalling pathway (Ghose *et al*., 2001) and inhibition of angiogenesis (Lu and Jiang, 2001).

Evidence from clinical intervention trials of cancer suppression by Se

Selenium is one of a very few nutritional compounds used in chemoprevention studies in which a successful intervention has been replicated (Young and Lee, 1999). Selenium supplementation has been reported to reduce hepatic cancer (Yu *et al*., 1991, 1997) and Se in combination with β-carotene and vitamin E reduced oesophageal cancer (Blot *et al*., 1995) (these studies should be viewed with caution as other health/dietary problems may have been confounding variables). The most robust of the cancer trials, first reported in 1996, found that 200 μg of Se d^{-1} (supplied as Se-enhanced yeast) reduced overall cancer incidence and mortality by as much as 50 %, and prostate cancer by >60 % (Clark *et al*., 1996). Although subsequent analysis of the data has changed some of the statistics, the data for chemoprotection against prostate cancer remain strong (Duffield-Lillico *et al*., 2002).

Confirming the results of Clark *et al*. (1996) is a high-priority research objective. Multiple small trials are currently being conducted, but the largest and most important trial is the selenium and vitamin E prostate cancer (SELECT) trial (National Institute of Health, 2004). This National Institute of Health-sponsored study has enrolled 32 400 male subjects 50 years or older in the United States, Puerto Rico and Canada and is the largest prostate cancer trial ever conducted. Subjects will be supplemented daily with either 200 μg of Se, 400 mg of vitamin E, vitamin E and Se, or a placebo, and is scheduled to last for 7 years. If results of the ongoing Se trials are positive it is likely that a strong world-wide demand for supplemental sources of Se will develop, and Se-enriched foods could potentially fill much of this demand (there is one potential problem with this study, in that the original intervention study used a natural product, Se-enriched yeast; the SELECT trial, however, is using purified selenomethionine, and so there is some question as to how results of this study can be extrapolated to intakes of Se through food).

Efficacy of Se-containing foods for cancer prevention; do foods contain consistent amounts and forms of Se?

Data collected in Finland has demonstrated that Se in soil can be reliably transferred to plants and ultimately to humans. Because of extremely low dietary Se intakes, Finland adopted a national policy in the mid-1980s of adding Se as sodium selenate to all agricultural fertilizers (Varo *et al*., 1988; Mäkelä *et al*., 1993). By 1989 the supplementation regimen had increased the human dietary intake of Se by Finnish people from 20–30 μg d^{-1} (in 1986) to 80–90 μg d^{-1} (in 1989), with the primary food source being wheat flour (Mäkelä *et al*., 1993). Within 2 years of beginning fertilization, markers of Se status in Finnish people were similar to people in the US. Other Se-enhanced foods that have been produced by Se fertilization include soybeans (Yang *et al*., 2003), tomatoes, strawberries, radishes and lettuce (Carvalho *et al*., 2003) and potatoes (Poggi *et al*., 2000). Arthur (2003) reviewed the evidence for increasing the content of Se in foods by addition of selenized fertilizer to the soil, and concluded that fertilization is safe and effective for increasing Se status in humans and animals.

Other countries have supplemented Se to their populations (either intentionally or unintentionally) by importing wheat grown on high-Se soils. For example, the blood Se concentrations of New Zealanders with very low intakes of Se increased 50 % following several years of importation of Australian wheat (Watkinson, 1981). Selenium-enriched wheat can be naturally produced when it is grown on soils naturally high in Se. The average concentration of Se in US wheat is approx. 0·3 mg Se kg^{-1}, but wheat produced in some areas of central South Dakota is consistently between 5 and 15 mg Se kg^{-1} (Finley, 1999*a*; Lawler *et al*., 2004; Soto-Navarro *et al*., 2004). Additional factors that affect Se accumulation in plants include soil type (Popijac and Prpic-Majic, 2002), potential ligands (Poggi *et al*., 2000), moisture (Tennant and Wu, 2000), sulfur status

(Baghour *et al*., 2002) and soil temperature (Baghour *et al*., 2002).

Is Se from Se-enriched foods bioavailable for the physiological functions of Se?

