

Current Knowledge and Challenges on the Development of a Dietary Glucosinolate Database in the United States

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ABSTRACT

Glucosinolates (GSLs) are a group of cancer chemopreventive sulfur-containing compounds found primarily in Brassica vegetables. The goals of this study were to summarize the current knowledge and discuss the challenges of developing a dietary GSL database for US foods. A systematic literature search was conducted for the period 1980–2020. Thirty articles were found to meet all inclusion and exclusion criteria; 27 GSLs were reported in 16 different vegetables. GSLs identified and quantified ranged from 3 for winter cress to 16 for cabbage. In general, the experimental designs of these 30 studies did not fully consider the factors related to the data quality. Enormous variations of GSLs are observed between different vegetables and in the same vegetables. In conclusion, the studies on GSLs in commonly consumed vegetables are still limited, and some data may be outdated. Currently available data are not sufficient to develop a valid GSL database in the United States. Curr Dev Nutr 2021;5:nzab102.

Keywords: Brassica, cruciferous, database, food composition, glucosinolate, isothiocyanate

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Introduction

Glucosinolates (GSLs) are sulfur-rich, anionic plant secondary metabolites (**[Figure 1](#page-1-0)**) found principally in the plant order Brassicales. The genus *Brassica* in Brassicaceae (older name, Cruciferae) contains most of the commonly consumed vegetables [\(1\)](#page-18-0), such as broccoli, kale, cabbages, cauliflower, and Brussels sprouts, which are often referred to as *Brassica* or cruciferous vegetables. In addition, a number of non-*Brassica* edible plants, such as radish, papaya, and winter cress, also contain GSLs. Evidence of human usage of GSL-containing plants as foods or condiments dates back thousands of years [\(2\)](#page-18-1). At present, some *Brassica* vegetables, including broccoli, cabbage, and cauliflower, are among the mostly commonly consumed vegetables in the world [\(3\)](#page-18-2).

In plants, GSLs coexist with an endogenous β -thioglucosidase called myrosinase; but they are stored separately in different cells or in different intracellular compartments. Upon plant tissue disruption, myrosinase comes into contact with GSLs resulting in rapid hydrolysis to form a variety of hydrolytic products [\(4\)](#page-18-3). The hydrolytic products exhibit toxicity or feeding deterrence to a wide range of potential insect herbivores. In humans, epidemiological evidence from prospective cohort studies and retrospective case-control studies has linked consumption of cruciferous vegetables to reduced risk of various types of cancer, including lung [\(5\)](#page-18-4), gastric [\(6\)](#page-18-5), colorectal [\(7\)](#page-18-6), breast [\(6\)](#page-18-5), bladder [\(8\)](#page-18-7), and

prostate cancer [\(9\)](#page-18-8). Large numbers of animal studies and human clinical trials have also been performed to reveal the possible molecular mechanisms, such as detoxification of carcinogens [\(10,](#page-18-9) [11\)](#page-18-10), antioxidant activities [\(12\)](#page-18-11), modulation of inflammation [\(13\)](#page-18-12), and induction of apoptosis [\(14\)](#page-18-13). The evidence accumulated in the past decades suggested that the cancer chemopreventive effects of cruciferous vegetables could largely be attributed to the hydrolytic products of GSLs such as isothiocyanates and indole-3-carbinol [\(15\)](#page-19-0).

Types of GSL and their concentrations vary enormously among different plants, both qualitatively and quantitatively, according to the species and cultivar, tissue type, growing stage, environmental factors, insect attack, and microorganism intrusion [\(16,](#page-19-1) [17\)](#page-19-2). The total GSL intake was estimated as 14.2 ± 1.1 mg/d for men and 14.8 ± 1.3 mg/d for women in a German population [\(18\)](#page-19-3); and 6.5 mg/d, among which 35% were of indole type, in a Spanish adult population [\(19\)](#page-19-4). The national mean daily intake in the United Kingdom was calculated to be 46.1 mg in fresh materials, and 29.4 mg from cooked foods [\(20\)](#page-19-5). However, these crude dietary intake estimates were made based on very limited data or dietary exposure to GSL-containing vegetables. There is currently no dietary intake estimation of GSLs in the United States, due to lack of adequate dietary GSL composition data.

