Special Article

Concentrations of thiocyanate and goitrin in human plasma, their precursor concentrations in brassica vegetables, and associated potential risk for hypothyroidism

Peter Felker, Ronald Bunch, and Angela M. Leung

*Brassica vegetables are common components of the diet and have beneficial as well as potentially adverse health effects. Following enzymatic breakdown, some glucosinolates in brassica vegetables produce sulforaphane, phenethyl, and indolylic isothiocyanates that possess anticarcinogenic activity. In contrast, progoitrin and indolylic glucosinolates degrade to goitrin and thiocyanate, respectively, and may decrease thyroid hormone production. Radioiodine uptake to the thyroid is inhibited by 194 μmol of goitrin, but not by 77 μmol of goitrin. Collards, Brussels sprouts, and some Russian kale (*Brassica napus*) contain sufficient goitrin to potentially decrease iodine uptake by the thyroid. However, turnip tops, commercial broccoli, broccoli rabe, and kale belonging to* Brassica oleracae *contain less than 10 μmol of goitrin per 100-g serving and can be considered of minimal risk. Using sulforaphane plasma levels following glucoraphanin ingestion as a surrogate for thiocyanate plasma concentrations after indole glucosinolate ingestion, the maximum thiocyanate contribution from indole glucosinolate degradation is estimated to be 10 μM, which is significantly lower than background plasma thiocyanate concentrations (40–69 μM). Thiocyanate generated from consumption of indole glucosinolate can be assumed to have minimal adverse risks for thyroid health.*

INTRODUCTION

Dietary intake of vegetables from the genus *Brassica*, which includes broccoli, broccoli rabe, kale, turnip, Brussels sprouts, Chinese cabbage, and cauliflower, has been associated with various health-promoting effects[1] that are attributed to the more than 100 diverse β-thioglucoside-N-hydroxysulfates, termed glucosinolates.[2,3] When these glucosinolates are acted upon by the enzyme myrosinase from adjacent plant cells, typically after mastication, they form isothiocyanates, some of which immediately break down to release a permanently charged thiocyanate ion. Extensive reviews of enzymatic products of glucosinolate breakdown by myrosinase and epithiospecifier protein and of nonenzymatic breakdown of glucosinolates into the major classes of isothiocyanates, thiocyanates, nitriles, indole-3-carbinol, various diindolylmethane dimers, etc., have been conducted previously.[2–4]

Through their pioneering work in the early 1990s, the research groups of Talalay and Zhang[5,6] identified broccoli as having significant anticarcinogenic activity and found the compound responsible for this activity to be glucoraphanin, a glucosinolate.[6] Using cell lines and animal studies, they demonstrated that the enzymatic degradation product of glucoraphanin, sulforaphane, has significant anticarcinogenic activity.[2] Sulforaphane

Affiliation: *P. Felker* and *R. Bunch* are with the D'Arrigo Bros. Co., of California, Salinas, California, USA. *A.M. Leung* is with the Division of Endocrinology, VA Greater Los Angeles Healthcare System, Los Angeles, California, and the Division of Endocrinology, David Geffen School of Medicine at UCLA, Los Angeles, California, USA.

