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Cruciferous Vegetables and Human Cancer Risk: Epidemiologic Evidence and Mechanistic Basis

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Abstract

Cruciferous vegetables are a rich source of glucosinolates and their hydrolysis products, including indoles and isothiocyanates, and high intake of cruciferous vegetables has been associated with lower risk of lung and colorectal cancer in some epidemiological studies. Glucosinolate hydrolysis products alter the metabolism or activity of sex hormones in ways that could inhibit the development of hormone-sensitive cancers, but evidence of an inverse association between cruciferous vegetable intake and breast or prostate cancer in humans is limited and inconsistent. Organizations such as the National Cancer Institute recommend the consumption of 5–9 servings of fruits and vegetables daily, but separate recommendations for cruciferous vegetables have not been established. Isothiocyanates and indoles derived from the hydrolysis of glucosinolates, such as sulforaphane and indole-3-carbinol (I3C), have been implicated in a variety of anticarcinogenic mechanisms, but deleterious effects also have been reported in some experimental protocols, including tumor promotion over prolonged periods of exposure. Epidemiological studies indicate that human exposure to isothiocyanates and indoles through cruciferous vegetable consumption may decrease cancer risk, but the protective effects may be influenced by individual genetic variation (polymorphisms) in the metabolism and elimination of isothiocyanates from the body. Cooking procedures also affect the bioavailability and intake of glucosinolates and their derivatives. Supplementation with I3C or the related dimer 3,3′-diindolylmethane (DIM) alters urinary estrogen metabolite profiles in women, but the effects of I3C and DIM on breast cancer risk are not known. Small preliminary trials in humans suggest that I3C supplementation may be beneficial in treating conditions related to human papilloma virus infection, such as cervical intraepithelial neoplasia and recurrent respiratory papillomatosis, but larger randomized controlled trials are needed.

Keywords

Brassica; glucosinolates; genetic polymorphisms; isothiocyanates; indole-3-carbinol; epigenetics

1. Introduction

Cruciferous or *Brassica* vegetables come from plants in the family known to botanists as Cruciferae or alternatively, Brassicaceae. Plants in the Cruciferae family have flowers with four equal-sized petals in the shape of a ‘crucifer’ cross. “Brassica” is the latin term for cabbage.

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Many commonly consumed cruciferous vegetables come from the *Brassica* genus, including broccoli, Brussels sprouts, cabbage, cauliflower, collard greens, kale, kohlrabi, mustard, rutabaga, turnips, bok choy and Chinese cabbage [1]. Although not in the *Brassica* genus, arugula, horseradish, radish, wasabi and watercress are also cruciferous vegetables. Like other vegetables, cruciferous vegetables contain a number of nutrients and phytochemicals with cancer chemopreventive properties, including folate, fiber, carotenoids and chlorophyll. However, cruciferous vegetables are unique in that they are rich sources of glucosinolates, sulfur-containing compounds that are responsible for their pungent aromas and spicy (some say bitter) taste [2]. The hydrolysis of glucosinolates by the plant enzyme myrosinase results in the formation of biologically active compounds, including indoles and isothiocyanates (Figure 1) [3]. More than 100 glucosinolates with unique hydrolysis products have been identified in plants. For example, broccoli is a good source of glucoraphanin, the glucosinolate precursor of sulforaphane (SFN), and glucobrassicin, the precursor of indole-3-carbinol (I3C) [4]. In contrast, watercress is a rich source of gluconasturtiin, the precursor of phenethyl isothiocyanate (PEITC). Table 1 lists some of the isothiocyanates and indoles that are currently under investigation for their cancer chemopreventive properties, along with their glucosinolate precursors. The purpose of this article is to review the available research on cruciferous vegetable intake and human cancer risk, with particular attention to those compounds that make cruciferous vegetables unique—glucosinolates and their biologically active hydrolysis products.

2. Cruciferous Vegetables and Human Cancer Risk: Epidemiologic Evidence

Like most other vegetables, cruciferous vegetables are good sources of a variety of nutrients and phytochemicals that may work synergistically to help prevent cancer [5]. One challenge in studying the relationships between cruciferous vegetable intake and cancer risk in humans is separating the benefits of diets that are generally rich in vegetables from those that are specifically rich in cruciferous vegetables [6]. An extensive review of epidemiologic studies published prior to 1996 reported that the majority (67%) of 87 case-control studies found an inverse association between some type of cruciferous vegetable intake and cancer risk [7]. At that time, the inverse association appeared to be most consistent for cancers of the lung and digestive tract. The results of retrospective case-control studies are more likely to be distorted by bias in the selection of participants (cases and controls) and dietary recall than prospective cohort studies, which collect dietary information from participants before they are diagnosed with cancer [8]. In the past decade, results of large prospective cohort studies and studies taking into account individual genetic variation suggest that the relationship between cruciferous vegetable intake and the risk of several types of cancer is more complex than previously thought. Findings for lung, colorectal, breast and prostate cancer, which are the four major causes of cancer-related death in the US, are summarized next,

2.1. Lung Cancer

When evaluating the effect of cruciferous vegetable consumption on lung cancer risk, it is important to remember that the benefit of increasing cruciferous vegetable intake is likely to be small compared to the benefit of smoking cessation [9,10]. Although a number of case-control studies found that people diagnosed with lung cancer had significantly lower intakes of cruciferous vegetables than people in cancer-free control groups [7], the findings of more recent prospective cohort studies have been mixed. Prospective studies of Dutch men and women [11], U.S. women [12] and Finnish men [13] found that higher intakes of cruciferous vegetables (more than three weekly servings) were associated with significant reductions in lung cancer risk, but prospective studies of U.S. men [12] and European men and women [9] found no inverse association. The results of several studies suggest that genetic variation affecting the metabolism of glucosinolate hydrolysis products may influence the effects of

cruciferous vegetable consumption on lung cancer risk[14–17] (see Genetic Polymorphisms below).