Food composition data are considered as the foundation of dietetic practice and nutritional research [\(21\)](#page-19-6). In the United States, our group

FIGURE 1 Core chemical structure of glucosinolates.

has developed several databases for the dietary bioactive compounds of public health interest such as flavonoids, which have been widely used as a valuable tool to assess their dietary intakes [\(22,](#page-19-7) [23\)](#page-19-8). Recent interests of the scientific community in dietary GSLs center on their chemoprotective effects against cancer [\(24–26\)](#page-19-9). The relations between consumption of *Brassica* vegetables and/or GSLs and the risk of cancers have been investigated in many nutritional epidemiological studies [\(27–30\)](#page-19-10). To better understand the association between GSL intake and the risk of cancer and other chronic diseases, it is critical to precisely estimate the dietary intake of GSLs. A valid composition database of dietary GSLs would be expected to provide fundamental information in calculating the GSL intake.

An extensive literature search found only one published study that attempted to develop a dietary GSL database [\(31\)](#page-19-11). This article was published in 2003 and has since been broadly cited in epidemiological studies and used to estimate the dietary intake of dietary GSLs. However, data from only 18 references were included in developing the database, and among them 10 studies used the glucose-release methods, 5 used HPLC, and 3 used GC-based methods. Because of the limitation of analytical methods, no data on individual GSLs were provided. With significantly more data generated in the last two decades, and most importantly, the advancement of new analytical methods, it is possible to develop a new dietary GSL database. Development of a food composition database is a multistep process, generally including data acquisition, data evaluation, data compilation, data aggregation, and data dissemination [\(32,](#page-19-12) [33\)](#page-19-13). Data collection and the quality evaluation are the core tasks of developing a valid food composition database [\(23,](#page-19-8) [34\)](#page-19-14). The

FIGURE 2 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram of literature search and screening.

Year	Sample location	Foods	Analytical method	Reference
2003	Urbana, IL, USA	Broccoli	HPLC after desulfation	(41)
1994	Washington DC, USA	Broccoli (green sprouting broccoli)	HPLC after desulfation	(47)
2002	IL, USA	Broccoli	HPLC after desulfation	(72)
1981	Madison, WI, USA	Rutabaga, turnip	GC	(73)
1987	Madison, WI, USA	Broccoli, Brussels sprouts, cauliflower, collards, kale, mustard greens, kohlrabi	GC	(74)
1985	Madison, WI, USA	Radish	GC	(75)
1987	Madison, WI, USA	Turnip	GC	(76)
2000	Knoxville, TN, USA	Broccoli	HPLC after desulfation	(77)
2005	Knoxville, TN, USA	Broccoli, Brussels sprouts, red cabbage, cauliflower, kale	HPLC after desulfation	(43)
1980	OR, NY, WI, USA	Cabbage	GC-MS	(78)
2014	USA	Brussels sprouts, cabbage	HPLC after desulfation	(79)
1988	Syracuse, NY, USA	Broccoli, Brussels sprouts, green cabbage	HPLC after desulfation	(53)
1989	USA	Brussels sprouts, broccoli	HPLC after desulfation	(52)
1995	Salinas, CA, USA	Broccoli	HPLC after desulfation	(80)
2011	USA	Broccoli	HPLC after desulfation	(81)
2014	IL, USA	Broccoli	HPLC after desulfation	(82)
2015	IL, USA	Horseradish	HPLC after desulfation	(83)
1999	Champaign, IL, USA	Cabbage, broccoli, kale, Brussels sprouts, cauliflower	HPLC after desulfation	(48)
2004	Champaign, IL, USA	Horseradish	HPLC after desulfation	(84)
2001	Champaign, IL, USA	Broccoli	GC-MS	(44)
2005	Wooster, OH, USA	Green cabbage	HPLC after desulfation	(85)
2014	ME, OR, USA	Broccoli	HPLC after desulfation	(86)
2005	CA, USA	Broccoli	GC-MS	(87)
2005	Becker, MN, USA	Red cabbage, green cabbage	HPLC after desulfation	(42)
1991	Ontario, Canada	Rutabaga	HPLC after desulfation	(88)
1993	Ontario, Canada	Broccoli	HPLC after desulfation	(54)
2017	College Station, TX, USA	Green kohlrabi, green cabbage	HPLC after desulfation	(89)
2015	MD, USA	Broccoli microgreens	UPLC after desulfation	(90)
2005	Columbus, OH, USA	Broccoli, broccoli sprouts, Brussels sprouts, cauliflower	HPLC-MS	(91)
1997	Canada; Harmony, NJ, USA	Winter cress	HPLC-MS	(92)

TABLE 1 Summary of the studies that contained the original data of glucosinolate contents of foods in the United States and Canada (1980–2020)1

1UPLC, ultra performance liquid chromatography.

primary goals of this study were to summarize the currently available GSL data, evaluate the data quality, and discuss the challenges on the development and application of a dietary GSL database for the foods grown and/or sold in the United States.