Correspondence: *P. Felker*, D'Arrigo Bros. Co., of California, 21777 Harris Rd, Salinas, California 93908, USA. Email: Peter.Felker@darrigo.com. Phone: +1-831-455-4500.
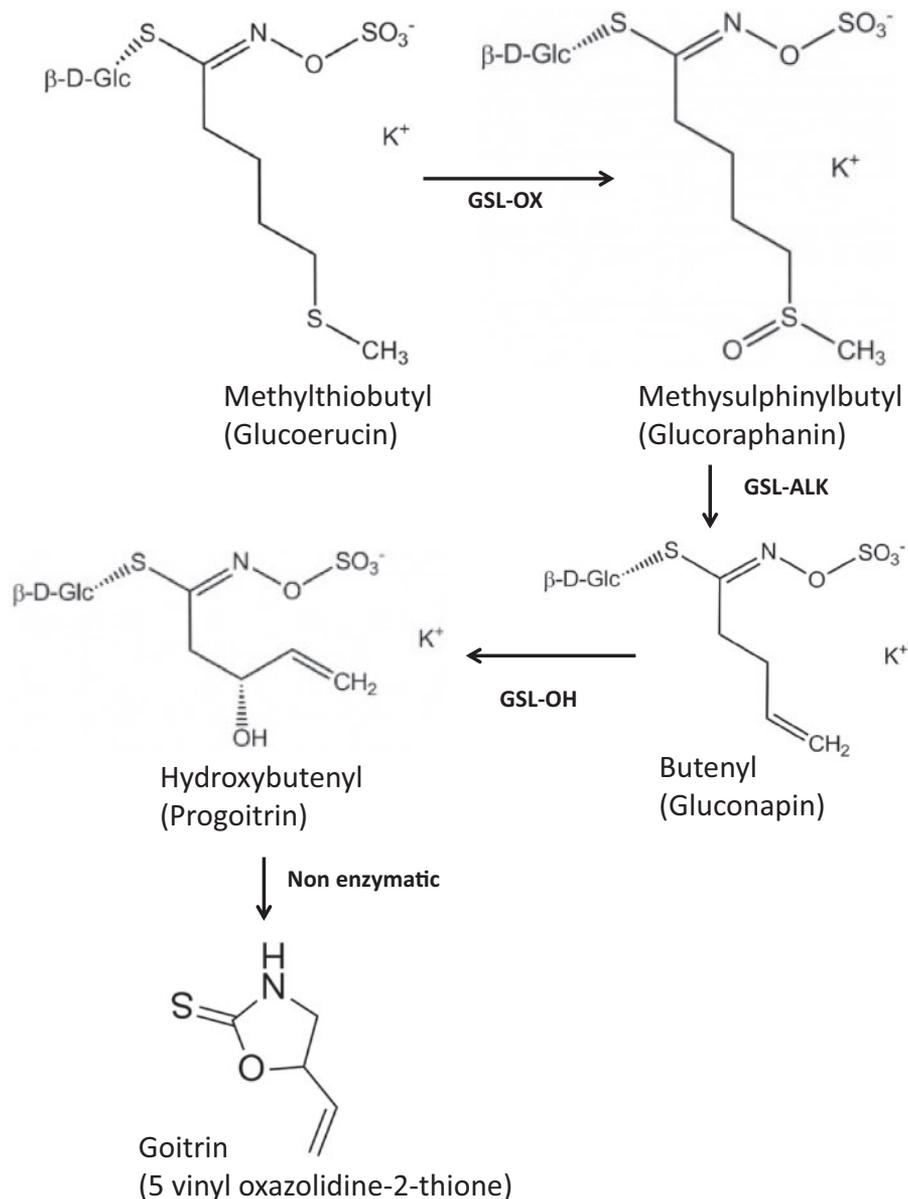
Figure 1 **Pathway for conversion of methylthiobutyl glucosinolate to goitrin.** The enzymes glucosinolate oxidase (NCBI accession no. J3760740) (GSL-OX), glucosinolate alkenylation (NCBI accession no. EF611253) (GSL-ALK), and glucosinolate hydroxylation (NCBI accession no. FJ376074) (GSL-OH) and the nonenzymatic reaction are indicated below or to the side of the arrows.

has been described as "the most potent inducer of phase II enzymes identified to date"[7] and has shown benefits in alleviating multiple chronic conditions, including allergic respiratory inflammation from oxidant stimuli in the upper airway caused by asthma or air pollutants.[7] It has also been associated with decreased risk of various cardiovascular diseases,[8] lung cancer,[9] prostate cancer,[10] urinary cancer,[11] and colon cancer.[12] In addition to sulforaphane, other products of myrosinase-induced degradation of glucosinolate have been demonstrated to have significant anticarcinogenic benefits. Such compounds include low-molecular-weight aliphatic isothiocyanates,[13] phenethyl isothiocyanate,[14,15] and two

products of indole glucosinolate degradation, indole-3-carbinol and diindolylmethane.[16,17]

In contrast to these beneficial effects of glucosinolates, there is evidence (primarily from animal studies) of adverse effects on the thyroid caused by the glucosinolate progoitrin, from which the product of myrosinase-induced degradation is goitrin (Figure 1).[18–21] Iodine is a dietary micronutrient required for the production of thyroid hormone, and the potential adverse effects of goitrin and thiocyanates are based on their ability to inhibit iodine utilization by the thyroid. Despite the development of high-performance liquid chromatography–mass spectrometry methods for
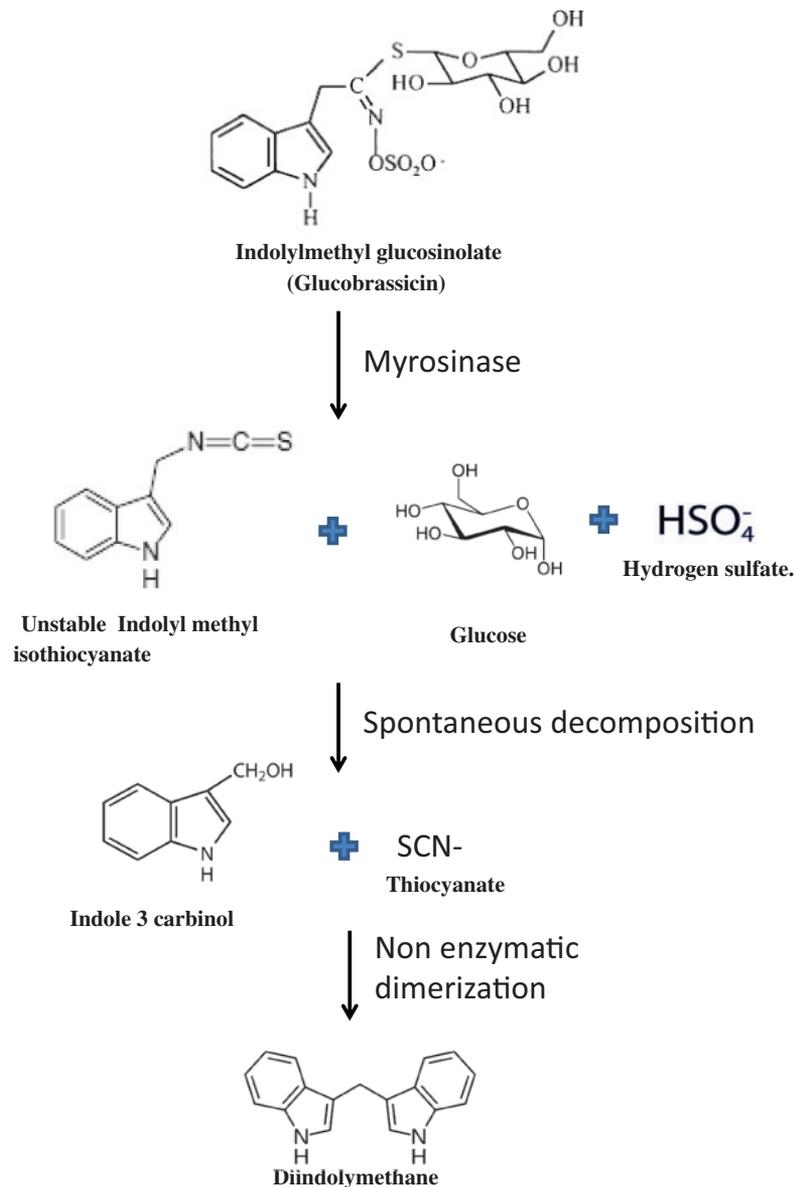
Figure 2 **Pathway for the enzymatic and nonenzymatic degradation of indole glucosinolates, which results in production of thiocyanate and the anticarcinogenic compounds indole-3-carbinol and diindolylmethane.** *Abbreviations*: HSO_4^-, hydrogen sulfate; SCN^-, thiocyanate.