2.2. Colorectal Cancer

A small clinical trial found that the consumption of 250 g/d (9 oz/d) of broccoli and 250 g/d of Brussels sprouts significantly increased the urinary excretion of a potential carcinogen found in well-done meat, namely 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) [18]. Walters *et al*. concluded that high cruciferous vegetable intake might decrease colorectal cancer risk by enhancing the elimination of PhIP and related dietary heterocyclic amine carcinogens. Although a number of case-control studies conducted prior to 1990 found that people diagnosed with colorectal cancer were more likely to have lower intakes of various cruciferous vegetables than people without colorectal cancer [19–22], most prospective cohort studies have not found significant inverse associations between cruciferous vegetable intake and the risk of developing colorectal cancer over time [23–26]. One exception was a prospective study of Dutch adults, which found that men and women with the highest intakes of cruciferous vegetables (averaging 58 g/d) were significantly less likely to develop colon cancer than those with the lowest intakes (averaging 11 g/d) [27]. Surprisingly, higher intakes of cruciferous vegetables were associated with increased risk of rectal cancer in women in that study. As with lung cancer, the relationship between cruciferous vegetable consumption and colorectal cancer risk may be complicated by genetic polymorphisms. The results of several recent epidemiological studies suggest that the protective effects of cruciferous vegetable consumption may be influenced by inherited differences in the capacity of individuals to metabolize and eliminate glucosinolate hydrolysis products [28–31] (see Genetic Polymorphisms, next).

2.3. Breast Cancer

The endogenous estrogen 17β-estradiol can be metabolized to 16α-hydroxyestrone ($16\alpha OHE_1$) or 2-hydroxyestrone ($2OHE_1$). In contrast to $2OHE_1$, $16\alpha OHE_1$ is highly estrogenic and has been found to enhance the proliferation of estrogen-sensitive breast cancer cells in culture [32,33]. It has been hypothesized that shifting the metabolism of 17β-estradiol toward $2OHE_1$ and away from $16\alpha OHE_1$ could decrease the risk of estrogen-sensitive cancers, such as breast cancer [34]. In a small clinical trial, increasing cruciferous vegetable intake of healthy postmenopausal women for four weeks increased urinary $2OHE_1$:$16\alpha OHE_1$ ratios, suggesting that high intakes of cruciferous vegetables can shift estrogen metabolism. However, the relationship between urinary $2OHE_1$:$16OHE_1$ ratios and breast cancer risk is not clear. Several small case-control studies found that women with breast cancer had lower urinary ratios of $2OHE_1$:$16\alpha OHE_1$ [35–37], but larger case-control and prospective cohort studies did not find significant associations between urinary $2OHE_1$:$16\alpha OHE_1$ ratios and breast cancer risk [38–40]. The results of epidemiological studies of cruciferous vegetable intake and breast cancer risk are also inconsistent. Several recent case-control studies in the US, Sweden and China found that measures of cruciferous vegetable intake were significantly lower in women diagnosed with breast cancer than in cancer-free control groups [41–43], but cruciferous vegetable intake was not associated with breast cancer risk in a pooled analysis of seven large prospective cohort studies [44].

2.4. Prostate Cancer

Although glucosinolate hydrolysis products have been found to inhibit growth and promote death (apoptosis) of cultured prostate cancer cells [45,46], the results of epidemiological studies of cruciferous vegetable intake and prostate cancer risk are inconsistent. Four out of eight case-control studies published since 1990 found that some measure of cruciferous vegetable intake was significantly lower in men diagnosed with prostate cancer than men in a cancer-free control group [47–50]. Of the 4 prospective cohort studies that have examined associations between

cruciferous vegetable intake and the risk of prostate cancer, none found statistically significant inverse associations overall [51–54]. However, the prospective study that included the longest follow-up period and the most cases of prostate cancer found a significant inverse association between cruciferous vegetable intake and the risk of prostate cancer when the analysis was limited to men who had a prostate specific antigen (PSA) test [51]. Since men who have PSA screening are more likely to be diagnosed with prostate cancer, limiting the analysis in this way is one way to reduce detection bias [55]. Presently, epidemiological studies provide only modest support for the hypothesis that high intakes of cruciferous vegetables reduce prostate cancer risk [1].

2.5. Other cancers

At the present time, there is no consistent epidemiological evidence to support a role for cruciferous vegetables as chemopreventive agents against cancers other than the four major types discussed above (lung, colorectal, breast and prostate). One exception may be pancreatic cancer. A recent multiethnic cohort study found no evidence for an inverse association between vegetable intake and pancreatic cancer overall, but inverse associations were seen for dark green vegetables in high-risk persons [56]. In another report [57], among specific subgroups of fruits and vegetables, a non-significant inverse association was observed with cruciferous vegetable consumption (3 or more servings per week *versus* less than one serving/wk: HR, 0.70; 95% CI, 0.43–1.13). Cabbage consumption was associated with a statistically significant lower risk of pancreatic cancer (1 or more servings/wk *versus* never consumption: HR, 0.62; 95% CI, 0.39–0.99). Findings from this prospective study do not support a relationship of overall fruit and vegetable consumption with pancreatic cancer risk, but the association between consumption of cruciferous vegetables and pancreatic cancer risk warrants further investigation. A prior study concluded that increasing vegetable and fruit consumption may impart some protection against developing pancreatic cancer [58].