Methods

A systemic literature search was conducted for the period 1980–2020 based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [\(35\)](#page-19-24). PubMed, SciFinder, and Google Scholar were selected as primary search databases. The following keywords were used in different combinations: glucosinolate, *Brassica*, cruciferous, food composition, analysis, isothiocyanate, vegetable. Reference lists of the retrieved research and review articles were reviewed to identify references not found using electronic search engines. Only data in original research articles were included; secondary data

in review articles or books were excluded. The detailed inclusion and exclusion criteria are described as follows:

- a) Because the database is intended to be developed for the US foods, plant materials obtained from retailers or fields in the United States were included. According to USDA's Economic Research Service, Mexico and Canada are the major exporters of GSL-containing vegetables to the United States (https://www.ers.usda.gov/data-products/vegetables-andpulses-data/by-commodity). Therefore, data from the studies conducted in these two countries, if any, were also included.
- b) Information on the source and identification, ideally full scientific names and cultivars, of samples were provided. Only the data on fresh raw samples were included.
- c) Only the data of commonly consumed plants and the edible parts of the samples were included. Wild plants or those used as folk medicine, animal feeds, nonedible parts, or by-products of the plants, such as seeds of certain vegetables [\(36\)](#page-19-25), were excluded.

FIGURE 3 Numbers of publications on glucosinolate-containing vegetables in the United States and Canada from 1980 to 2020.

- d) The analytical methods must be carefully described. Because studies have shown that different GSLs and isothiocyanates had different biological effects, only the methods that quantify individual GSLs were included. Thus, the publications that only reported total GSLs were excluded.
- e) For the publications that aimed to study the factors or the effects of certain treatments or manipulations on the concentrations of GSLs, only the data from untreated or control groups were included.

Data extracted from the studies were converted to the unit of milligrams per 100 g fresh weight (FW). Values reported based on dry weight were converted into an FW basis using the water content provided in the specific study, or the moisture level reported in USDA National Nutrient Database for Standard Reference, Legacy Release [\(37\)](#page-19-26) if such information is not available in the literature.

Results

Overview of the publications

After several sequential stages of screening [\(35\)](#page-19-24), 30 articles were found to meet all inclusion and exclusion criteria (**[Figure 2](#page-1-1)**), including 28 articles published by US researchers and 2 by Canadian researchers. The summary of these 30 studies, including the year of publication, foods being analyzed, location of samples, and the analytical method,

TABLE 2 Scientific and common names of 16 glucosinolate-containing plant foods

Scientific name	Common name	
Armoracia rusticana	Horseradish	
Barbarea vulgaris	Winter cress	
Brassica campestris ssp. rapifera	Turnip	
Brassica juncea var. rugosa	Mustard greens	
Brassica napus ssp. rapifera	Rutabaga	
Brassica oleracea var. acephala	Kale	
Brassica oleracea L. acephala group (var. sabellica)	Collard	
Brassica oleracea var. botrytis	Cauliflower	
Brassica oleracea var. botrytis subvar. cymosa Lam.	Broccoli, calabrese, green sprouting broccoli	
Brassica oleracea var. capitata	Cabbage, red cabbage, green cabbage	
Brassica oleracea var. gemmifera	Brussels sprouts	
Brassica oleracea var. gongylodes	Kohlrabi	
Brassica oleracea var. italica	Broccoli	
Brassica oleracea var. italica	Broccoli microgreens	
Brassica oleracea var. italica	Broccoli sprouts	
Raphanus sativus ssp. radicola	Radish (red, white, and black)	

TABLE 3 Glucosinolates reported in 16 glucosinolate-containing plant foods and their trivial and semisystematic names¹

1GSL, glucosinolate; N/A, not available.

is presented in **[Table 1](#page-2-0)**. The number of publications almost equally distributed in each decade from 1980 to 2020. But the number of publications for different foods is unequal. Broccoli is the most studied one, which appeared in 16 studies. Cabbage and Brussels sprouts were each reported in 7 studies, cauliflower was reported in 4 studies, and kale in 3 studies. The rest of the foods were only presented in 1–2 studies (**[Figure 3](#page-3-0)**).