measuring goitrin and other products of glucosinolate hydrolysis in plasma,[22] there are no data on concentrations of goitrin in human plasma following the ingestion of brassica vegetables, which could be helpful in establishing nutritional safety guidelines. One study assessed the change in radioactive iodine uptake by the thyroid glands of human subjects following the administration of recrystallized goitrin.[23] The authors reported that 25 mg (194 μmol) was the minimal amount of goitrin required to decrease the uptake of radioiodine; in contrast, a smaller ingested amount, 10 mg (70 μmol), caused no inhibition of uptake.

Additional adverse effects on the thyroid have been suggested to result from the thiocyanate ion that is produced by myrosinase-induced degradation of indole glucosinolates (Figure 2).[4] The thiocyanate ion is a competitive inhibitor of the sodium/iodide symporter located on the basolateral membrane of the thyroid follicular cell.[24,25] Thus, exposure to thiocyanate can reduce iodide uptake by the thyroid gland[26] and has the potential to result in decreased synthesis of thyroid hormone (hypothyroidism). The thiocyanate ion also occurs naturally in humans, in whom its oxidation by peroxidase in saliva constitutes part of the innate defense in the oral cavity.[27] Hurst[28] has reviewed the chemistry of thiocyanate oxidation by myeloperoxidase to enhance the bactericidal effect of phagocytic killing by neutrophils. One study examined the addition of

thiocyanate to raw cow's milk, since stimulation of oxidation of exogenous thiocyanate to the short-lived hypothiocyanite anion ($OSCN^-$) by lactoperoxidase from milk has bactericidal effects, assisting in milk preservation.[29] To test whether these thiocyanate concentrations could have an effect on thyroid function in humans, Dahlberg et al.[29] added 8 mg of thiocyanate each day to the milk consumed by human volunteers and reported no effect on serum thyroxine, triiodothyronine, or thyrotropin concentrations after 12 weeks. Serum thiocyanate concentrations were 69 μM and 121 μM before and after milk ingestion, respectively, among nonsmokers after the 12-week study period.[30] These data confirm other reports demonstrating serum thiocyanate concentrations among nonsmokers,[30,31] as thiocyanate is also produced from cigarette smoke as a metabolite of cyanide.

From a plant-breeding perspective, it is important to understand if it would be useful to breed for enhanced levels of indole glucosinolates in brassica vegetables to maximize the anticarcinogenic benefits described above. Alternatively if the thiocyanate concentrations resulting from myrosinase degradation of indoles are detrimental to thyroid function, plant breeders should seek to decrease indole glucosinolate concentrations in brassica vegetables. There is extensive literature on the effects of high goitrin concentrations in *Brassica napus* seed meal fed to animals (as a byproduct of oil production) and the breeding of low-goitrin lines for use in animal feed.[19] Since synthesis of the glucosinolate responsible for goitrin is only a 2-step enzymatic process from synthesis of the highly beneficial glucoraphanin that yields sulforaphane,[32] it is important to identify the health risks and benefits of glucosinolate ingestion. Accordingly, it would be desirable to have brassica vegetables with high glucoraphanin concentrations and the lowest possible progoitrin concentrations.