3. Genetic Polymorphisms

There is increasing evidence that genetic differences in humans may influence the effects of cruciferous vegetable intake on cancer risk [59]. Glutathione *S*-transferases (GSTs) are a family of enzymes that metabolize a variety of compounds, including isothiocyanates, in a way that augments their elimination from the body. Genetic polymorphisms that affect the activity of GST enzymes have been identified in humans. Null variants of the *GSTM1* gene and *GSTT1* gene contain large deletions, and individuals who are homozygous for the *GSTM1*-null or *GSTT1*-null gene cannot produce the corresponding GST enzyme [60]. Lower GST activity in such individuals could result in slower elimination and longer exposure to isothiocyanates after cruciferous vegetable consumption [61]. In support of this idea, several epidemiological studies have found that inverse associations between isothiocyanate intake from cruciferous vegetables and the risk of lung cancer [14–17] or colon cancer [28–30] were more pronounced in *GSTM1*-null and/or *GSTT1*-null individuals. These findings suggest that the protective effects of high intakes of cruciferous vegetables may be enhanced in individuals that eliminate potentially protective compounds like isothiocyanates more slowly.

4. Cooking and Bioavailability of Glucosinolate Hydrolysis Products

Because most glucosinolates are chemically and thermally stable, enzymatic hydrolysis is required for the formation of biologically active isothiocyanates and indoles [3]. Myrosinase, a β-thioglucosidase, is physically separated from glucosinolates in intact plant cells. However, when raw cruciferous vegetables are chopped or chewed, myrosinase comes in contact with glucosinolates and catalyzes the hydrolysis of the glucosidic bond, yielding glucose and an unstable thiohydroxamate-*O*-sulfonate, which undergoes spontaneous rearrangement to form a number of possible products depending on the side chain structure and the reaction conditions

(Figure 1). At neutral pH (6–7), the major glucosinolate hydrolysis products are stable isothiocyanates, with the exception of those with an indole moiety or a β-hydroxy side chain. Unstable β-hydroxy-isothiocyanates undergo spontaneous cyclization to form oxazolidine-2-thiones (e.g., goitrin), and indole isothiocyanates undergo lysis to their corresponding alcohols (e.g., I3C). Thorough chewing of raw cruciferous vegetables increases glucosinolate contact with plant myrosinase and increases the amount of isothiocyanates absorbed [62]. Even when plant myrosinase is completely inactivated by heat, the myrosinase activity of human intestinal bacteria allows for some formation and absorption of isothiocyanates [63].

Glucosinolates are water-soluble compounds that may be leached into cooking water. Boiling cruciferous vegetables for 9–15 minutes resulted in an 18–59% decrease in the total glucosinolate content of cruciferous vegetables [6]. Cooking methods that use less water, such as steaming or microwaving, may reduce glucosinolate losses. However, some cooking practices, including boiling [62], steaming [64] and microwaving at high power (850–900 watts) [65,66] can inactivate myrosinase, the enzyme that catalyzes glucosinolate hydrolysis. Several studies in humans have found that inactivation of myrosinase in cruciferous vegetables substantially decreases the bioavailability of isothiocyanates [62,64,65].

5. Fate of Isothiocyanates and Indoles

Isothiocyanates are metabolized primarily through the mercapturic acid pathway. Conjugation of isothiocyanates to glutathione is facilitated by GSTs. The glutathione conjugates are further metabolized to mercapturic acids by the sequential activity of γ-glutamyltranspeptidase, cysteinylglycinase and *N*-acetyltransferase (Figure 2). Isothiocyanate metabolites can be measured in the urine, and are highly correlated with dietary intake of cruciferous vegetables [61]. There is growing evidence that metabolites generated via the mercapturic acid pathway can contribute to the biological activity of dietary isothiocyanates, such as SFN (see below) [67,68].

Indoles from cruciferous vegetables have received considerable interest as cancer chemoprotective agents, including studies of their bioavailability *in vivo*. In the acidic environment of the stomach, I3C molecules can combine with each other to form a complex mixture of biologically active compounds, known collectively as acid condensation products [69]. Although numerous acid condensation products of I3C have been identified, some of the most prominent include the dimer 3,3′-diindolylmethane (DIM) and a cyclic trimer (CT) (Figure 3). The biological activities of individual acid condensation products differ from those of I3C and may be responsible for most of the biological effects attributed to I3C [70,71]. Information about the metabolism of I3C and its acid condensation products is limited. Oxidative metabolites indole-3-carboxylic acid and lesser amounts of indole-3-carboxaldehyde have been detected in the plasma of mice fed I3C [71].

