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Review Article

Chemoprotective effects of broccoli and other *Brassica* vegetables

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Abstract

Numerous epidemiological studies indicate that *Brassica* vegetables protect humans against cancer. The family Cruciferae genus *Brassica* consists of the species *Brassica oleracea* (*e.g.*, broccoli, cabbage, cauliflower, Brussels sprouts, kale, turnips, collards), which are the most frequently consumed vegetables worldwide. *Brassica* vegetables are the richest sources of glucosinolates (β-thioglycoside-*N*-hydroxysulfates), which have considerable anticarcinogenic properties. Glucosinolates are hydrolyzed to biologically active isothiocyanates by the plant enzyme myrosinase when the cells in the plant are damaged (*e.g.*, cut, ground or chewed). Glucoraphanin, which accounts for 35-50% of broccoli glucosinolates, is converted to sulforaphane (1-isothiocyannato-4-(methylsulfinyl)butane), a potent monofunctional phase II enzyme inducer. Several animal studies demonstrated that *Brassica* vegetables are protective against various classes of DNA-reactive carcinogens. They can reduce chemically induced tumor formation, attenuate the effects of polycyclic aromatic hydrocarbons and nitrosoamines and have a protective effect against heterocyclic amines. This review outlines *Brassica* vegetables and their metabolism, chemoprotective mechanisms of action, pharmacogenomics and adverse effects. In addition, the overview of recently conducted epidemiological studies on risk reduction of different cancers due to consumption of *Brassica* vegetables is provided.

Introduction

According to the World Cancer Research Fund and the American Institute for Cancer Research, an estimated 40% of all human cancers are related to diet. It is believed that the relationship of diet to cancer is not necessarily due to the inclusion of carcinogens in our diet, but rather may be a consequence of excluding anticarcinogens from our meals.

Numerous epidemiological studies indicate that *Brassica* vegetables protect humans against cancer. The family Cruciferae, genus *Brassica,* consists of the species *Brassica oleracea* (*e.g.*, broccoli, cabbage, cauliflower, Brussels sprouts, kale, turnips, collards) (Fig. 1), which are the most frequently consumed vegetables worldwide. The unique effectiveness of *Brassica* vegetables in protecting against neoplastic disease is attributed to the fact that they are the richest sources of glucosinolates (β-thioglycoside-*N*-hydroxysulfates), which have considerable anticarcinogenic properties.

Glucosinolates and their metabolism

There are more than 120 known different glucosinolates (1), all of which have a common structure comprising a β-D-thioglucose group, a sulfonated oxime moiety

Fig. 1. Broccoli.

and variable side-chains derived from methionine, tryptophan, phenylalanine or branched-chain amino acids (2). They are nonvolatile, hydrophilic compounds that occur as unstable anions due to their highly acidic sulfate group. Glucosinolates are generally sequestered as potassium salts in plant vacuoles (1). In *Brassica* vegetables they occur at concentrations of 500-2000 μg/g, with the highest amounts in Brussels sprouts, cabbage and broccoli. Condiments made from *Brassica* plants (*i.e.,* horseradish and mustard) contain glucosinolates in concentrations as high as 75,000 μg/g (3).

Glucosinolates are hydrolyzed to biologically active isothiocyanates by the plant enzyme myrosinase (EC 3.2.3.1) when the cells in the plant are damaged (*e.g.*, cut, ground or chewed). Cooking of vegetables inactivates myrosinase, but intestinal microbial metabolism also contributes to isothiocyanate production. It has been documented that isothiocyanates are about 6 times more available than glucosinolates, which must be hydrolyzed first (4).

In *Brassica* species, isothiocyanates and indoles are the most frequently studied breakdown products of glucosinolates (mostly brassinin, sinigrin, sulforaphane, indole-3-carbinol and phenethylisothiocyanate) (5). Apart from glucosinolates, *Brassica* plants contain other potentially protective agents, such as flavonoids, polyphenols, vitamins, fiber and pigments.