While it may not be necessary to replicate cancer trials with candidate Se-enriched plant foods, it is important to demonstrate that the Se is bioavailable. This has traditionally been determined by measuring the relative efficacy (usually by comparison to selenite or SeMet) of a selenocompound for improvement of Se status of Se-depleted animals or humans; Se status is usually assessed by measuring blood Se and glutathione peroxidase enzyme activity (Levander, 1983). However, reduction of cancer risk may not be associated with improvement of these variables; thus studies need to specifically demonstrate the bioavailability of Se for chemoprotection against cancer. High-Se garlic has been reported to inhibit mammary cancer in rats and mice (Ip *et al*., 1992; Ip and Lisk, 1994*a*, 1995; Ip *et al*., 2000; Dong *et al*., 2001), Se-enriched soy reduced metastasis of melanoma in mice (Li *et al*., 2004), Brazil nuts protected against mammary cancer in rats (Ip and Lisk, 1994*b*) and Se-enriched ramps inhibited mammary tumours in rats (Whanger *et al*., 2000). Selenium-enriched broccoli reduced preneoplastic lesions in rat colon (Finley *et al*., 2000; Finley, 2003*a*), spontaneous intestinal tumours in the Multiple Intestinal Neoplasia (Min) mouse line (Finley, 2003*a*) and carcinogen-induced mammary tumours in mice (Finley *et al*., 2001), and increased the activity of pro-apoptotic genes in mice (Zeng *et al*., 2003). However, the bioavailability of Se from broccoli, when determined by improvement of Se status in rats, was much lower than for selenite or SeMet (Finley, 1998; Finley *et al*., 2004); studies in humans gave similar results (Finley, 1999*b*).

While the only natural substance that has been demonstrated to decrease cancer in humans is the Se-enriched yeast that was used in the cancer trial of Clark *et al*. (1996), there have been numerous Se bioavailability trials in humans. Selenium-enriched wheat improved Se status in US men (Longnecker *et al*., 1993), Dutch men (van der Torre *et al*., 1991), New Zealanders (Watkinson, 1981), Finnish medical students (Mäkelä *et al*., 1993), Norwegian women (Bibow *et al*., 1993) and adults in Yugoslavia with low Se intakes (Djujic *et al*., 2000). High-Se wheat has been used as a component of cattle rations and short-term (3–4 months) feeding increased the content of Se in beef almost 10-fold above the US average (Hintze *et al*., 2001; Soto-Navarro *et al*., 2004). Selenium from soy protein isolate was reported to be more bioavailable to preschool children than Se from milk (Solomons *et al*., 1986). Low bioavailability has been reported for Se from mushrooms (Mutanen, 1986).

Does Se enrichment of a plant cause any unintended interaction?

Selenium and Se-enriched foods have been investigated rigorously, but the enrichment of foods with Se has been done without consideration of interactions with other nutritive and/or non-nutritive components. However, reports of a novel interaction between Se and glucosinolates in broccoli provide an example of an unintended consequence of manipulation of a single bioactive compound. Selenium-enriched broccoli used in animal cancer trials (Davis *et al*., 1999; Finley *et al*., 2000; Finley, 2003*a*) is from a commercially available variety produced by fertilization with Se during the period when the floret develops and matures (Finley, 1998). Although no other growth conditions were altered, Se fertilization potently inhibited Sf production (by as much as 75 %, compared with unfertilized controls) (Charron *et al*., 2001; Robbins *et al*., 2004), and changed the profile and decreased the total amount of polyphenols (Robbins *et al*., 2004).

Rats that consumed Se-enriched broccoli also had an unexpected metabolic alteration brought about by the interaction of Se and Sf. Thioredoxin reductase (TR) is a selenoprotein (Mustacich and Powis, 2000); the production of TR is highly regulated by Se availability at the translational level, and beyond a certain point, additional dietary Se does not increase selenoprotein production (Burk and Hill, 1993). However, broccoli and/or Sf induces TR protein and activity beyond the maximum normally induced by Se alone (Hintze *et al*., 2003*a*; Zhang *et al*., 2003*a*). The proposed mechanism for this induction is that SF activates TR transcription by activating an ARE on the TR promoter (Hintze *et al*., 2003*b*). Thus feeding Se and Sf simultaneously causes a simultaneous increase in transcription and translation, synergistically increasing TR activity beyond the maximum induced by either compound alone (Hintze *et al*., 2003*b*; Zhang *et al*., 2003*a*). The functional consequences for cancer reduction are unclear as TR is a powerful antioxidant whereas reduced thioredoxin is a potent activator of many growth genes; therefore upregulation of thioredoxin reductase has the potential to induce as well as inhibit cancer (Mustacich and Powis, 2000; Powis *et al*., 2000).