6. Effects on Xenobiotic Metabolism

Biotransformation enzymes play important roles in the metabolism and elimination of a variety of xenobiotics, including drugs, toxins and carcinogens. In general, phase I biotransformation enzymes catalyze reactions that increase the reactivity of hydrophobic (fat-soluble) compounds, preparing them for reactions catalyzed by phase II biotransformation enzymes. Reactions catalyzed by phase II enzymes generally increase water solubility and promote the elimination of the compound from the body [1]. Bifunctional and monofunctional inducers, including those from cruciferous vegetables, have been reviewed by Kensler and colleagues [72].

6.1. Modulation of Biotransformation by Isothiocyanates

Some chemical carcinogens require biotransformation by phase I enzymes, such as those of the cytochrome P450 (CYP) family, in order to become active carcinogens that are capable of binding DNA and inducing mutations. Inhibition of specific CYP enzymes involved in carcinogen activation inhibits the development of cancer in animal models [64]. Isothiocyanates, including PEITC and BITC, have been found to inhibit carcinogen activation by CYP enzymes in animal studies [73,74]. In human volunteers, ingestion of a watercress homogenate, which is rich in the glucosinolate precursor of PEITC, increased the area under the plasma concentration-time curve for the drug chlorzoxazone, suggesting that CYP2E1 activity was inhibited [74].

Many isothiocyanates, particularly SFN, are potent inducers of phase II enzymes in cultured human cells [4]. Phase II enzymes, including GSTs, UDP-glucuronosyl transferases (UGTs), NADPH quinone oxidoreductase (NQO) and glutamate cysteine ligase play important roles in protecting cells from DNA damage by carcinogens and reactive oxygen species [75]. The genes for these and other phase II enzymes contain a specific sequence of DNA called an antioxidant response element (ARE). Isothiocyanates have been shown to increase phase II enzyme activity by increasing the transcription of genes that contain an ARE [76]. Limited data from clinical trials suggests that glucosinolate-rich foods can increase phase II enzyme activity in humans. When smokers consumed 170 g/d (6 oz/d) of watercress, urinary excretion of glucuronidated nicotine metabolites increased significantly, suggesting UGT activity increased [77]. Brussels sprouts are rich in a number of glucosinolates, including precursors of AITC and SFN. Consumption of 300 g/d (11 oz/d) of Brussels sprouts for a week significantly increased plasma and intestinal GST levels in nonsmoking men [78].

6.2. Modulation of Biotransformation by Indoles

Aggarwal and Ichikawa recently reviewed [79] the molecular targets and anticancer potential of I3C and its indole derivatives, as well as the evidence for tumor promotion in some studies (see below). Acid condensation products of I3C, particularly DIM and indole[3,2-*b*]carbazole (ICZ), can bind in the cytoplasm to the aryl hydrocarbon receptor (Ahr) [70,80]. Binding allows the Ahr to enter the nucleus where it forms a complex with the AhR nuclear translocator (Arnt) protein. This Ahr/Arnt complex binds to specific DNA sequences in genes known as xenobiotic response elements (XRE) and enhances their transcription [81]. Genes for a number of CYP enzymes and several phase II enzymes are known to contain XREs. Thus, oral consumption of I3C results in the formation of acid condensation products that can increase the activity of certain phase I and phase II enzymes [80–83]. Increasing the activity of biotransformation enzymes is generally considered a beneficial effect because the elimination of potential carcinogens or toxins is enhanced. However, there is a potential for adverse effects, because some procarcinogens require biotransformation by phase I enzymes to become active carcinogens [84].

7. Effects on Estrogen Metabolism and Activity

Estrogens, including 17β-estradiol, exert their estrogenic effects by binding to estrogen receptors (ERs). Within the nucleus, the estrogen-ER complex can bind to DNA sequences in genes known as estrogen response elements (EREs), and enhance the transcription of estrogen-responsive genes [85]. Some ER-mediated effects, such as those that promote cellular proliferation in the breast and uterus, can increase the risk of developing estrogen-sensitive cancers [86].

7.1. Effects of Indole-3-Carbinol on Estrogen Receptor Activity

When added to breast cancer cells in culture, I3C has been found to inhibit the transcription of estrogen-responsive genes stimulated by 17β-estradiol [87,88]. Acid condensation products of I3C that bind and activate Ahr may also inhibit the transcription of ER-responsive genes by competing for coactivators or increasing ER degradation [81,89]. In contrast, some studies in cell culture [90,91] and animal models [92] have found that acid condensation products of I3C enhance the transcription of ER-responsive genes. Further research is needed to determine the nature of the stimulatory and inhibitory effects of I3C and its acid-condensation products on ER-responsive gene transcription under conditions that are relevant to human cancer risk.

7.2. Effects of Indole-3-Carbinol on Estrogen Metabolism

As mentioned above, 17β-estradiol can be converted to 16α-hydroxyestrone ($16\alpha OHE_1$) or 2-hydroxyestrone ($2OHE_1$), sometimes viewed as ‘bad’ and ‘good’ estrogenic metabolites, respectively. In controlled clinical trials, oral supplementation with 300–400 mg/d of I3C has consistently increased urinary $2OHE_1$ levels or urinary $2OHE_1$:$16\alpha OHE_1$ ratios in women [93–97]. Supplementation with 108 mg/day of DIM also increased urinary $2OHE_1$ levels in postmenopausal women [98]. However, the relationship between urinary $2OHE_1$:$16OHE_1$ ratios and breast cancer risk is not clear. Although women with breast cancer had lower urinary ratios of $2OHE_1$:$16\alpha OHE_1$ in several small case-control studies [35–37], larger case-control and prospective cohort studies have not found significant associations between urinary $2OHE_1$:$16\alpha OHE_1$ ratios and breast cancer risk [38–40].

