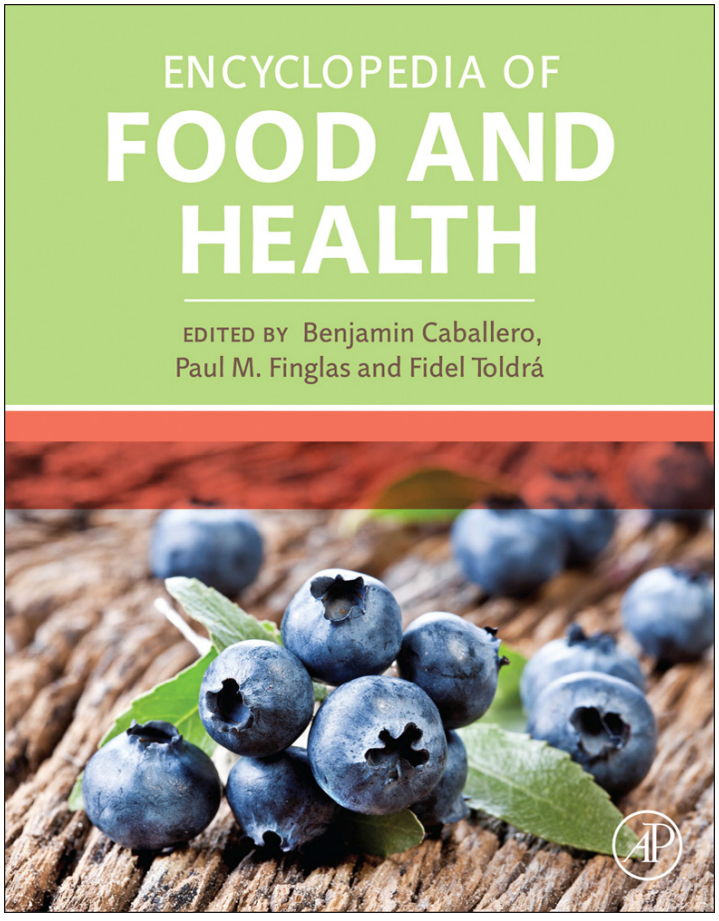

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Glucosinolates from the *Brassica* Vegetables and Their Health Effects

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Background

Glucosinolates impart a sharp, somewhat fiery character to many edible plants. Efforts to understand the chemical origin of these pungent flavors led to the first observations on the unique properties of glucosinolates when, in 1831, French chemists Pierre-Jean Robiquet and Antoine François Boutron-Charlard isolated the glucosinolate sinalbin from white mustard (*Sinapis alba*). Formerly referred to as mustard oil glycosides, the glucosinolates comprise a relatively small but chemically diverse group of defensive plant secondary metabolites. Plants employ intact glucosinolates as host recognition cues (as both deterrents and attractants) for specialist insect and generalist insect oviposition. Glucosinolates further serve as potent biopesticides, participating in a binary chemical defense system that protects plants from environmental stressors such as insect herbivory and microbial pathogen attacks. Glucosinolate-producing plants are of major economic and nutritional importance, demonstrating versatility in a wide range of applications that encompass industrial, agricultural, and phytopharmacological usages. In particular, glucosinolate degradation products have been identified as potent chemoprotective agents in humans against the pathogenesis of many chronic diseases such as cancer, cardiovascular disease, and neurodegenerative disease, among others. The diminutive, glucosinolate-rich thale cress (or, mouse-ear cress, *Arabidopsis thaliana*), a member of the Brassicaceae family, is highly valued as a model organism for plant biology and genetics research.

Sources and Production

The majority of glucosinolate-producing plants are members of the Brassicales order (formerly Capparales), which includes the cruciferous families Brassicaceae, Capparaceae, and Caricaceae. The Brassicales alone comprise more than 350 genera and 3000 species. Of the many hundreds of cruciferous species investigated, all are able to synthesize glucosinolates. Glucosinolates have also been identified in more than 500 species of diverse noncruciferous dicotyledonous angiosperms; of note are the singular glucosinolate-producing genus *Drypetes* (syn. *Putranjiva*) of the Euphorbiaceae family (no other members of this vast plant family have been shown to contain them) and the monogeneric family Moringaceae, which contains 13 species that all produce chemically unique glucosinolates.

Glucosinolates are decidedly stable compounds with little biological activity. Conversely, some glucosinolate degradation products, such as the isothiocyanates, are unstable electrophilic compounds that react spontaneously with biological nucleophiles. Glucosinolates and their degradation products lend a pungent aroma and sharp flavor (sometimes described as bitter) to edible plants and are responsible for the 'heat' most notably associated with wasabi and horseradish.

Glucosinolate distribution in plant structures varies widely between roots, leaves, stems, and seeds and is strongly influenced by plant age. As such, the highest concentrations of glucosinolates are usually found in the reproductive organs of plants, with substantially higher concentrations present in young plants versus mature plants. Following *de novo* synthesis in siliques, glucosinolates are transported from mature to developing plant structures where they are stored as inert, hydrophilic precursors to toxic isothiocyanates and other biologically active compounds. Glucosinolates coexist with but are compartmentally segregated from plant myrosinases, an endogenous family of β-thioglucosidase enzymes (EC 3.2.1.147), whose sole known substrates are glucosinolates. These enzymes are strategically sequestered throughout plants in specialized idioblast, or myrosin, cells of plant leaves, stems, and flower clusters in a species-specific manner; multiple forms of myrosinase can exist within the same plant.