The genetics of the synthesis of glucosinolates in general[33] and, specifically, of the enzymes responsible for synthesis of the positive and negative health attributes described above (i.e., indole glucosinolates, glucoraphanin, and progoitrin)[32,34,35] have been well studied. Natural variation in these synthetic enzymes in the major commercial hybridizing *Brassica* species (i.e., *Brassica oleracae*, *B. napus,* and *Brassica rapa*) has resulted in considerable variation in progoitrin and indole glucosinolate concentrations in common brassica vegetables, suggesting that breeding studies could produce more healthful cultivars. However, Agerbirk et al.[4] have suggested that, despite the benefits of the indole glucosinolates indole-3-carbinol and diindolylmethane, high concentrations of indole glucosinolates might have adverse effects.

In contrast to considerable research on broccoli (*B. oleracae*) and glucoraphanin, much less work has been done on *B. rapa*, which is widely consumed in China as bok choy and Chinese cabbage, in Italy as Cima di rapa, and in the United States as broccoli rabe. Chinese studies show that bok choy (*B. rapa*) is the most widely consumed brassica vegetable in China and that brassica vegetable consumption, as measured by urinary isothiocyanate concentrations, is associated with a significant reduction in breast cancer risk among Chinese women.[36,37]

This review aims to summarize the concentrations of goitrin found in various brassica vegetables, to assess human exposure to dietary goitrogens as measured by plasma goitrin and thiocyanate concentrations, and to outline the potential health benefits and adverse effects of brassica vegetable ingestion.

LITERATURE SEARCH

In this narrative review, a systematic search strategy of the Web of Science and Google Scholar databases for articles published from 1950 to 2014 was employed, using combinations of the search terms "indole glucosinolate," "progoitrin," "goitrin," "plasma thiocyanate," and "thyroid," to include all studies that measured goitrin concentrations. Units were converted to micromoles per 100 grams of fresh weight to compare measured values from the published papers. If dry matter percentages were provided in the papers, they were used to convert dry weights to fresh weights, and if no moisture content was available, a dry matter content of 13.6% was used, since this was found to be typical of the ratio of fresh to lyophilized brassica vegetables (P.F. and R.B, unpublished data, 2012).

In many cases, the maturity of the plant tissues was not provided. Yang and Quiros[32] only analyzed leaves from 6-week-old greenhouse plants. The leaves and floral heads from the D'Arrigo Bros. Co. were obtained from 4- to 6-week-old field-grown plants that were probably larger because of greater soil volume.

In this review, the species designation of the vegetables is as reported in the original articles. It should be noted that the Latin binomial *Brassica campestris*, a diploid with 2n=20, has been replaced by *B. rapa*. *B. oleracae* is also a diploid, with 2n=18, but *B. napus* is an amphidipoid, with 4n=38, probably arising from an ancient combination of the *B. oleracae* and *B. rapa* genomes.[38]

Glucosinolate concentrations in brassica vegetables

Some of the glucosinolates examined here were measured from plants that were seeded in D'Arrigo Bros.

fields, harvested, stored for no more than several days under refrigeration as is done for commercial crops, and analyzed using large sample sizes of fresh material (2–4 g). The glucosinolate analyses were performed using an in-house proprietary method based on the intact glucosinolate method of Wade et al.,[39] which utilizes a hydrophilic interaction liquid chromatography column. These modifications were made for several reasons: to shorten the processing time and avoid possible indole degradation, to eliminate interfering phenolic compounds by anion exchange pretreatment, to obtain separation of 13 peaks, and to increase throughput. As the B. rapa samples did not contain sinigrin, this glucosinolate was used as an internal standard after measuring the concentration using the molar extinction coefficient of twice-crystallized sinigrin[40] and after calibrating the spectrophotometer with the alkaline dichromate solution of Haupt.[41] The relative absorption coefficient of sinigrin at 229 was taken from Agerbirk et al.[42] Using the fragmentation patterns described by Clarke,[3] the identity of the peaks was confirmed using high-performance liquid chromatography–mass spectrometry at the University of California Davis Genome Center and at the Stanford University Vincent Coates Foundation Mass Spectrometry Laboratory.

Estimation of human plasma thiocyanate concentrations derived from brassica vegetables

Population data from the United States have shown urinary thiocyanate concentrations resulting from low-level environmental exposure[43] but have not been systemically evaluated on a similar scale for thiocyanate levels in plasma. As thiocyanate concentrations in human plasma derived from the action of myrosinase on indole glucosinolates have not been reported in the literature, the ratio of the glucoraphanin ingested to the sulforaphane plasma concentration was used as a surrogate, taking into account that 1 μmol of indole glucosinolate can produce no more than 1 μmol of thiocyanate.[42] As further support for the use of the glucoraphanin to sulforaphane ratio as a surrogate for indole glucosinolate to thiocyanate ratios, the demonstration by Cramer and Jeffery[44] that 74% of the ingested sulforaphane could be accounted for in the urine in 24 hours as N-acetyl-cysteine, (which results from the conjugation of sulforaphane with glutathione), implies that, while the thiocyanate to indole glucosinolate ratio could be lower than the sulforaphane to glucoraphanin ratio, it could not be more than 26% higher.