8. Other Anticarcinogenic Properties

8.1. Induction of Cell Cycle Arrest and/or Apoptosis

After a cell divides, it passes through a sequence of stages known as the cell cycle before dividing again. Following DNA damage, the cell cycle can be transiently arrested to allow for DNA repair, or alternatively there can be activation of pathways leading to apoptosis if the damage cannot be repaired [99]. Defective cell cycle regulation may result in the propagation of mutations that contribute to the development of cancer. A number of isothiocyanates, including AITC, BITC, PEITC and SFN, have been found to induce cell cycle arrest in cultured cells [4], and similar findings have been reported for indoles, such as I3C [100,101]. However, the physiological relevance of these cell culture studies is unclear since it is not known how much I3C is available to the tissue after oral administration in humans (see Metabolism and Bioavailability above) [102]. Unlike normal cells, cancer cells proliferate rapidly and lose the ability to respond to cell death signals by undergoing apoptosis. I3C and DIM have been found to induce apoptosis when added to cultured prostate [100], breast [103,104] and cervical cancer cells [105]. Isothiocyanates has been found to inhibit proliferation and induce apoptosis in a number of cancer cell lines, as reviewed by Hecht [74].

8.2. Inhibition of Tumor Invasion and Angiogenesis

Limited evidence in cell culture experiments suggests that I3C and DIM can inhibit the invasion of normal tissue by cancer cells [88] and inhibit the development of new blood vessels (angiogenesis) required by tumors to fuel their rapid growth [106]. There is some evidence that isothiocyanates, such as SFN, might similarly affect tumor invasion and angiogenesis (reviewed in [107]), although further studies are warranted *in vivo*.

8.3. Anti-inflammatory Activity

The nuclear factor kappa B (NF-κB) has a pivotal role in cancer chemoprevention due to its involvement in tumor cell growth, proliferation, angiogenesis, invasion, apoptosis, and survival [108]. Inflammation promotes cellular proliferation and inhibits apoptosis, increasing the risk

of developing cancer [109]. Among their reported anticancer properties, SFN and PEITC have been found to decrease the secretion of inflammatory signaling molecules by white blood cells and to decrease DNA binding of NF-κB, a pro-inflammatory transcription factor [107,110, 111].

8.4. Epigenetic Modulation

In the nucleus, DNA is associated with basic proteins called histones. In general, acetylation of histones by histone acetyltransferases (HATs) makes DNA more accessible to transcription factors, which bind DNA and activate gene transcription. Deacetylation of histones by histone deacetylases (HDACs) restricts the access of transcription factors to DNA. Acetylation and deacetylation of nuclear histones is an important cellular mechanism for regulating gene transcription [112]. However, the balance between HAT and HDAC activities that exists in normal cells may be disrupted in cancer cells. Compounds that inhibit HDACs have the potential to induce the transcription of tumor suppressor proteins that promote differentiation and apoptosis in transformed (precancerous) cells [6]. AITC and SFN metabolites inhibit HDAC activity in cultured cancer cells and in animals models, and SFN-rich broccoli sprouts inhibited HDAC activity in peripheral blood mononuclear cells of human volunteers [68, 113–116].

There also is growing evidence for transplacental cancer chemopreventive effects of I3C and other dietary modulators, involving changes in DNA promoter methylation, chromatin remodeling, and expression of imprinted genes [117–120]. This avenue of epigenetic research is likely to gain increasing interest in the future.

8.5. Anti-viral and Anti-bacterial Effects

Infection with certain strains of human papilloma virus (HPV) is an important risk factor for cervical cancer [19]. Transgenic mice that express cancer-promoting HPV genes develop cervical cancer with chronic 17β-estradiol administration. In this model, feeding I3C markedly reduced the number of mice that developed cervical cancer [121]. A small placebo-controlled trial in women examined the effect of oral I3C supplementation on the progression of precancerous cervical lesions classified as cervical intraepithelial neoplasia (CIN) [122]. After 12 weeks, 4 out of the 8 women who took 200 mg/d had complete regression of CIN and 4 out of the 9 who took 400 mg/d had complete regression, while none of the 10 women who took a placebo had complete regression. Although these preliminary results are encouraging, larger controlled clinical trials are needed to determine the efficacy of I3C supplementation for preventing the progression of precancerous lesions of the cervix [123].

Bacterial infection with *H. pylori* is associated with a marked increase in the risk of gastric cancer [124]. Purified SFN inhibited the growth and killed multiple strains of *H. pylori* in the test tube and in tissue culture, including antibiotic resistant strains [125]. In an animal model of *H. pylori* infection, SFN administration for 5 days eradicated *H. pylori* from 8 out of 11 xenografts of human gastric tissue implanted in immune-compromised mice [126]. However, in a small clinical trial, consumption of up to 56 g/d (2 oz/d) of glucoraphanin-rich broccoli sprouts for a week was associated with *H. pylori* eradication in only 3 out of 9 gastritis patients [127]. Further research is needed to determine whether SFN or foods rich in its precursor glucobrassicin will be helpful in the treatment of *H. pylori* infection in humans [128–130].