Upon plant injury and subsequent loss of cellular integrity due to insect herbivory, human mastication, or food processing techniques, myrosinase hydrolyzes the neighboring glucosinolates to yield glucose, hydrogen sulfate, and unstable aglycones that spontaneously rearrange to form a diverse group of bioactive compounds that vary depending on chemical structure, environmental conditions (such as pH and presence of ferrous ions), and the coexistence of other factors present in the plant, such as epithiospecifier proteins (ESP) or epithiospecifier modifier proteins (ESM). Whereas the presence of ESP favors epithionitrile formation (primarily from aliphatic glucosinolates), ESM promotes isothiocyanate formation. Myrosinases have also been identified, but little characterized, in fungi and bacteria including many bacteria commonly associated with human and animal gut microflora. Myrosinases are activated to various degrees by ascorbic acid, and in some instances, the enzyme is almost inactive in its absence. Activation is not dependent on the redox reactivity of ascorbate, rather, it has been suggested that ascorbate provides a nucleophilic catalytic group to the reaction.

In humans, myrosinase-producing intestinal microflora convert unhydrolyzed glucosinolates to their cognate isothiocyanates. Microbial-induced conversion is highly variable and subject to interindividual differences in commensal microbiota population. As such, conversion is distinguished by high converters (individuals with high elimination profiles) and low converters (individuals with low elimination profiles). Antimicrobial agents or mechanical bowel cleansing abrogates microbial conversion (see Figure 1).

Chemical Structure

The defining characteristic of glucosinolate structure is a β-D-glucopyranose moiety, sulfur-linked to a β-thioglucoside-*N*-hydroxysulfate and an amino acid-derived side chain (R).

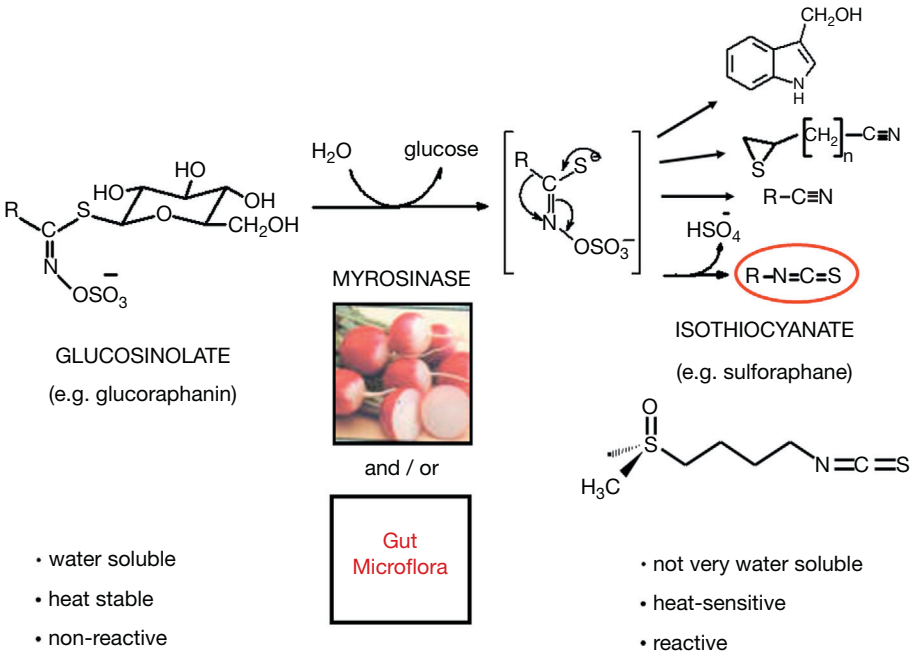

Figure 1 Glucosinolates are hydrolyzed to isothiocyanates by the enzyme myrosinase, which is physically segregated within the plant from its substrate. Myrosinase-like activity is also present in the gut microflora but is highly variable between persons.

During glucosinolate biosynthesis, the precursor side chains undergo extensive modification and may contain straight or branched carbon chains and aromatic or indole moieties. Further side-chain modifications include glycosylation, desaturation, or functionalization with hydroxyl groups, carbonyl groups, or sulfur linkages in various oxidation states, the latter occurring in more than one-third of all nonsynthetic glucosinolates. About 120 distinct glucosinolate structures have been identified and are broadly classified as aliphatic, aromatic, or heterocyclic (or indole), based upon structural similarities of their amino acid precursor and the extent to which their side chain is modified. Aliphatic glucosinolates are derived from alanine, isoleucine, leucine, methionine, or valine; aromatic glucosinolates are derived from phenylalanine or tyrosine; indole glucosinolates are derived from tryptophan. Methionine, in particular, undergoes the most extensive transformation and gives rise to the large family of methylthioalkyl-, methylsulfinyl-, and methylsulfonyl-glucosinolates. Some aromatic benzyl glucosinolates have an additional sugar moiety (rhamnose or arabinose) in glycosidic linkage to the aromatic ring. The implication of these sugars' presence has only been speculated upon to date, but their occurrence in two families of plants (Moringaceae and Resedaceae) whose genera are widely exploited for their pharmacological properties is significant.