Indole glucosinolate concentrations in brassica vegetables that produce the thiocyanate ion upon mastication

There are 4 indole glucosinolates that could produce thiocyanates by the action of myrosinase: (1) the unsubstituted indole glucosinolate known as glucobrassicin; (2) an indole glucosinolate with a methoxy group on the N in the heterocycle, known as neoglucobrassicin; (3) an indole glucosinolate with a methoxy group on the 4 position of the 6-membered ring, known as 4-methoxy-glucobrassicin; and (4) an indole glucosinolate with a hydroxyl group on the 4 position of the 6-membered ring, known as 4-hydroxyglucobrassicin. Table 1[22,32,45–52] summarizes the data available for these indole glucosinolates.

The brassica vegetables with the highest total indole glucosinolate concentrations are kale (B. oleracae leaves; 840 μmol/100 g), kale (B. napus leaves; 465 μmol/100 g), and Brussels sprouts (392 μmol/100 g). Those with intermediate concentrations are B. rapa tsoi-sim heads (134 μmol/100 g), choho (149 μmol/100 g), broccoli rabe commercial varieties (187 μmol/100 g), and broccoli heads (188 μmol/100 g). The brassica vegetables with the lowest values are Chinese cabbages, turnips, pak choi, and Chinese greens, which contain glucosinolate concentrations that range from 5 to 99 μmol/100 g of fresh weight.

The two species of kale sold commercially (i.e., kale of the species B. oleracae and Russian/Siberian kale of the species B. napus) contain markedly different glucosinolate profiles. The brassica with the highest total indole glucosinolate concentration was B. oleracae kale (840 μmol/100 g), which did not contain any progoitrin, but the B. napus kale (465 μmol/100 g) had a progoitrin concentration of 176 μmol/100 g, putting it into the category of potential risk for thyroid damage.[23]

Progoitrin concentrations in brassica vegetables that produce goitrin upon mastication

Based on the measurements by Langer et al.,[23] who investigated goitrin doses that impaired thyroidal iodine uptake (i.e., 70 μmol was associated with no inhibition, whereas 194 μmol inhibited iodine uptake), the brassica vegetables were categorized into 3 classes: (1) those with progoitrin concentrations over 100 μmol/100 g of fresh weight [i.e., the Siberian (Russian) kales belonging to the species B. napus, one collard (B. oleracae), and one Brussels sprout (B. oleracae)]; (2) those with goitrin concentrations between 10 and 100 μmol/100 g of fresh weight [i.e., Chinese cabbages (B. rapa), some collards, and Brussels sprouts]; and (3) those with goitrin concentrations less than 10 μmol/100 g of fresh weight [i.e., turnip tops, some Chinese and Japanese greens, commercial broccoli rabe (B. rapa), commercial

Table 1 **Glucosinolate concentrations in brassica vegetables**

Species	Common name	Tissue type	No. tested	Mean concentration (range) in μmol/100 g of fresh weight				Reference/source
				Glucoraphanin (may provide anticancer benefits)	Thiocyanate-producing indole glucosinolates (may have adverse thyroidal effects)	Progoitrin (may have adverse thyroidal effects)	Total glucosinolates	
Brassica rapa	Napo (turnip)	Flower buds and leaves	20	0	17 (5–32)	1.49 (0–5)	489 (63–1157)	Rosa (1997)[47]
B. rapa ssp *rapa*	Turnip tops	Leaves	45	0	9 (1–26)	9.96 (1–72)	400 (133–644)	Cartea et al 2012[48]
B. rapa	Turnip	Young leaves	18	0	5	0	533	Yang & Quiros (2010)[32]
Brassica rapa L. ssp *pekinensis*	Chinese cabbage	Leaves	62	0	28 (7–72)	20.44 (8–78)	194 (39–662)	Lee et al. (2014)[49]
B. rapa	Chinese cabbage	Young leaves	39	0	27 (5–139)	2.24 (0–20)	42 (6–254)	Yang & Quiros (2010)[32]
Brassica campestris L. ssp *chinensis* var *tai-tsai* Hort.	Tai tsai	Leaves	1	0	65	31.17	175	He et al. (2003)[50]
Brassica campestris L. ssp *chinensis* var *communis* Tsen et Lee	Pak choi	Leaves	1	0	37	2.18	85	He et al. (2003)[50]
B. rapa	Broccoletto	Young leaves	2	0	16 (15–17)	0.90 (0.80–1.0)	151 (145–157)	Yang & Quiros (2010)[32]
B. rapa	Japanese green	Young leaves	1	0	19	2	68	Yang & Quiros (2010)[32]
B. rapa	Oriental green	Young leaves	1	0	2	0.09	19	Yang & Quiros (2010)[32]
B. rapa	Pak choi	Young leaves	3	16	21 (9–6.77)	8 (0–4)	56 (43–77)	Yang & Quiros (2010)[32]
B. rapa	Chiifu	Young leaves	1	0	68	37	135	D'Arrigo Bros.
B. rapa	Chiifu	Floral heads	1	5	99	68	256	D'Arrigo Bros.
B. rapa	Tsoi-sim	Young leaves	1	1	72	17.22	177	D'Arrigo Bros.
B. rapa	Tsoi-sim	Floral heads	1	2	134	23.78	328	D'Arrigo Bros.
B. rapa	Choho	Young leaves	1	1	149	2.36	209	D'Arrigo Bros.
B. rapa	Broccoli rabe (commercial varieties)	Young leaves	10	1 (0–6)	83 (53–118)	3.27 (0–7)	601 (853–317)	D'Arrigo Bros.
B. rapa	Broccoli rabe (commercial varieties)	Floral heads	10	13 (2–42)	187 (115–208)	4.55 (2–10)	809 (649–1003)	D'Arrigo Bros.
B. rapa	European (Cima de rapa varieties)	Young leaves	11	0 (0–1)	79 (40–136)	1.45 (0–3)	526 (303–820)	D'Arrigo Bros.