Glucosinolate profiles and concentrations vary in *Brassica* species under different environmental, growth, storage and preparation conditions. Glucosinolates and their breakdown products are hydrophilic, and as much as 63% of the glucosinolate content may leach into the cooking water during boiling (6). Frozen broccoli samples may contain a significantly lower glucosinolate content due to unfavorable storage conditions or to their removal during the blanching process (7).

In broccoli, different glucosinolates were quantified (4) (Table I). The content of the four major glucosinolates glucoraphanin, glucoiberin, glucobrassicin and neoglucobrassicin varies significantly among broccoli samples. Glucoraphanin, which accounts for 35-50% of broccoli glucosinolates, is converted to the isothiocyanate sulforaphane, a potent monofunctional phase II enzyme inducer mainly responsible for the anticancer activity of broccoli and *Brassica* spp. (7). Glucobrassicin is broken down to indole-3-carbinol, which is further converted to several polyaromatic indolic metabolites under acidic conditions in the stomach (Fig. 2).

Isothiocyanates are metabolized *in vivo* via the mercapturic acid pathway. Indole glucosinolates are degraded by myrosinase to a variety of indole structures, including indole-3-carbinol, 3,3'-diindolylmethane, indole-3-acetonitrile, indole-3-acetic acid and ascorbigen (5). In the body, these indole-containing compounds are converted to indolo[3,2-*b*]carbazole. Thiol conjugates of isothiocyanates are formed by conjugation with glutathione, a reaction catalyzed by glutathione *S*-transferase (GST). Subsequent stepwise cleavage of glutamine and glycine yields L-cysteine-isothiocyanates, which are acetylated to produce *N*-acetyl-L-cysteine conjugates (mercapturic acids) and excreted in urine as dithiocarbamates.

Broccoli sprouts have been shown to be an exceptionally rich source of chemoprotective glucoraphanin (the glucosinolate of sulforaphane), with levels up to 50 times higher that those found in mature plants (4, 7, 8). In animal studies, broccoli sprouts demonstrated very efficient inhibition of rat tumorigenesis (7). Studies comparing glucosinolate profiles of mature broccoli and broccoli sprouts have shown that the sprouts contain mainly alkylthioglucosinolates (glucoraphanin, glucoerucin and glucoiberin). The major glucosinolates in mature broccoli are typically indoles (glucobrassicin, neoglucobrassicin and smaller quantities of 4-hydroxyglucobrassicin), which account for about 68% of the total glucosinolates. In broccoli sprouts, indole glucosinolates account, on average, for only 3% of total glucosinolates (7).

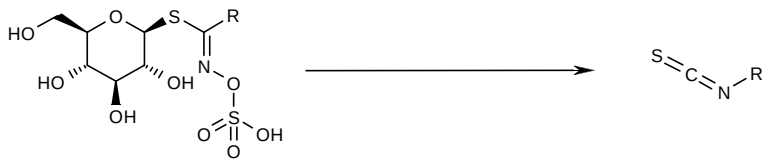
The chemical structures of other components of broccoli are shown in Figure 3.

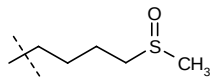
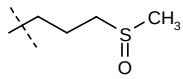
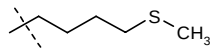
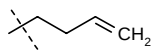
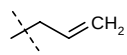
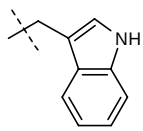
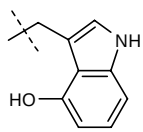
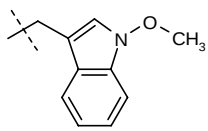
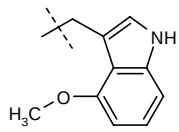
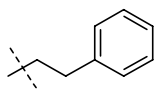
Chemoprotective mechanisms of *Brassica* plants