Biosynthesis

The biosynthetic pathway of glucosinolates shares homology with that of the cyanogenic glycosides and likely occurs sequentially in the cytoplasm, endoplasmic reticulum, and chloroplast. The universally accepted model of this pathway transpires in a stepwise fashion involving side-chain elongation, core (glycone) biosynthesis, and side-chain modification. Side-chain elongation proceeds by *N*-hydroxylation of select precursor amino acids followed by decarboxylation. Multifunctional cytochrome P450 enzymes (CYP79s and CYP83s), glucosyltransferases, and sulfotransferases mediate biosynthesis of the core glycone structure, with aldoximes, aci-nitro or nitrile oxides, S-alkyl -thiohydroximates, thiohydroximic acids, and desulfoglucosinolates serving as transitory intermediates. The precise mechanism of side-chain modification, the terminal step of glucosinolate biosynthesis, has been the source of much speculation and little experimental work; however, during side-chain modification, oxidations, hydroxylations, and desaturations give rise to the wide range of structural diversity present in glucosinolates. This diversity further influences reactivity, solubility, and human biological activity of not only the glucosinolates but also the resultant products (isothiocyanates). For example, whereas initial oxidation of the side chain sulfur of methionine and its chain-elongated homologues likely gives rise to the large family of methylsulfinyl- and methylsulfonyl-glucosinolates, conjugation of a hydroxyalkyl glucosinolate with benzoic acid is expected to give rise to the benzoyloxyalkyl glucosinolates. Furthermore, oxidation of glucoerucin, the major glucosinolate present in arugula, gives rise to glucoraphanin, the precursor to the potent anticarcinogenic isothiocyanate sulforaphane. However, other metabolic modifications of glucoerucin may give rise to the goitrogenic glucosinolate progoitrin, with no demonstrated anticarcinogenic activity, but with the potential to cause dietary goiter (hence its name) if consumed at high levels. It is noteworthy that at least ten of the aromatic and indole glucosinolates, which exhibit high bioactivity, are singly or multiply methoxylated.

Hydrolysis of aliphatic and aromatic glucosinolates generally yields isothiocyanates while indole hydrolysis yields nitriles and unstable isothiocyanates that rapidly form indole-3-carbinol (I3C), indoleacetonitrile, thiocyanate ions, and 3,3′-diindolylmethane (DIM). I3C may then spontaneously condense under

the acid conditions of the stomach to form compounds that closely resemble 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD or dioxin) in structure, toxicity, and carcinogenicity. Despite this toxicity, indole glucosinolate metabolites, in particular, I3C and DIM, have been investigated for their potential as cancer chemoprotective agents.

Glucosinolate biosynthesis within individual cruciferous vegetables is both constitutive and inducible and varies considerably in response to genetic factors and environmental influences, such as jasmonate and salicylic acid signaling, pathogenic attack, insect herbivory, and soil nitrogen or sulfur content. As many as 25 glucosinolates may be present in a single plant (*Arabidopsis* spp.), although a census in the range of five to ten is far more typical; total content in plants can range from 1% of dry weight in vegetative tissues of many *Brassica* vegetables to as much as 10% in the seeds of some plants.

Patterns of Consumption

Cruciferous vegetables from the *Brassica* genus are the primary dietary source of glucosinolates in humans and include broccoli, bok choy, Brussels sprouts, cabbage, cauliflower, collard, kale, kohlrabi, mustard, rutabaga, and turnips. In addition, edible plants from other genera including arugula, capers, cress, moringa, papaya, radish, wasabi, and watercress contribute substantially to glucosinolate intake. (See Figure 2 and Table 1.)

Regional and cultural dietary patterns strongly influence glucosinolate consumption. Food disappearance data indicate Asian populations consume markedly higher quantities of cruciferous vegetables, many of which are consumed rarely in Western cultures. For example, whereas daikon, or Japanese white radish, is the most commonly consumed cruciferous vegetable in Japan (20 kg year^{-1}), broccoli ranks the highest in the United States (2.5 kg year^{-1}). Total crucifer intake varies considerably by country but average intake is estimated at 7.6 kg year^{-1}; the highest total intake is observed in China (37 kg year^{-1}) and the lowest in Greece (2.1 kg year^{-1}). Although selective breeding to increase glucosinolate content is advocated for health reasons, efforts to decrease glucosinolate content have been promoted as means to reduce bitterness, a quality perceived (by some) as an obstacle to palatability. (See Box 1.)

Accurate assessment of dietary glucosinolate intake has proved problematic. Traditional dietary assessment methods such as food frequency questionnaires and dietary recalls may be susceptible to reporting bias and fail to consider differences in glucosinolate content in plants, which demonstrates remarkable variability across species and cultivars. Environmental factors such as soil and growing conditions, cultivar, harvest time, and postharvest storage further modulate glucosinolate levels in foods. In addition, food processing and preparation methods may reduce exposure of humans to glucosinolate degradation products by 60–90%. For example, glucosinolates are water-soluble and may leach into cooking water; alternatively, heat-labile myrosinases are readily inactivated at most cooking temperatures. Furthermore, in light of the complex interactions between gut microbiota and host metabolism, circadian rhythms may influence exposure as well: Recent work suggests that whereas conversion of intact glucosinolates to bioactive metabolites predominates during the day, metabolism of isothiocyanates is more efficient during the night.