Assessment of evidence of efficacy for selenium. Based on proposed decision criteria (Fig. 1), in many ways the strongest argument for cancer prevention can be made for Se-enhanced foods. Many cellular and animal studies have been conducted under the umbrella of a strong mechanistic hypothesis (however, again diverse techniques, cell and animal models and multiple hypotheses dilute these findings somewhat). These data combined with epidemiologic evidence have been the basis for multiple human clinical trials, and all of the reported trials have found a chemoprotective effect of Se. However, there are problems with the clinical trials; several trials are not readily applicable to healthy subjects eating balanced diets, and the strongest trial was done in a specialized subset of subjects (subjects that had a prior incidence of skin cancer) (Clark *et al*., 1996). Moreover, a trial that will hopefully produce definitive results is currently underway and even interim results are not anticipated for several years.

Thus the process proposed in Fig. 1 would suggest that the data are probably strong enough to begin production of candidate foods, but a marketable food product depends on positive results from the current prostate cancer intervention

trial. The production of Se-enriched foods is challenging, however, as both the chemical form and total amount of Se may be influenced by many variables. Additionally the methods traditionally used to assess Se bioavailability may have little relevance to cancer reduction. Finally, studies with Se-enriched foods have demonstrated that enrichment of one bioactive compound may cause a concomitant decrease in other important compounds, indicating that it may be very difficult to produce 'super-fortified' plants. The proposed decision process (Fig. 1) would indicate that, in addition to waiting on the results of the current cancer trial, further product and method development and further experimentation are warranted before Se-enriched plant foods may truly be marketed for cancer-inhibiting properties.

SUMMARY

Evidence is increasing that the consumption of bioactive compounds in vegetables reduces the risk of cancer. The possibilities of designing foods that will help reduce the risks of specific cancers have been a great impetus to the 'functional food' industry. However, there are major obstacles and if they are not overcome they could erode consumer confidence and dampen enthusiasm for nutritionally enhanced plant foods. Criteria have been proposed (Fig. 1) to evaluate (*a*) the evidence for reduction of cancer risk by the bioactive compound, and (*b*) the ability of food containing a bioactive compound to reduce cancer risk without compromising the function of other compounds in the food.

Polyphenolic compounds are the source of intense research interest but, aside from specific compounds such as ECGC found in green tea, the emerging data are not providing sufficient evidence to warrant production of even candidate polyphenol-enhanced plant foods. Glucosinolates and lycopene are compounds with emerging, but as of the present, inconsistent evidence of efficacy. Although the proposed decision process would question production of foods enhanced with these compounds at this stage, foods are being produced and marketed. The evidence for efficacy of lycopene and GS from foods therefore needs to be evaluated and, at present, the variability between products is a major obstacle that must be overcome. β-Carotene is an example of a compound that circumvented much of the proposed decision process, and consequently the non-nutritive functions of β-carotene are still questionable, and enhancing β-carotene in plants (for non-nutritive benefits) is not warranted at this point. There is strong initial evidence, as well as evidence from clinical trials, for the chemopreventive benefits of selenocompounds. However, the largest and most comprehensive clinical trial is still in progress and interim results are not anticipated for several years. Additionally, there are methodological problems associated with the production of Se-enhanced foods, and many methods used to evaluate Se bioavailability may not be applicable to its cancer-preventive function. Thus at this point, production of Se-enriched candidate compounds seem warranted, but marketing such foods should be postponed until results of the ongoing clinical trial are known. Additionally, it remains to be demonstrated that foods can be enriched with consistent amounts and chemical forms of bioavailable Se.

Thus a review of the literature regarding carotenoids, glucosinolates, polyphenols and selenocompounds finds many gaps in our knowledge of how such compounds affect the cancer process and how they can be enhanced in foods. While such gaps should serve to slow down the rush to develop and market such foods, the available evidence indicates that they have the potential to help reduce the risk of our primary health problems, especially heart disease and cancer. Consequently the gaps in our current understanding of these compounds and plants that produce them should not dampen enthusiasm for work in this area, but instead should serve as an incentive for plant and nutritional scientists to develop joint strategies for improvement of health through food.

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