(continued)

Table 1 **Continued**

Species	Common name	Tissue type	No. tested	Mean concentration (range) in μmol/100 g of fresh weight				Reference/source
				Glucoraphanin (may provide anticancer benefits)	Thiocyanate-producing indole glucosinolates (may have adverse thyroidal effects)	Progoitrin (may have adverse thyroidal effects)	Total glucosinolates	
B. rapa	European (Cima de rapa varieties)	Floral heads	11	3 (0–9)	122 (63–181)	1.39 (0–5)	674 (480–888)	D'Arrigo Bros.
Brassica oleracea	Broccoli	Heads	6	64 (30–88)	59 (42–72)	0.87 (0–4)	188 (102–263)	Carlson et al. (1987)[51]
B. oleracea	Broccoli	Heads	50	96 (11–296)	26 (5–85)	13.68 (0–108)	178 (35–486)	Kushad et al. (1999)[52]
B. oleracea	Broccoli (Bravado cultivar)	Heads	1	97	61	0	159	D'Arrigo Bros.
B. oleracea	Broccoli	Heads		105		0.09		Tian et al. (2005)[53]
B. oleracea	Broccoli	Heads		30		3.90		Song et al. (2005)[22]
Brassica alboglabra Bailey	Chinese kale	Leaves	1	127	28.63	119	421	He et al. (2003)[50]
Brassica oleracea L. convar acephala var sabellica	Kale	Leaves	1	270	6.77	2	429	He et al. (2003)[50]
B. oleracea var acephala	Galega kale	Flower buds and leaves	20	117 (49–211)	16.11 (2–60)	0	468 (353–672)	Rosa (1997)[47]
B. oleracea L. var acephala	Kale	Leaves	4	55 (44–70)	5.90 (0–23)	4 (0–8)	220 (64–307)	Carlson et al. (1987)[51]
B. oleracea	Kale	Leaves	1	274	7	5	584	Ku et al. (2014)[54]
B. oleracea	Kale	Leaves	5	42 (15–62)	840 (446–1172)	0	884 (478–884)	D'Arrigo Bros.
B. oleracea	Kale (Siberian)	Leaves	1	29	102	158.1	694	Carlson et al. (1987)[51]
Brassica napus ssp pabularia	Kale (Russian)	Leaves	1	53	202	365.9	667	Ku et al. (2014)[54]
B. napus	Kale	Leaves	3	55 (3–118)	465 (216–803)	176.6 (29–368)	828 (330–1241)	D'Arrigo Bros.
B. oleracea convar acephala var medullosa Thell	Collards	Leaves	1	3	198	314.16	731	He et al. (2003)[50]
B. oleracea	Collards	Leaves	5	5 (0–13)	108 (67–165)	70.08 (17–130)	439 (316–600)	Carlson et al. (1987)[51]
B. oleracea	Brussels sprouts	Button	6	8 (0–23)	392 (328–469)	8.33 (1–25)	553 (466–601)	Carlson et al. (1987)[51]
B. oleracea	Brussels sprouts	Heads	4	13 (5–19)	84 (63–108)	42.66 (15–100)	329 (227–504)	Kushad et al. (1999)[52]
B. oleracea	Brussels sprouts	Heads		0.6		2.40		Song et al. (2005)[22]
B. oleracea	Brussels sprouts	Heads		15		133.0		Tian et al. (2005)[53]

Table 2 Studies reporting human plasma concentrations of thiocyanate and sulforaphane

Compound	No. of subjects	Condition	Reference
Thiocyanate ion (μM)			
69	37	Nonsmokers before consuming 8 mg of thiocyanate daily	Dahlberg et al. (1984)[29]
121	37	Nonsmokers after consuming 8 mg of thiocyanate daily for 12 wk	Dahlberg et al. (1984)[29]
Mean (SD) 40±24	16	Nonsmokers	Morgan et al. (2011),[30] Nedoboy et al. (2014)[31]
Mean (SD) 47±26	74	Nonsmokers	Nedoboy et al. (2014)[31]
Sulforaphane (μM)			
1.9 (mean); 0.37–7.3 (range)	14	After consumption of fresh, steamed, and microwaved broccoli and Brussels sprouts	Angelino & Jeffrey (2014)[54]
0.4	12	After consumption of fresh broccoli in crossover study	Conaway et al. (2000)[55]
0.3	18	After consumption of raw broccoli	Saha et al. (2012)[57]
0.114	5	After consumption of 200 g of raw broccoli	Song et al. (2005)[22]
0.07 (raw); 0.04 (cooked)	1	After consumption of 200 g of raw or microwaved broccoli	Vermeulen et al. (2008)[57]

broccoli (*B. oleracae*) varieties, and the kales belonging to the species *B. oleracae*].