Cruciferous vegetable intake can serve as a proxy for glucosinolate intake but biomarkers such as urinary isothiocyanate metabolites may provide more definitive, complementary measures of habitual intake as well as metabolite-specific

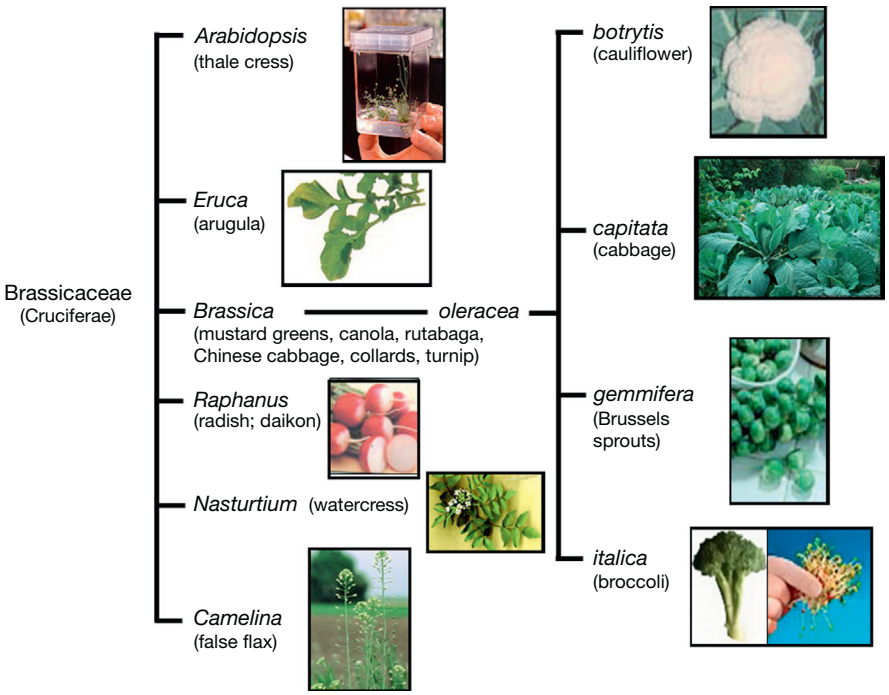

Figure 2 Commonly consumed vegetables in the family Brassicaceae (Cruciferae).

Table 1 Common dietary sources of glucosinolates

Dietary vegetable	*Latin binomial*
Aspiration™ (Chinese kale x broccoli)	–
Arugula (rocket)	*Eruca sativa*
Broccoli (Calabrese; sprouting broccoli)	*Brassica oleracea* var. *italica*
Broccoli raab (rapini; brocoletto; taitcat)	*Brassica rapa* var. *rapa*
Broccolini™ (Chinese kale x broccoli)	–
Broccoflower™ (bright green cauliflower)	*Brassica oleracea* var. *botrytis*
Brussels sprouts	*Brassica oleracea* var. *gemmifera*
Cabbage	*Brassica oleracea* var. *capitata*
Capers	*Capparis spinosa*
Cauliflower	*Brassica oleracea* var. *botrytis*
Charlock (kaber)	*Brassica kaber; Brassica arvensis; Sinapis arvensis*
Chinese cabbage	*Brassica rapa* var. *pekinensis*
Chinese kale (gai lan; gai lon)	*Brassica oleracea* var. *alboglabra*
Chinese mustards (including bok choy, pak choi, choy sum, and celery mustard)	*Brassica rapa* ssp. *chinensis*
Choy sum (mock pak choi; cai tai; saishin)	*Brassica rapa* ssp. *parachinensis*
Collards (collard greens)	*Brassica oleracea* var. *sabellica*
Colza	*Brassica napus* ssp. *napus*
Cress	*Lepidium sativum*
Daikon (mooli; Chinese radish; Korean radish; lo bok)	*Raphanus sativus* var. *longipinnatus*
Horseradish	*Armoracia rusticana*
Kale	*Brassica oleracea* var. *acephala*
Branching bush kale	*(also Brassica oleracea* var. *fruticosa)*
Borecole	*(also Brassica oleracea* var. *selenesia)*
Galega kale (couve galega)	
Marrow stem kale	*(also Brassica oleracea* var. *medullosa)*
Siberian kale (Hanover salad)	*(also Brassica napus* var. *pabularia)*
Thousand-headed kale	*(also Brassica oleracea* var. *ramosa; B. oleracea* var. *millecapitata)*
Kohlrabi (knol khol; turnip cabbage)	*Brassica oleracea* var. *gongylodes*
Maca	*Lepidium meyenii*
Mashua	*Tropaeolum tuberosum*
Mizuna (curled mustard; Japanese greens)	*Brassica rapa* ssp. *japonica*
Mustard (greens; brown mustard)	*Brassica juncea* varieties (many)
Mustard seed	
White mustard	*Sinapis alba*
Yellow mustard	*Sinapis hirta*
Chinese mustard	*Brassica juncea*
Black mustard	*Brassica nigra*
Field mustard	*Brassica campestris*
Ethiopian mustard	*Brassica carinata*
Papaya (pawpaw)	*Carica papaya*
Radish	*Raphanus sativus*
Swede (rutabaga)	*Brassica napus* var. *napobrassica*
Tendergreen (spinach mustard; komatsuna)	*Brassica rapa* var. *perviridis*
Texsel greens	*Brassica carinata*
Tronchuda cabbage (Portuguese cabbage; couve tronchuda; Galician cabbage; braganza; sea-kale cabbage)	*Brassica oleracea* var. *costata*
Turnip rape	*Brassica campestris* var. *oleifera*
Turnips	*Brassica rapa* var. *rapifera*
Wasabi	*Wasabia japonica*
Watercress	*Nasturtium officinale*