9. Adverse effects

In vivo, naturally occurring isothiocyanates and their metabolites have been found to inhibit the development of chemically-induced cancers of the lung, liver, esophagus, stomach, small intestine, colon and mammary gland (breast) in a variety of animal models [67,73]. When

administered before or at the same time as the carcinogen, oral I3C has been found to inhibit the development of cancer in a variety of animal models and tissues, including cancers of the mammary gland (breast) [131,132], stomach [133], colon [134,135], lung [136] and liver [137]. However, a number of studies found that I3C actually promoted or enhanced the development of cancer when administered chronically after the carcinogen (post initiation). The cancer promoting effects of I3C were first reported in a trout model of liver cancer [138, 139]. However, I3C also has been found to promote or enhance cancer of the liver [140,141], thyroid [142], colon [143] and uterus [144] in rats. The long-term effects of I3C supplementation on cancer risk in humans are not known, but the contradictory results of animal studies have led some to caution against the widespread use of I3C and DIM supplements in humans until the potential risks versus benefits are better understood [141,145,146].

10. Future Perspectives

Although epidemiological studies provide some evidence that higher intakes of cruciferous vegetables are associated with decreased cancer risk in humans [7], it is difficult to determine whether such protective effects are related to isothiocyanates or other factors associated with cruciferous vegetable consumption. Investigators have attempted to calculate human isothiocyanate exposure based on assessments of cruciferous vegetable intake and measurements of the maximal amounts of isothiocyanates that can be liberated from various cruciferous vegetables in the laboratory [61]. Case-control studies using this technique found that dietary isothiocyanate intakes were significantly lower in Chinese women [14] and US men [16] diagnosed with lung cancer than in cancer-free control groups. Assessing dietary intake of cruciferous vegetables may not accurately measure an individual's exposure to isothiocyanates, since other factors may alter the amount of isothiocyanates formed and absorbed (see Metabolism and Bioavailability above). Measuring urinary excretion of isothiocyanates and their metabolites may provide a better assessment of isothiocyanate exposure [42,61], but few studies have examined relationships between urinary isothiocyanate excretion and cancer risk. In a prospective study, Chinese men with detectable levels of urinary isothiocyanates at baseline were at significantly lower risk of developing lung cancer over the next ten years than men with undetectable levels [17]. A case-control study found that urinary isothiocyanate excretion was significantly lower in Chinese women diagnosed with breast cancer than in a cancer-free control group [147]. In contrast, cruciferous vegetable intake estimated from a food frequency questionnaire was not associated with breast cancer risk in the same study. One important frontier in the study of cruciferous vegetables and health is to obtain better assessment of physiologically-relevant concentrations of individual vegetable constituents and their tissue metabolites. This difficult area is being developed in human trails, facilitated by such analytical techniques as LC-MS-MS and accelerator mass spectrometry [148–150].

11. Intake Recommendations

Although many organizations, including the National Cancer Institute, recommend the consumption of 5–9 servings (2½–4½ cups) of fruits and vegetables daily [151], separate recommendations for cruciferous vegetables have not been established. Much remains to be learned regarding cruciferous vegetable consumption and cancer prevention, but the results of some prospective cohort studies suggest that adults should aim for at least 5 weekly servings of cruciferous vegetables [12,51,152].

12. Summary

Cruciferous vegetables are unique in that they are rich sources of sulfur-containing compounds known as glucosinolates. Chopping or chewing cruciferous vegetables results in the formation

of bioactive glucosinolate hydrolysis products, such as isothiocyanates and indole-3-carbinol. High intake of cruciferous vegetables has been associated with lower risk of lung and colorectal cancer in some epidemiological studies, but there is evidence that genetic polymorphisms may influence the effective of cruciferous vegetables on human cancer risk. Although glucosinolate hydrolysis products may alter the metabolism or activity of sex hormones in ways that could inhibit the development of hormone-sensitive cancers, evidence of an inverse association between cruciferous vegetable intake and breast or prostate cancer in humans is limited and inconsistent. Many organizations, including the National Cancer Institute, recommend the consumption of 5–9 servings (2½–4 ½ cups) of fruits and vegetables daily, but separate recommendations for cruciferous vegetables have not been established.

For specific constituents of cruciferous vegetables, isothiocyanates are derived from the hydrolysis of glucosinolates, and compounds such as sulforaphane may help prevent cancer by enhancing the elimination of potential carcinogens from the body and increasing the transcription of tumor suppressor proteins, including those silenced by epigenetic mechanisms. Epidemiological studies provide some evidence that human exposure to isothiocyanates through cruciferous vegetable consumption may decrease cancer risk, but the protective effects may be influenced by individual genetic variation in the metabolism and elimination of isothiocyanates from the body. Glucosinolates are present in relatively high concentrations in cruciferous vegetables, but cooking, particularly boiling and microwaving at high power, may decrease the bioavailability of isothiocyanates.

Among indoles, indole-3-carbinol is derived from the hydrolysis of glucobrassicin, and in the acidic environment of the stomach it forms a number of biologically active indole acid condensation products, such as 3,3′-diindolylmethane (DIM) and related oligomers. I3C has been found to inhibit the development of cancer in animals when given before or at the same time as a carcinogen. However, in some cases, I3C enhanced the development of cancer in animals when administered after a carcinogen. The contradictory results of animal studies have led some experts to caution against the widespread use of I3C and DIM supplements for cancer prevention in humans until their potential risks and benefits are better understood. Although I3C and DIM supplementation have been found to alter urinary estrogen metabolite profiles in women, the effects of I3C and DIM on breast cancer risk are not known. Small preliminary trials in humans suggest that I3C supplementation may be beneficial in treating conditions related to human papilloma virus infection, such as cervical intraepithelial neoplasia and recurrent respiratory papillomatosis, but larger randomized controlled trials are needed.