Glucoraphanin concentrations that produce beneficial effects

Glucoraphanin was reported in large concentrations only in the *B. oleracae* species or in *B. napus* that includes the *B. oleracae* genome. None of the *B. rapa* varieties have large quantities of glucoraphanin; *B. rapa* Chiifu and some *B. rapa* broccoli rabe varieties have small levels of glucoraphanin, concentrated only in the head.

The anticarcinogenic benefits of sulforaphane, phenethyl isothiocyanate, and myrosinase-induced degradation products of indole glucosinolates can be maximized by raw consumption of the vegetables mentioned above or by the addition of functional myrosinase to denatured myrosinase (which results from cooking or blanching of frozen vegetables).[53]

Comparison between isothiocyanate plasma concentrations from brassica vegetable ingestion and endogenous thiocyanate plasma concentrations

In Table 2,[29–31,54–57] plasma concentrations of thiocyanate in smokers and nonsmokers are compared with plasma concentrations of the isothiocyanate sulforaphane for use in estimating the potential thiocyanate plasma concentration resulting from ingestion of indole glucosinolate. After consuming as much as 200 g of raw broccoli, the plasma concentration of sulforaphane (i.e., the glucoraphanin degradation product) was 0.114 μM,[22] while other reports demonstrated mean plasma levels of 0.04–0.07, 0.3, 0.4, and 1.9 μM.[54–56] Broccoli that was fed raw had a higher sulforaphane

concentration, as would be expected.[57] The maximum plasma thiocyanate value reported for smokers was 121 μM.[29] Choi et al.[58] measured the concentrations of thiocyanate, cyanide, and organic isothiocyanates in the serum of rats after administration of a single dose of 50 μmol of various glucosinolates. As shown in Table 1, a dose of 50 μmol is comparable to the amount of glucoraphanin present in a 100-g serving of broccoli for a human, and thus the maximum value of 50 μM of sinigrin-derived thiocyanate they measured in rat plasma is an overestimation of what could be expected in humans.

If 60–120 μmol of glucoraphanin per serving results in a sulforaphane concentration of less than 5 μM, an indole glucosinolate amount of 120 μmol might also result in no more than an approximately 5 μM increase in thiocyanate ion. This may be on the high side, since Agerbirk et al.[4] reported that the myrosinase-catalyzed indole glucosinolate degradation pathway was very complex and likely leads to a lower stoichiometric conversion of indole glucosinolate to thiocyanate than glucoraphanin to sulforaphane. Another complicated issue in the conversion of glucosinolates to isothiocyanates is the genetic variation in myrosinase specificity for glucosinolate epimers and the variation in the epithiospecifier protein that results in nitrile, rather than thiocyanate, formation.[59] Since the indole concentrations in many brassica vegetables are 200 μmol/100 g of fresh weight, this suggests the maximum thiocyanate increase would be on the order of 10 μM. Since this concentration is much less than 40–121 μM, the naturally occurring "control" thiocyanate concentration reported in various studies (Table 2), it is unlikely that thiocyanate arising from indole glucosinolates from normal serving sizes of common brassica vegetables would have

significant adverse effects on thyroid function. However, it would be useful to confirm this with a study in which plasma thiocyanate levels are measured before and after consumption of a meal rich in indole glucosinolates.

Progoitrin concentrations that result in decreased thyroid hormone production in animals

It is useful to compare progoitrin concentrations in brassica vegetables for human consumption with the progoitrin concentrations in defatted rapeseed meal that have caused thyroid or other metabolic dysfunction when fed to livestock. Kelley and Bjeldanes[60] found that, when a continuous diet of 154 μmol of progoitrin per 100 g was fed to rats, a hypothyroid state was produced, negatively affecting the glutathione S-transferase activities that are responsible for the conjugation and subsequent detoxification of many potentially toxic, electrophilic substances. In a comparison of two progoitrin-containing diets (71 μmol/100 g and 226 μmol/100 g) in lambs, progoitrin concentrations increased significantly in the lung and thyroid.[61] Thomke et al.[62] fed two diets containing goitrin concentrations of 383 μmol/100 g and 755 μmol/100 g to pigs and found that only the higher concentration diet significantly suppressed live weight gain and increased liver and thyroid masses.