exposure levels. However, human exposure to nondietary sources of synthetic analogs of isothiocyanate metabolites such as those used extensively in agricultural and industrial applications can further distort quantification of exposure. Examples include the dithiocarbamates, which are used as pesticides against insects, fungi, and bacteria; accelerators in the vulcanization of natural rubber and synthetic rubber; precursors in the synthesis of sulfide materials and nanoparticles; and flocculants for the remediation of heavy metal-contaminated wastewater.

Availability, Absorption, and Metabolism

The chemical structure and properties of intact glucosinolates preclude absorption, and little, if any, of the parent

Box 1 Glucosinolates as Modulators of Bitter Taste Receptors

Glucosinolates have been described alternately as sharp, pungent, acrid, astringent, or lacrimatory, but they are most widely regarded – in some cases inaccurately – as bitter. Whereas the glucosinolates sinigrin and gluconapin (prominent in Brussels sprouts, cabbage, cauliflower, turnip, rutabaga, and kale) are associated with strong bitterness, glucobrassicin and neoglucobrassicin (prominent in mature broccoli) confer moderate bitterness. The glucosinolates glucoerucin, glucoiberverin, and glucoraphanin do not contribute appreciably to bitterness.

Isothiocyanates typically are not associated with bitterness but they do contribute to overall flavor. One notable exception is goitrin, the hydrolytic degradation product of the nonbitter glucosinolate progoitrin. Goitrin contributes to the bitterness of rutabaga, Brussels sprouts, cabbage, turnip, and cauliflower and presents a significant barrier to consumer acceptance.

Humans' innate aversive reaction to bitterness is a highly conserved protective measure against ingestion of toxic substances. Oral perception of bitterness entails the binding of hydrophilic molecules to G protein-coupled receptors encoded by the TAS2R family of taste receptor genes and ensues via subsequent initiation of attendant downstream signaling pathways. This activation of oral chemosensors elicits a cascade of events that induces local epithelial changes or alterations in autonomic responses, such as excessive salivation or increased gut motility, respectively. TAS2R receptors are expressed in humans in oral taste receptor cells and extraoral sites including tissues of the gut, airways, and testes, where they exhibit variable ligand specificity. Although their functions in these tissues have not been clearly identified, TAS2Rs have been implicated in a diverse portfolio of activities that mediate reflexive actions to protect humans (or their reproductive capacity), including neuroendocrine signaling, airway patency, and spermatogenesis. Whether bitter-tasting compounds such as some glucosinolates modulate cell signaling pathways regulated by TAS2Rs to elicit beneficial (or deleterious) health effects remains to be seen.

glucosinolates is recovered in urine or feces. As such, nearly all glucosinolate bioactivity in humans is attributable to their respective hydrolytic degradation products, of which the isothiocyanates are prominent examples. Only a small fraction of the 120 or so known glucosinolates, and even fewer isothiocyanates, occur frequently in the diet, however.

Isothiocyanates, as lipophilic nonnutrients, encounter the same biotransformative events as other xenobiotic compounds and are subject to a vigorous metabolic response in the small intestine and liver. Nevertheless, isothiocyanates exhibit variable absorption kinetics, high levels of metabolic disposition, strong modulatory effects on ATP-binding cassette (ABC) transporters, and an expansive range of bioavailability. The liver is the predominant site of isothiocyanate metabolism due to high hepatocellular expression of glutathione S-transferases (GST), phase 2 cytoprotective enzymes central to isothiocyanate biotransformation; however, the rapid rate of isothiocyanate clearance – within hours of ingestion – implicates other tissues in metabolism, including the oral, gastric, and intestinal epithelia. The small intestine, in particular, demonstrates uniform and abundant expression of GST and actively participates in isothiocyanate metabolism. Although traditional models of xenobiotic metabolism rarely consider extraintestinal uptake, sublingual and buccal tissues in the oral cavity demonstrate moderate permeability and absorption of other lipophilic compounds. Furthermore, they express the requisite enzymes and efflux transporters involved in isothiocyanate metabolism, raising the question regarding the location of these activities.

Although the sites wherein isothiocyanate biotransformation occurs have not been fully characterized, the processes that mediate the compounds' metabolic disposition likely transpire in this fashion: Following passive diffusion into cells, CYP450 enzymes may oxidize isothiocyanates to their cognate isocyanates, unstable intermediates of amines, carbamates, or benzene derivatives. More frequently, however, isothiocyanates undergo spontaneous or enzyme-enhanced conjugation to glutathione and subsequent cellular efflux via ABC transporters. The isothiocyanates are exceptionally avid substrates of multidrug resistance proteins (MRP2); as such, isothiocyanate conjugates are readily deconjugated and may be shuttled in and out of cells multiple times via MRP2, providing repeated exposures to cells. Intact glucosinolates, isothiocyanates, and their glutathione conjugates may enter the enterohepatic circulation, the bidirectional cycling of xenobiotic compounds and their metabolites between the intestine and liver via bile transporters. Enterohepatic circulation subjects these compounds to repeated bouts of recycling and may prolong the compounds' half-life; alternatively, they may be transported to the distal gut where they are subject to microbial hydrolysis and subsequent absorption or elimination in feces.