The chemoprotective mechanism of action of *Brassica* plants is due to their high content of glucosinolates and the ability of glucosinolate metabolites, isothiocyanates and indoles to modulate the biotransformation of phase I and II enzyme activities (9, 10). Phase I enzymes (cytochrome P-450 and flavin-dependent monooxygenases) convert hydrophobic compounds to reactive electrophiles by oxidation, hydroxylation and reduction reactions to prepare them for reaction with water-soluble moieties. Phase II enzymes (*e.g.,* GSTs, UDP-glucuronosyltransferase [UDPGT], sulfotransferases, *N*-acetyltransferases) catalyze conjugation reactions.

Isothiocyanates and indoles from *Brassica* spp. can affect biotransformation enzyme activity by several mechanisms; they induce the expression of phase I and phase II enzymes and, to a lesser extent, directly inhibit the cytochrome P-450 pathway (9). However, the mechanisms of action of indoles and isothiocyanates differ. Indoles, as bifunctional inducers, affect phase I as well as phase II enzyme activities and can act via an interaction with arylhydrocarbon receptors (AhR) (2). In the body, indoles are either chemically or enzymatically converted to indolo[3,2-*b*]carbazole, a moderate AhR agonist. The activated AhR binds to specific sites on DNA and induces the expression of P-450 enzymes of the CYP1 family in hepatic and extrahepatic tissue. Isothiocyanates are considered monofunctional inducers; however, some of them can induce phase I or II enzymes, and some can induce both phase I and II enzymes. Generally, compounds that induce both phase I and phase II steps are thought to speed carcinogenic compounds through the metabolic pathway toward elimination, whereas agents that induce only phase I are thought to accelerate chemical carcinogenesis (9, 10).

Table I: Broccoli glucosinolates.



Glucosinolate	R	Isothiocyanate
Glucoraphanin		Sulforaphane
Glucoiberin		3-(Methylsulfinyl)propylisothiocyanate
Glucoerucin		
Gluconapin		
Sinigrin		Allyl isothiocyanate
Glucobrassicin		
4-Hydroxyglucobrassicin		
Neoglucobrassicin		
4-Methoxyglucobrassicin		
Gluconasturtiin		Phenethyl isothiocyanate

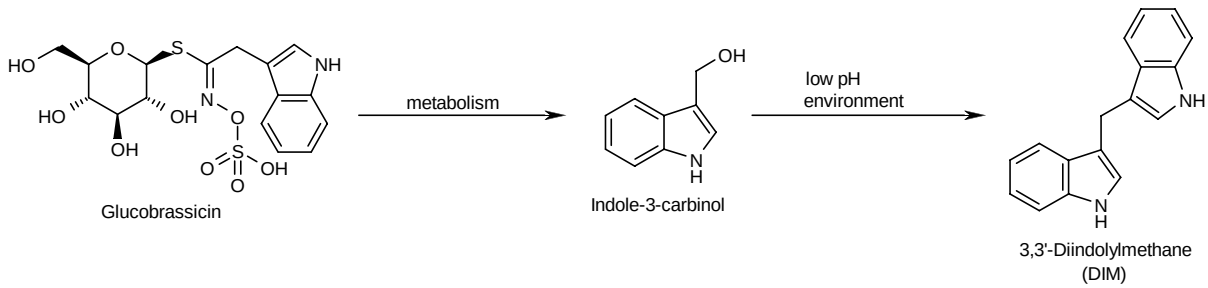
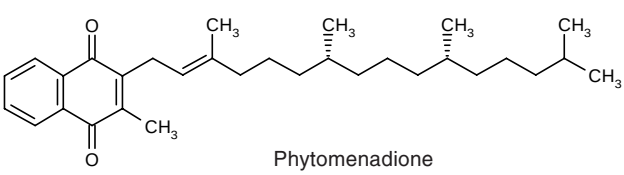
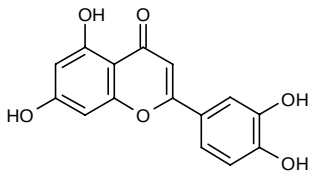


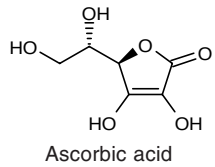
Fig. 2. Metabolism of glucobrassicin.