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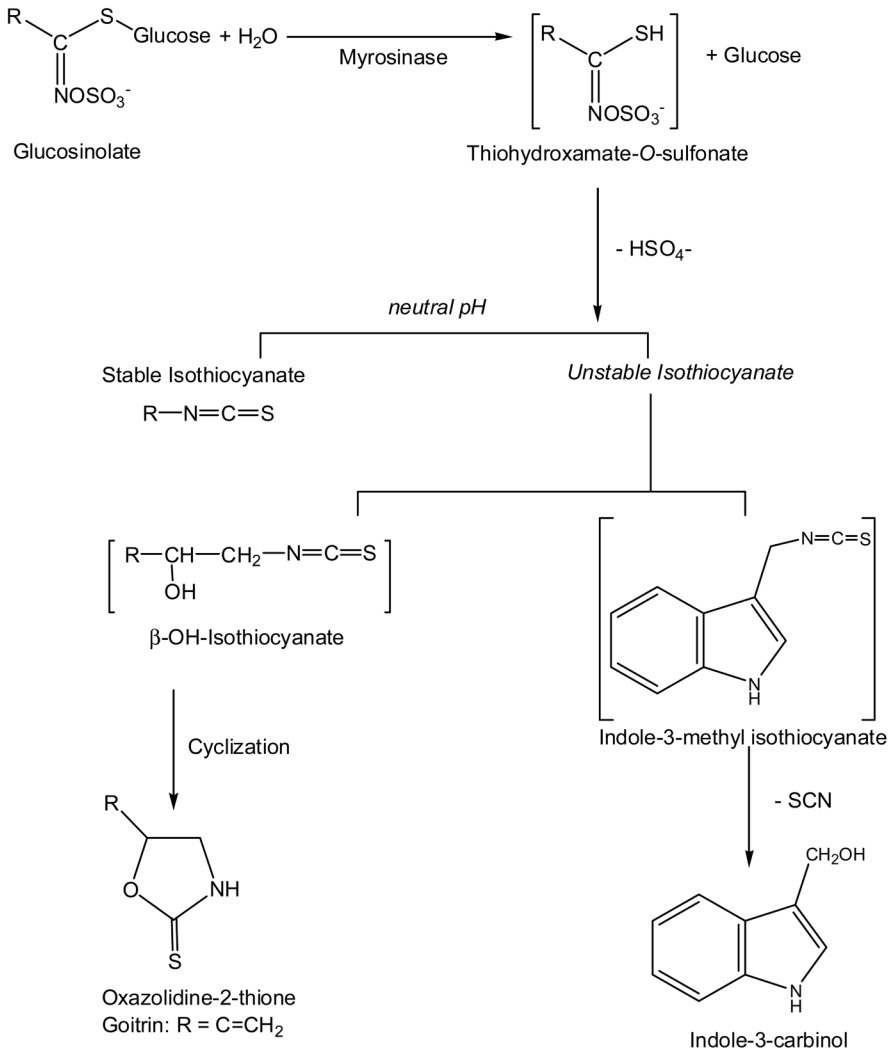


Figure 1. Breakdown of glucosinolates (adapted from [3])

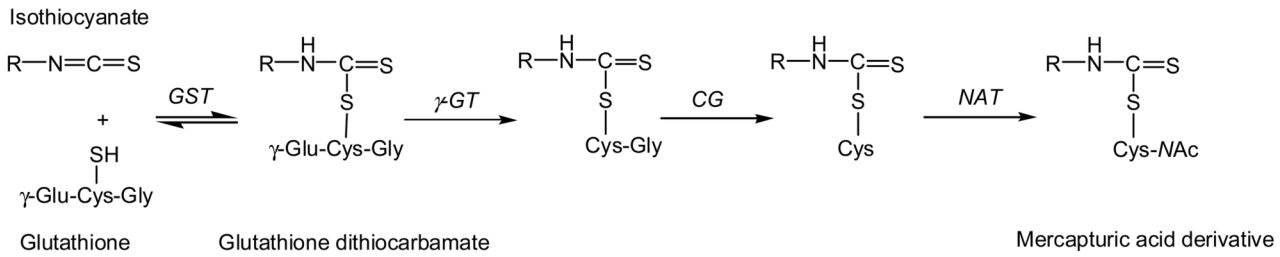


Figure 2. Metabolism of isothiocyanates via the mercapturic acid pathway (adapted from [3,4])
Abbreviations: GST, glutathione *S*-transferase; γ-TP, γ-glutamyltranspeptidase; CG, cysteinylglycinase; NAT, *N*-acetyltransferase.