Absorbed isothiocyanate species are metabolized primarily by the mercapturic acid pathway, during which nonenzymatic and enzymatic conversions yield the dithiocarbamates cysteinylglycine, cysteine, and N-acetylcysteine conjugates. These metabolites are preferentially eliminated in urine, likely due to their low molecular weight, which is generally less than 500–600 Da. Generally speaking, glucosinolates, isothiocyanates, and their metabolites are cleared from urine within 72 h of ingestion. Over half of ingested glucosinolates, which require initial degradation to isothiocyanates by myrosinase, are cleared in the urine within 24 h, and they are fully cleared within 72 h. Over half of ingested isothiocyanates are cleared in the urine within 8 h, and they are fully cleared within 24–48 h. As such, N-acetylcysteine conjugates in urine serve as a biomarker of isothiocyanate (and glucosinolate) uptake and metabolism. However, interindividual differences in cellular metabolic competence, gut microbiota population, bowel transit time, and the presence of other dietary components may influence elimination rates.

The collective processes of MRP2 transport, enterohepatic circulation, and mercapturic metabolism likely play a key role in isothiocyanate bioavailability. Indeed, isothiocyanates demonstrate biphasic serum concentrations and urinary output rates, which may have implications for health effects as well as extrapolation of data across individuals. These redundant, iterative processes speak to the toxicity of isothiocyanates yet ultimately they may be responsible for the protective cellular responses induced by exposure to these compounds.

Health Effects

The health effects associated with the consumption of glucosinolates are incumbent upon conversion to their bioactive form, the isothiocyanates. Postabsorptive metabolism of isothiocyanates may be responsible for producing therapeutic moieties that mediate these beneficial effects; however, toxicities have been reported from consumption of some

non-isothiocyanate metabolites of certain glucosinolates not commonly consumed. Nevertheless, compelling evidence links increased consumption of fruits and vegetables, especially cruciferous vegetables, to reduced incidence of many chronic diseases, including cancer, cardiovascular disease, neurodegenerative disease, metabolic dysregulation and its pathological sequelae, and autoimmune dysfunction. A variety of clinical trials addressing the role of isothiocyanates in the prevention and management of these and other chronic diseases are under way as this article goes to press, with new data promising further insight into the mechanistic actions of this unique class of bioactive plant compounds.

Anti-Inflammatory and Immunomodulatory Agents

Inflammation and altered immune function are prominent features in the pathogenesis of many chronic diseases. The anti-inflammatory and immunomodulatory activities of isothiocyanates likely contribute to the putative protective effects associated with these compounds. For example, in rodents, sulforaphane, one of the most extensively studied isothiocyanates, has been shown to inhibit endothelial cell response to the vascular endothelial growth factor and downregulate the expression of cyclooxygenase-2 and proinflammatory cytokines such as interleukin (IL)-1β, IL-6, granulocyte macrophage colony-stimulating factor, and tumor necrosis factor-α (TNF-α). Sulforaphane also inhibits activation of the transcription factor NFκB, thus suppressing gene expression of proinflammatory mediators and reducing the number of activated macrophages in rodent models. Recent evidence suggests that through direct covalent binding to the N-terminal proline, isothiocyanates potently inhibit the tautomerase activity of the macrophage migration inhibitory factor, a proinflammatory cytokine implicated in the pathogenesis of inflammatory and autoimmune diseases.

Cancer

Isothiocyanates have been studied extensively for their role in preventing carcinogenesis. Ingestion of approximately two servings per day of cruciferous vegetables may reduce the relative risk for cancer by as much as 50% at certain sites. At least some, and perhaps most, of the cancer chemoprotective activity of these vegetables is believed to be due to their content of minor dietary components such as glucosinolates, a role supported by *in vitro* animal and human data. In particular, epidemiological data indicate that aliphatic and indolyl glucosinolate intakes are inversely associated with risk of some types of cancer; however, genetic polymorphisms in isothiocyanate metabolism and elimination may mitigate the protective effects. In rodent models of both spontaneous and chemically induced cancers, isothiocyanates confer protection at multiple sites including the lungs, esophagus, stomach, colon, mammary glands, bladder, pancreas, and skin. In addition, the isothiocyanates inhibit growth of human tumor cells in xenograft models of prostate, colorectal, esophageal, and breast cancer. Multiple mechanisms are likely at play including altered carcinogen metabolism due to modifications in the activities of drug-metabolizing enzymes, induction of cell cycle arrest and apoptosis, inhibition of angiogenesis and metastasis, altered histone acetylation status, and antioxidant, anti-inflammatory, and immunomodulatory activities. Critically, it is this ability of a single agent to exert such diverse effects on events that are intimately involved in the pathogenesis of cancer that makes the isothiocyanates such efficient protective agents. Early discoveries that isothiocyanates were capable of reducing the activation of procarcinogens by inhibiting phase 1 drug-metabolizing enzymes and inducing the transcription of cytoprotective phase 2 enzymes, ultimately decreasing the levels of the carcinogens available to mutate DNA, provided the impetus for the development of a quantitative bioassay for screening induction capabilities based on increased transcription of NAD(P)H: quinone oxidoreductase 1 (NQO1). Induction of this widely distributed NAD(P)H-dependent flavoprotein, which promotes obligatory 2-electron reductions of quinones to hydroquinones, is a salient feature of the phase 2 response and is of central importance in protecting cells against oxidative stress.