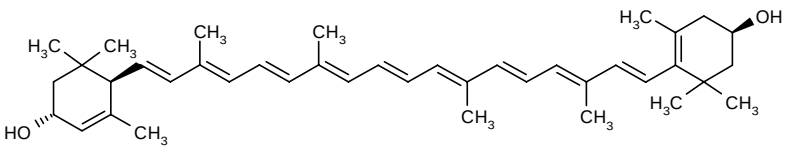
Phytomenadione



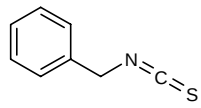
Luteolin



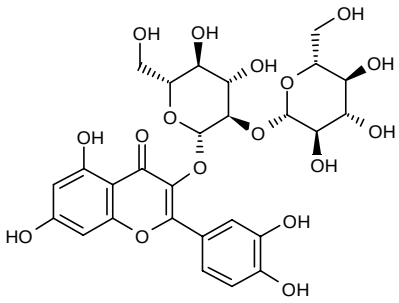
Ascorbic acid



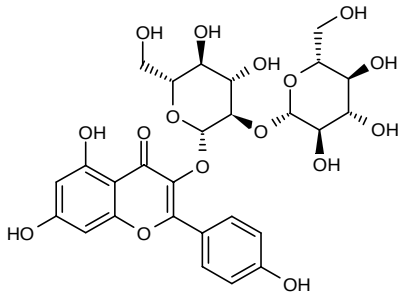
Lutein



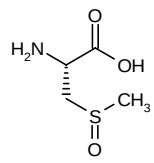
BITC



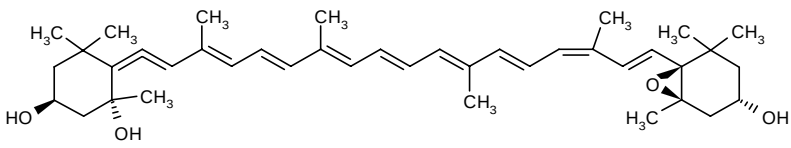
Quercetin 3-*O*-sophoroside



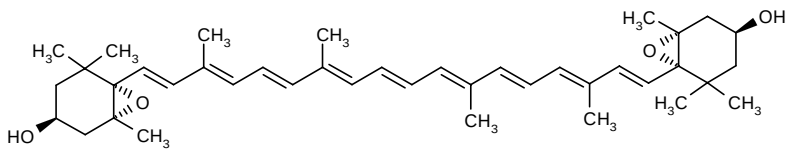
Kaempferol 3-*O*-sophoroside



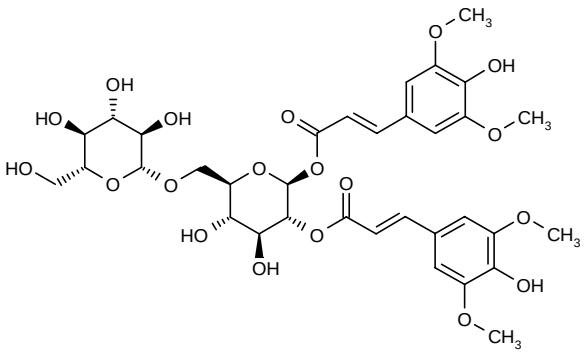
S-Methylcysteine sulfoxide



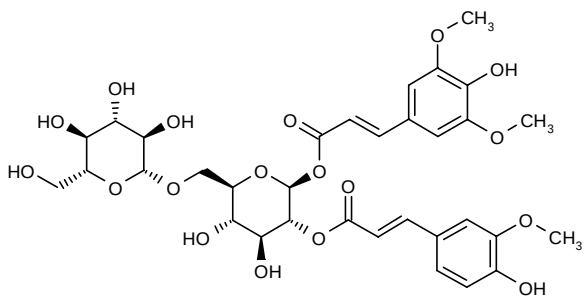
Neoxanthin



Violaxanthin



1,2-Disinapolylgentiobioside



1-Sinapoyl-2-feruloylgentiobioside

Fig. 3. Other components of broccoli.