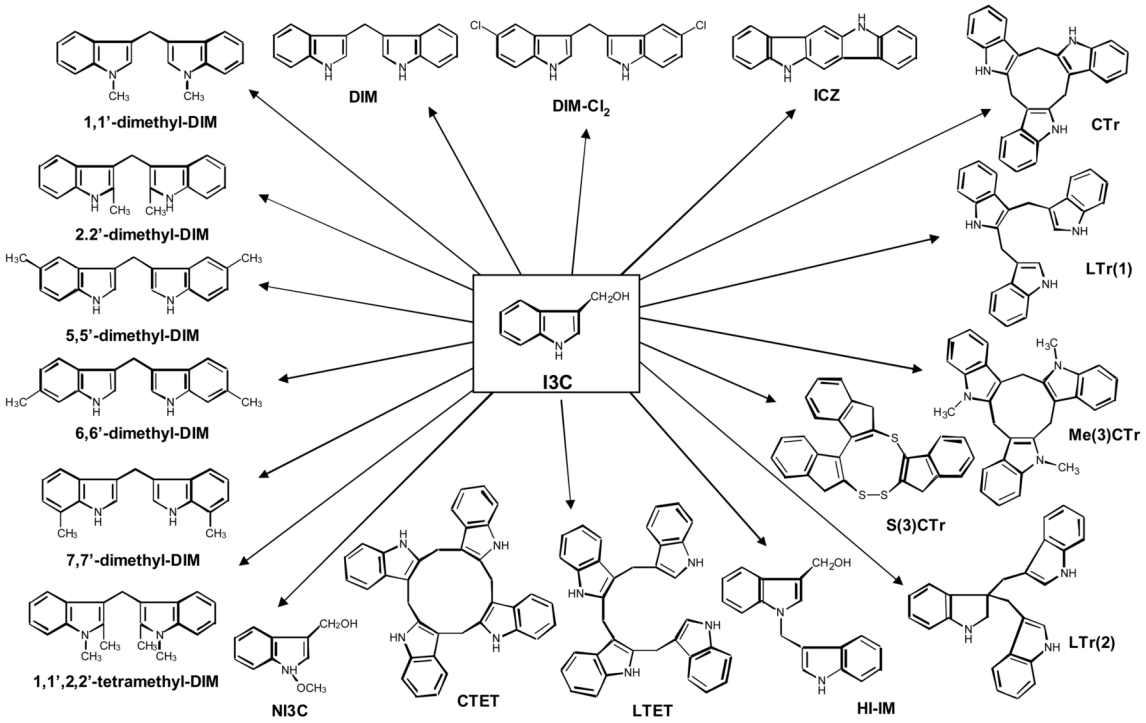
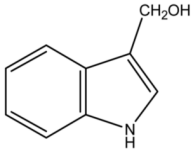
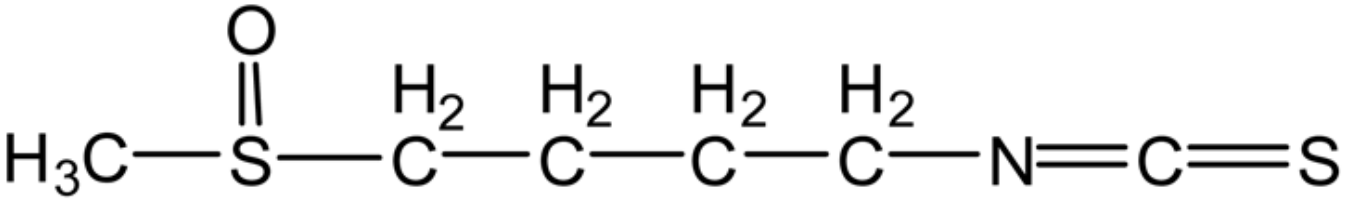
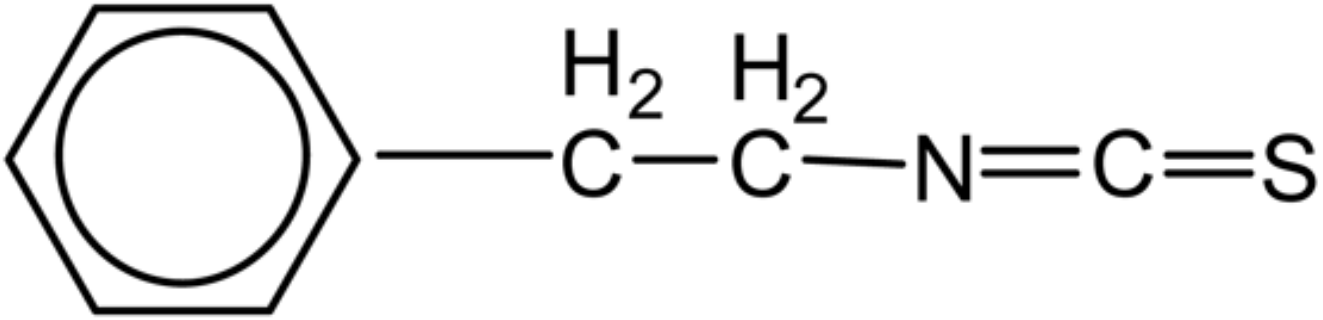
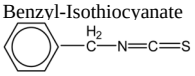


Figure 3. Indole-3-carbinol and its acid condensation products
Figure courtesy of Bharat B Aggarwal, with slight modification from [79]

Table 1

Some food sources of selected isothiocyanates and their glucosinolate precursors that are under investigation for their cancer chemopreventive properties.

Glucosinolate (precursor)	**Indole or Isothiocyanate**
Glucobrassicin	Indole-3-Carbinol 
Glucoraphanin	Sulforaphane 
Gluconasturtiin	Phenethyl-Isothiocyanate 
Glucotropaeolin	Benzyl-Isothiocyanate 
Sinigrin	Allyl-Isothiocyanate