From the previous data of Langer et al.,[23] who reported that 77 μmol of goitrin did not decrease thyroidal radioiodine uptake,[23] it is reasonable to infer that a single serving of brassica vegetables with less than 70 μmol of progoitrin would be unlikely to result in decreased thyroid hormone production in humans. Specifically, ingestion of commercial broccoli and broccoli rabe, which each contain less than 10 μmol progoitrin per 100-g serving, would appear to pose minimal risk of thyroidal toxicity. Excess consumption should be avoided, as an elderly woman in New York presented with myxedema coma (the most severe and a life-threatening form of hypothyroidism) after ingesting up to 1.5 kg of raw bok choy daily for several months.[63]

Animal feeding trials investigating the safety of rapeseed meal[19] typically do not distinguish the concentration of glucosinolate progoitrin, whose degradation product isothiocyanate goitrin has known antithyroid effects, from the total glucosinolate concentration of the meal. Since the glucosinolates glucoraphanin and phenethyl glucosinolate have well-established anticarcinogenic properties, this broad selection against all glucosinolates might be counterproductive. Similarly, the work of Tang et al.,[64] who used the benzene dithiol reagent to measure the sum of all isothiocyanates, cannot discriminate between profiles with or without high concentrations of goitrin or sulforaphane.

The major potential negative effects of brassica consumption on thyroid hormone production have been suggested to result from the goitrin and thiocyanate content of these vegetables, as described above. However, decreased thyroidal radioiodine uptake has been also demonstrated to occur when allyl and methyl isothiocyanates (from mustards) were incubated with thyroid tissue slices at concentrations of 10^{-3} and 10^{-4}M.[18] Whereas the concentrations of isothiocyanates in the tissue slices with diminished iodine uptake were much higher than the background levels found in human plasma (Table 2), intracellular isothiocyanate concentrations can be orders of magnitude higher (i.e., mM). These high increased intracellular concentrations are due to diffusion of the isothiocyanates across the cell membrane. Once inside the cell, the isothiocyanates spontaneously form adducts with the cysteine thiol of glutathione.[65] Following secretion, these conjugates are unstable and readily disassociate to their parent compounds,[65] and therefore, conjugate concentrations in the millimolar range would also expose the tissues to the same range of isothiocyanate concentrations that Ermans and Bourdoux[18] demonstrated in thyroid slices and reported as evidence of decreased iodine uptake.

Genetics of glucoraphanin and progoitrin synthesis

Progoitrin is the terminal glucosinolate produced in a chain of glucosinolate syntheses that start with methionine (Figure 1).[32,34] After removal of the carboxylic acid and amine group, the methionine side chain is elongated to produce a methyl thiobutyl group. The thioether in this group is first oxidized by the enzyme glucosinolate oxidase (NCBI [National Center for Biotechnology Information] accession no. FJ3760740) (GSL-OX) to produce the methylsulfinyl group (i.e., glucoraphanin) and is then oxidized – through the loss of the CH3CO – by the enzyme glucosinolate alkenylase (GSL-ALK) (NCBI accession no. EF611253) to produce the unsaturated butenyl (gluconapin). Lastly, it is oxidized by the enzyme glucosinolate hydroxylase (GSL-OH) (NCBI accession no. FJ376074) to produce hydroxybutenyl, which, after a nonenzymatic reaction, cyclizes to produce goitrin.

In broccoli, the GSL-ALK enzyme is nonfunctional due to a 2-bp deletion.[66] This results in termination of the pathway and, consequently, high glucoraphanin concentrations. The majority of the *B. rapa* species have a functional *GSL-ALK* gene, and thus the glucosinolate present at the highest concentration by far is the butenyl glucosinolate (D'Arrigo Bros. Co., unpublished data and Yang and Quiros[32]). This highly functional *GSL-*

ALK gene is also responsible for very low glucoraphanin concentrations in *B. rapa*, since the glucoraphanin is converted in the next step of the pathway (i.e., to butenyl glucosinolate).

Li et al.[34] examined crosses of broccoli × cauliflower, collard × broccoli, and collard × cauliflower and found that the hydroxylating gene *GSL-OH* segregated with high progoitrin concentrations in the collard × broccoli progeny. This indicates that progoitrin production is single-gene controlled and, due to common ancestry among *Brassica* species, could occur in most commercial brassica vegetable species. Fortunately, it only occurs in high concentrations in *B. napus* kale and some collards.

Liu et al.[66] have utilized knowledge of this pathway and RNA silencing techniques to downregulate expression of the *GSL-ALK* genes in the problematic *B. napus* species used for oilseed production. Not only was the progoitrin reduced by 65% in the seeds, but the glucoraphanin concentration was increased to a high concentration of 4226 μmol/100 g of seeds. It would be interesting to examine the glucoraphanin concentration in the sprouts of this transgenic plant.

CONCLUSION

Components of brassica vegetables demonstrate important anticarcinogenic properties that must be balanced against the potential adverse effects of progoitrin and thiocyanate on thyroid hormone production. The consumption of typical serving sizes of raw, commercial *B. oleracae* and *B. rapa* varieties (i.e., broccoli, Chinese cabbage, bok choy, broccoli rabe) correspond to progoitrin- and thiocyanate-generating indole glucosinolate exposures at concentrations far lower than those likely to impair thyroid function. In contrast, excessive consumption (e.g., >1 kg/d for several months) of raw Russian/Siberian kale of the species *B. napus*, some collards, and Brussels sprouts, all of which have high progoitrin concentrations and thus can decrease iodine uptake into the thyroid to affect the synthesis of thyroid hormone, should be avoided.

Acknowledgments

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