Continued

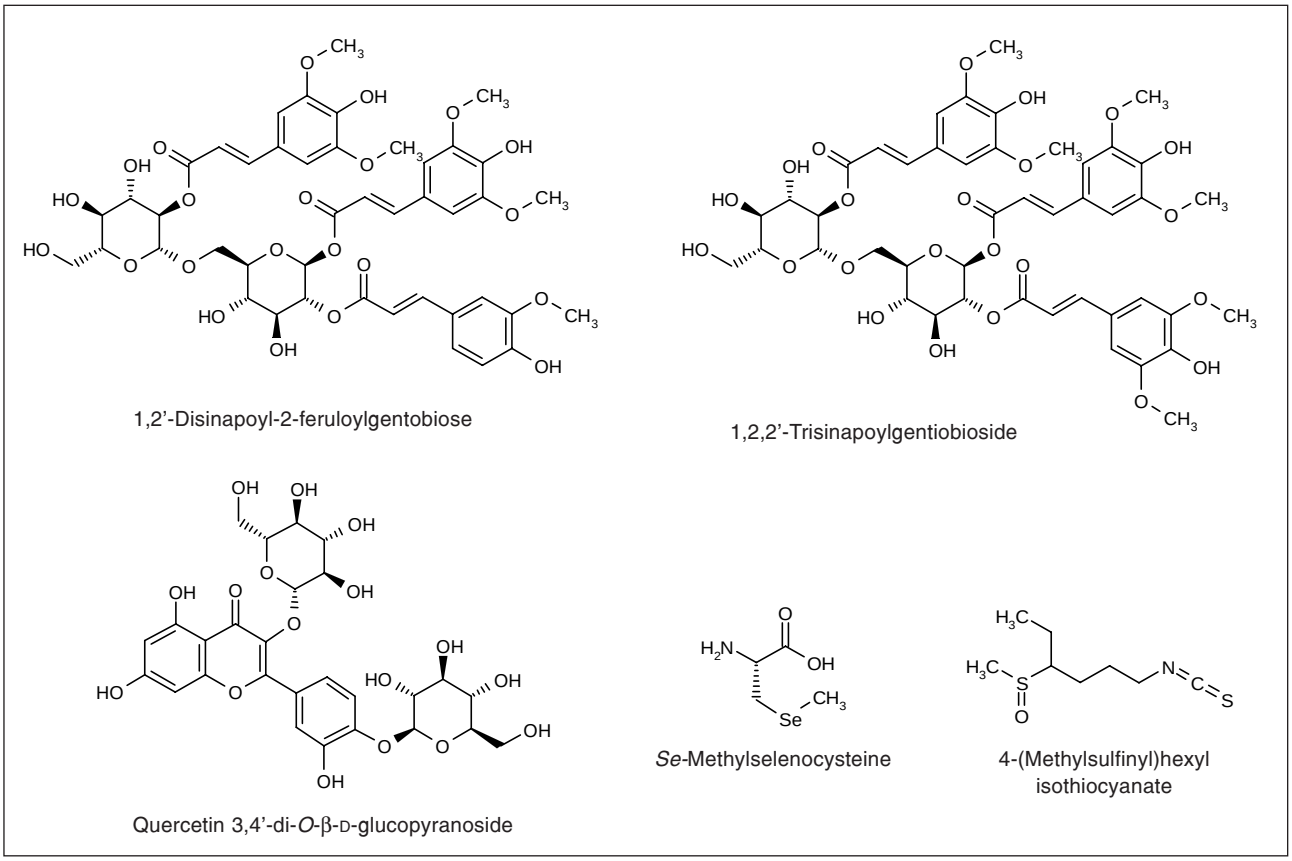


Fig. 3. (Cont.) Other components of broccoli.