Broccoli extracts demonstrate the greatest capacity for NQO1 induction of any glucosinolate-producing plants evaluated thus far, and sulforaphane has been isolated as the principal active compound. In fact, no other phytochemicals in broccoli extracts have been shown to contribute to this activity. In addition, induction of other cytoprotective proteins, including both subunits of glutamate–cysteine ligase, the rate-limiting enzyme of glutathione biosynthesis, heme oxygenase-1, and GSTs, has been observed. Importantly, induction has long-lasting protective effects because the cytoprotective proteins typically have long half-lives of several days. Global gene expression profiling has confirmed that isothiocyanates serve as broad-spectrum chemopreventive agents and modulate extensive networks of cytoprotective genes and signaling pathways.

Many of the genes encoding cytoprotective proteins share a common transcriptional regulation through the Keap1–Nrf2–ARE pathway, a key mediator of cytoprotective responses to oxidative stress and electrophilic stress. Kelch-like ECH-associated protein 1 (Keap1) serves as the ubiquitin ligase substrate adaptor that presents the nuclear factor-erythroid 2 p45-related factor-2 (Nrf2) for ubiquitination and subsequent proteasomal degradation. Isothiocyanates react with specific cysteine residues of Keap1, eliminating Keap1's ability to target the Nrf2 for degradation. Consequently, the Nrf2 accumulates and translocates to the nucleus where it binds to antioxidant response elements (AREs), specific DNA sequences in the upstream regulatory regions of cytoprotective genes, and activates transcription. Nrf2 activation induces the coordinate transcription of a diverse group of cytoprotective enzymes, altering the metabolism and elimination of environmental procarcinogens in humans and affording protection against the damaging effects of environmental toxins by enhancing their detoxification. Sulforaphane is the most potent naturally occurring small-molecule inducer of cytoprotective proteins. In effect, isothiocyanates such as sulforaphane expedite their own metabolism and elimination through upregulation of phase 2 enzymes and subsequent formation of readily excretable metabolites. Isothiocyanates further reduce cancer risk through modulation of epigenetic marks via inhibition of histone deacetylase,

promotion of global histone acetylation, and the induction of cell cycle arrest and apoptosis of cancer cells, thus reducing tumor growth. Further, sulforaphane induces perturbations in several signaling pathways, such as transforming growth factor-β receptor, endothelial growth factor receptor, and insulin signaling. Because these pathways are associated with carcinogenesis and inflammation, it is reasonable to assume that the net effect of their modulation will be reduction in cell proliferation and maintenance of tissue homeostasis.

Cardiovascular Disease

Similarly, isothiocyanates' ability to induce cytoprotective proteins and prevent chronic inflammation reduces oxidative stress in cardiovascular tissues, as evidenced by increased glutathione, glutathione reductase, and glutathione peroxidase levels and decreased protein nitrosation. In rodent models of hypertension and stroke, endothelial-dependent relaxation of the aorta is improved, the number of infiltrating activated macrophages is reduced, and blood pressure is decreased. Similarly, in rodent models of cardiovascular ischemia and reperfusion, oral delivery of isothiocyanate-containing extracts improves postischemic ventricular function, reduces myocardial infarct size, and decreases cardiomyocyte apoptosis.

Nervous Tissue Injury and Neurodegenerative Disease

Oxidative stress and chronic inflammation are also central to the pathogenesis of diseases of the central nervous system, and the protective effects of isothiocyanates are evident in models of nervous tissue injury and neurodegeneration. In models of spinal cord injury, sulforaphane administration decreases infarct size, brain (or spinal cord) edema, and cortical apoptosis, reduces inflammatory markers and tissue damage, attenuates loss of endothelial cell markers and tight junction proteins, preserves blood–brain barrier function, and decreases the presence of neurological deficits. In addition, sulforaphane decreases both microglial activation and the upregulation of inflammatory markers following endotoxin injection. Sulforaphane also demonstrates a protective effect in rodent models of brain ischemia and reperfusion, traumatic brain and spinal cord injury, intracerebral hemorrhage, and contusion.

In the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease, sulforaphane protects nigral dopaminergic neurons against cell death and reduces astrogliosis, microgliosis, and the release of proinflammatory mediators in the basal ganglia. Intrathecal administration of sulforaphane reduces oxidative stress and proinflammatory cytokine expression caused by spinal nerve transection and inhibits the development of neuropathic pain. Following exposure to methylmercury, decreased mercury accumulation was observed in the brain of sulforaphane-treated mice, suggesting the potential of isothiocyanates to accelerate the metabolism and elimination of neurotoxins. In most cases, the neuroprotective effects of sulforaphane are accompanied by activation of the transcription factor Nrf2 and upregulation of its target genes. Furthermore, mice that are deficient in Nrf2 (knockout mutants) are more sensitive to damage and cannot be protected by sulforaphane, strongly suggesting that the transcriptional program orchestrated by Nrf2, and not the isothiocyanate itself, is largely responsible for the protective effects of sulforaphane.