More detailed studies on the molecular mechanism of action of indoles and isothiocyanates have been described recently by Lampe and Peterson (9). Binding of indole derivatives (*e.g.,* 3,3'-diindolylmethane) to the AhR leads to translocation of the AhR complex to the nucleus and interaction with xenobiotic response elements (XRE) in the gene promoter. The recruitment of transcription factors and coactivators results in transactivation. Induction of CYP1A, CYP1B, GSTs, NAD(P)H:quinone oxidoreductase (NQO1) and UDPGT is mediated by AhR. The inducing potency of indoles depends on their affinity for AhR. Isothiocyanates (*e.g.*, sulforaphane or phenethyllisothiocyanate) activate genes through the antioxidant/electrophile response element (ARE/EpRE). Isothiocyanates dissociate the cytoplasmic protein Kelch-like ECH-associated protein (Keap1) from the transcription factor Nrf2 (nuclear factor-erythroid 2-related factor 2), allowing it to translocate to the nucleus and to form Nrf2/Maf (musculoaponeurotic fibrosarcoma virus) heterodimers, which activate transcription through the ARE/EpRE. Regulation of NQO1, γ-glutamylcysteine synthase and several GSTs is mediated by ARE/EpRe.

Recent studies point to different mechanisms of action of glucosinolates that may further help explain the protective effects of *Brassica* vegetables against cancer. Studies of the molecular events involved in the activation of a gene promoter called the antioxidant responsive element (ARE) suggest that substances such as sulforaphane may induce activity and protein expression of thioredoxin reductase (TR), a selenium-dependent protein that determines intracellular oxidative status (1). Several indoles and isothiocyanates from *Brassica* have been found to induce apoptosis and regulate cellular proliferation in human colon adenocarcinoma cells and breast tumor cells (5, 11). Some studies suggest that indoles downregulate the epidermal growth factor receptor (EGFR) and reduce the activity of the antiapoptotic proto-oncogene Akt. Indole exposure may shift estrogen metabolism to favor catechol estrogens with less affinity for the estrogen receptor and which exhibit tamoxifen-like properties (6, 11).

Pharmacogenomics of glucosinolates

It has been documented that the relationship between *Brassica* intake and cancer risk is influenced by the genetic polymorphisms in biotransformation enzymes that metabolize isothiocyanates, as well as in receptors and transcription factors that interact with these compounds (9). Data from molecular epidemiological studies suggest that genetic and associated functional variations in biotransformation enzymes lead to individual differences in cancer risk in response to *Brassica* vegetables.

For example, one hypothesis sustains that individuals with the GST-null genotypes are at higher risk for cancer because of a reduced capacity to dispose of activated carcinogens. Other studies focusing on the relationship between GST polymorphism and exposure to isothiocyanates suggest that the polymorphisms associated with reduced GST activity may result in longer circulating half-lives of isothiocyanates and potentially greater chemoprotective effects of *Brassica* vegetables.

There is accumulating evidence that *Brassica* vegetables are protective towards various classes of DNA-reactive carcinogens (2). The development of *in vitro* models with metabolically competent cell lines led to the detection of potent enzyme inducers such as sulforaphane. *Brassica* juices induce GST and cytochrome P-450 1A2 in human hepatoma cells and protect against the genotoxic effects of benzo[*a*]pyrene and other carcinogens. Animal data showed that indoles and isothiocyanates attenuate the effects of polycyclic aromatic hydrocarbons and nitrosoamines via the induction of GST and inhibition of cytochrome P-450 isozymes.

Studies showed that *Brassica* vegetables are also protective against heterocyclic aromatic amines and that they attenuate DNA damage and preneoplastic lesions in the colon and liver of rats (2). These effects are accompanied by the induction of UDPGT, which is considered as the mechanism of protection against heterocyclic aromatic amines by *Brassica* vegetables.

Adverse effects of glucosinolates

Glucosinolates and products of their hydrolysis can result in adverse effects in experimental animals when administered at doses exceeding the normal daily consumption (10). Indoles, especially when tested as pure substances, have an ability to form carcinogenic and mutagenic *N*-nitroso compounds with nitrites. In experimental animals, after isothiocyanate administration, a decrease in food consumption, body weight gain and renal dysfunction, and an increase in serum cholesterol levels were reported. Indole-3-carbinol elicited hepatotoxicity and neurological impairment in mice. In humans, however, no adverse effects have been identified at doses close to normal daily consumption. *Brassica* vegetables have the potential to cause goiters, which can be ascribed to the hydrolysis products of glucosinolates. In experimental animals, the thiocyanate ion may compete with iodine for uptake by the thyroid gland, and goitrin may interfere with thyroid hormone synthesis. However, in humans, no effect of a high but realistic intake of *Brassica* on thyroid function was found.

Anticarcinogenic effects of broccoli and other *Brassica* vegetables

Chemoprotective effects of broccoli and other *Brassica* plants should be considered from the prevention standpoint. Cancer chemoprevention is defined as pharmacological intervention with synthetic or naturally occurring compounds that may prevent, inhibit or reverse carcinogenesis, or prevent the development of invasive cancer (5). There is significant evidence that human dietary patterns are associated with cancer risk. This complex association may be a function of interactions of dietary components with each other, other environmental exposures, and genetic factors that affect regulation of gene expression and cell growth. Most evidence for the anticarcinogenic effects of *Brassica* vegetables and glucosinolates has come from animal studies, although increasing data are emerging from human studies.

Epidemiological data on cancer risk reduction due to the consumption of *Brassica* vegetables have been summarized in several comprehensive reports. Van Poppel *et al*. (12) reviewed 6 cohort and 74 case-control studies on the association between *Brassica* consumption and cancer risk. The cohort studies showed inverse associations between *Brassica* consumption and the risk of lung, stomach and all cancers taken together. In the case-control studies, 64% of all studies found significant inverse associations between *Brassica* intake and cancer risk, mostly for lung, stomach, colon and rectal cancer, and least consistent for prostate, endometrial and ovarian cancer. The authors stated that, although these findings might have been distorted by bias, it can be concluded that a high consumption of *Brassica* vegetables is associated with a decreased risk of cancer.

In animal models of breast cancer, administration of isothiocyanates or indole-3-carbinol, or *Brassica* consumption, reduces tumor incidence or delays tumor onset. Broccoli sprouts were highly effective in reducing the incidence, multiplicity and rate of development of mammary tumors in dimethylbenz[*a*]anthracene-treated animals (7). Although human studies do not show a consistent association between *Brassica* intake and breast cancer risk, some studies postulated that consumption of 1-2 servings of *Brassica* vegetables per day may reduce the risk of breast cancer by as much as 20-40% (5). It has been demonstrated that *Brassica* vegetables consumption was associated with higher values of an endocrine biomarker, the urinary ratio of 2-hydroxyestrone to 16-hydroxyestrone, in healthy postmenopausal women (6). This suggests that *Brassica* consumption can shift estrogen profiles towards reducing breast cancer risk (6, 11). Recent case-control studies in Chinese women with breast cancer (11) demonstrated that urinary isothiocyanate levels (a biological measure of glucosinolate intake) were inversely associated with breast cancer (OR = 0.5).