Metabolic Dysregulation and Pathological Sequelae

Diabetes is a metabolic disorder characterized by a chronic, subacute state of inflammation. As such, anti-inflammatory therapeutic approaches are critical in the prevention and management of its pathological sequelae. Sulforaphane has been shown to reduce the serum C-reactive protein, IL-6, and TNF-α, common biomarkers of inflammation observed in individuals with type 2 diabetes. In addition, sulforaphane improves renal performance and minimizes pathological changes in the glomerulus while improving motor nerve conduction velocity and blood flow and reducing pain. These very recent findings encourage the potential development of dietary isothiocyanates as therapeutic agents to alleviate metabolic disorders and protect against renal damage and pain associated with diabetes. Interestingly, intervention in humans with diets rich in glucosinolates causes a decrease in the acylcarnitine levels in plasma and urine, indicating enhanced integration of fatty acid oxidation with the activity of the tricarboxylic acid cycle, which may have implications for metabolic derangements, as well as cancer and neurodegeneration.

Antimicrobial Activity

The antimicrobial activities of isothiocyanates against human pathogens such as *Escherichia coli*, *Salmonella typhimurium*, and *Candida* spp. are well documented. For example, the long history of use of cruciferous vegetables such as cabbage and mustard as wound poultices and antitumor agents likely is attributable to isothiocyanates. Sulforaphane, in particular, is bactericidal and bacteriostatic to *Helicobacter pylori*, and it decreases markers of colonization and inflammation associated with *H. pylori* infection in humans and rodents, which is strongly associated with the development of gastric cancer. In addition, isothiocyanate activity against a range of soil-borne fungal, bacterial, and nematode plant pathogens is profound and has been extensively characterized. It underpins the burgeoning use of cruciferous plants as green manure and soil biofumigation to exploit the fungicidal and nematicidal activities of isothiocyanates.

Antinutritional Characteristics

Some antinutritional effects of isothiocyanates have been reported. In particular, hydrolysis of ß-hydroxyalkenyl glucosinolates (e.g., progoitrin and epi-progoitrin) gives rise to ß-hydroxyalkenyl isothiocyanates, which further cyclize to oxazolidine-2-thiones. These compounds may elicit goitrogenic effects in mammals, a phenomenon first observed in rabbits and described as 'cabbage' goiter. Efforts to suppress the goitrogenicity of rapeseed (*Brassica napus*), an oilseed crop of significant economic and agricultural importance, have led to the highly successful development of the oilseed crop canola in Canada and the 00-rapeseed in northern Europe. The

goitrogenicity of dietary glucosinolates and isothiocyanates in humans is much less clear in the context of normal dietary intake or even enthusiastic consumption.

Conclusion

Glucosinolates are amino acid-derived plant secondary metabolites whose role in plant defense has been well characterized. A wealth of evidence supports the assertion that glucosinolates and their isothiocyanate metabolites provide myriad beneficial health effects in humans as well. In the human dietary context, it is not abundantly clear whether delivery of the glucosinolate (which might be viewed as a prodrug in pharmaceutical terminology) leads to a very different outcome, but clearly, tissue exposure is different with ingestion of isothiocyanate versus glucosinolate. The induction of cytoprotective enzymes, inhibition of inflammatory processes, and modulation of signaling pathways are among the many diverse pharmacological outcomes ascribed to isothiocyanate exposure. Unlike most small-molecule pharmacological agents that affect single targets, the intracellular targets of isothiocyanates are multiple. The activation of transcription factor Nrf2 alone, a major outcome of isothiocyanate exposure, leads to an orchestrated regulation of a vast network of genes comprising approximately 2% of the human genome, with cytoprotective, antioxidant, and anti-inflammatory functions. It is this ability to induce versatile and long-lasting responses, which ultimately protect against oxidative stress, electrophilic stress, and chronic inflammation (the fundamental underlying causes of most chronic diseases), that makes these unique phytochemicals exceedingly efficient protective agents. Although cell culture and animal studies strongly support the beneficial health effects of glucosinolates and isothiocyanates, it is challenging yet critical to translate the findings to human populations.

It is noteworthy that many glucosinolate-containing genera comprise plants that have been used for food or medicinal purposes by various cultures for many centuries. In addition, many tropical and subtropical species have such a compelling ethnopharmacology and such manifold food and medicinal uses that future, more rigorous investigations of their glucosinolates' properties seem promising. Undoubtedly, research in the area of functional genomics will serve to enhance our knowledge and understanding of the diverse biological roles of these unique compounds.

See also: Aflatoxin: A Global Public Health Problem; Antinutritional Factors in Legume Seeds: Characteristics and Determination; Antioxidants: Role on Health and Prevention; Brassica: Characteristics and Properties; Cancer: Diet in Cancer Prevention; Mustard; Papayas; Pesticides and Herbicides: Residue Determination; Pesticides and Herbicides: Types of Pesticide; Pesticides and Herbicides: Types, Uses, and Determination of Herbicides; Pesticides and Herbicides; Rapeseed Oil/Canola.

Further Reading

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