There is also a large body of evidence indicating that dietary patterns are associated with prostate cancer risk (3). Sulforaphane induces growth arrest in several prostate cancer cell lines, and the chemopreventive effect of indole-3-carbinol involves cellular mechanisms such as

blockade of cell cycle progression or the induction of apoptosis. Epidemiological studies provide modest support for the hypothesis that high intake of *Brassica* vegetables reduces prostate cancer risk. Of 12 studies, 3 reported a statistically significantly reduced risk and 1 reported a borderline significantly reduced risk with high *Brassica* vegetable consumption (3). The consumption of three or more servings of cruciferous vegetables per week was inversely related to disease, showing a decrease in risk of 41% (5), especially in more advanced cases, as compared to an intake of 1 serving per week. It has also been shown that the intake of cruciferous vegetables was inversely associated with the risk of bladder cancer (RR = 0.49) when more than 5 servings per week were consumed in males (5).

Data from molecular epidemiological studies suggest that genetic and associated functional variations in biotransformation enzymes lead to individual differences in cancer risk in response to *Brassica* vegetables. Heterocyclic aromatic amines can be possible risk factors for colon cancer. Several studies showed that consumption of cruciferous vegetables increases the metabolism of food-derived heterocyclic aromatic amines , thereby reducing the levels of unchanged amines excreted in urine (5).

Approximately 50% of the general population is unable to express glutathione *S*-transferase M1 (GSTM1), an enzyme which conjugates isothiocyanates, due to gene deletion. The null genotype was related to an increased risk of colorectal cancer. Broccoli consumption, in combination with the GSTM1-null genotype, was associated with a lower prevalence of colorectal adenoma due to high levels of isothiocyanates (5).

Low isothiocyanates intake in combination with the GSTM1-null genotype was associated with an increased risk of lung cancer in smokers (OR = 2.22), whereas the odds ratio for the GSTT1-null phenotype was 3.19, and for both GSTs it increased to 5.45 (5). The consumption of cruciferous vegetables significantly reduced the risk of lung cancer in smokers with both GSTM1 and GSTT1 (OR = 0.31 *vs*. OR = 0.70 in nonsmokers). The risk in nonsmokers with the GSTM1-null genotype was also reduced with a higher intake of isothiocyanates (OR = 0.54), whereas this effect was not observed among individuals with detectable GSTM1. In a prospective case-control study in men in Shanghai using the urinary isothiocyanate biomarker, a protective interaction between *Brassica* consumption and lung cancer was observed (13). The reduction in risk was strongest among persons genetically deficient in GSTM1 and GSTT1, enzymes that rapidly eliminate isothiocyanates.

Sulforaphane has been found to have bactericidal properties against both extracellular and intracellular forms of *Helicobacter pylori* (14). In animal studies, sulforaphane also prevented benzo[*a*]pyrene-induced stomach tumors, an effect dependent on induction of phase II enzymes because it was abolished in mice deficient in the Nrf2 gene, which controls this induction. Human studies demonstrated that *H. pylori* eradication in patients with *H. pylori*-associated gastritis elevated or restored GST activity and glutathione levels in the antral mucosa. The dual action of sulforaphane in inhibiting *Helicobacter* infections and blocking gastric tumor formation suggests that these mechanisms might function synergistically and supports the concept that sulforaphane has a chemopreventive effect in humans.

In conclusion, the last two decades of research have demonstrated that broccoli and *Brassica* vegetables are among the most promising chemoprotective dietary constituents. Further studies on their mechanisms of action and identification of active agents may contribute to the development of new directions in chemoprevention, as well as new anticancer therapies.

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Online links

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An advanced search of the Prous Science **Integrity**® drug discovery and development portal using the common name "broccoli" in the Drugs & Biologics knowledge area retrieves 32 compounds derived from this vegetable. A stream-lined chart format of the therapeutic impact of these compounds is swiftly displayed and reveals 23 chemopreventive agents, 11 oncolytics and 1 anti-*Helicobacter* compound. More than 490 references as well as patents and chemical structures can also be displayed via Integrity (http://integrity.prous.com/).