Overview of the data

GSLs in 16 different vegetables were reported, including 13 *Brassica* vegetables and 3 non-*Brassica* vegetables. Of the 13 *Brassica* vegetables, 10 of them are different varieties of *Brassica oleracea* L., which represent the most commonly consumed cruciferous vegetable in the United States. The common names of these vegetables and their scientific names are listed in**[Table 2](#page-3-1)**. In terms of the analytical method, HPLC analysis of desulfated GSLs [\(38\)](#page-19-27) was found to be the primary quantification method. GC or GC-MS were used in several early studies, and HPLC-MS was adopted in 2 studies. Although >100 GSLs have been identified or tentatively identified in plants thus far [\(39\)](#page-19-28), only 27 GSLs were reported in all 16 vegetables, included 19 aliphatic, 4 aromatic, and 4 indole GSLs (**[Table 3](#page-4-0)**). The concentrations of individual GSLs in each food are presented in **[Tables 4–8](#page-5-0)**. For studies with >1 sample, the range of the values is presented. The number of GSLs identified and quanti-

fied varied considerably between different vegetables, ranging from 3 for winter cress to 16 for cabbage (**[Table 9](#page-17-0)**).

Discussion

Data source: domestic compared with international data

Before developing a database of certain dietary component(s) based on the literature data, the first question to ask is, "What data should be included?" This question should be answered based on the purpose of developing such a database. GSLs are not essential nutrients for human beings. The main purpose of developing a dietary GSL database is to estimate the dietary intake, which can then be used in the nutritional epidemiological studies to evaluate the diet-disease relations [\(31\)](#page-19-11). Notably, almost all nutritional epidemiological studies were conducted in specific countries or regions, as was indicated in a recent review article [\(24\)](#page-19-9). Hence, calculating the GSL composition in 1 country or region, rather than combining all international data, would be more relevant in assessing the dietary intake of these compounds. Furthermore, types and concentrations of GSLs are highly variable based on the genetic background and various environmental factors [\(17\)](#page-19-2). Inclusion of international data will add unnecessary variations to the dataset. Therefore, only the

TABLE 4 Glucosinolates identified in broccoli grown in the Unites States¹

(Continued)

TABLE 4 (Continued)

TABLE 4 (Continued)

¹Data reported as dry weight in the literature were converted into fresh weight using the water content in USDA National Nutrient Database for Standard Reference, Legacy Release [\(37\)](#page-19-26), unless they were provided in the literature. FW, fresh weight; GSL, glucosinolate; N/A, not available; Refs, references.

data of the vegetables that people in the United States have access to, including those obtained from the markets in the United States or grown in the United States, Canada, and Mexico, were included in this study.

Evaluation of data quality

Many factors need to be considered when discussing the quality of food composition data. To ensure data quality, the USDA Methods and Application of Food Composition Laboratory (formerly the Nutrient Data Laboratory) has developed a comprehensive data quality evaluation system [\(34,](#page-19-14) [40\)](#page-19-29). Five categories, namely, sampling plan, number of samples, sample handling, analytical method, and analytical quality control, must be carefully assessed to ensure data quality. It is noteworthy that none of the 30 articles included in this study was designed to produce data for the purpose of developing a composition database. These studies were carried out for various other purposes, such as the comparison between different cultivars [\(41\)](#page-19-15), effects of fertilization [\(42\)](#page-19-22), or the seasonal variation [\(43\)](#page-19-17). Hence, no standard procedure was followed, and the experimental design generally did not fully consider the factors discussed above. For a certain study [\(44\)](#page-19-21), only GSLs of interest, not the full profile, were analyzed and reported.

As for the 5 categories, sampling plans of these studies were generally not comprehensive. Samples in some studies were collected from the field with information about cultivars, location, and growing conditions, whereas others were obtained from the local supermarket without further information. Another commonly neglected issue is the insufficient or incorrect description of the plant materials. For example, in 5 studies, only common names of the samples were provided. Because many GSL-containing vegetables have been consumed by human beings for hundreds or even thousands of years, it is very common that one species/variety has multiple common names, and one common name can refer to different varieties or even species [\(15,](#page-19-0) [45,](#page-19-30) [46\)](#page-19-31). As an example, *Brassica oleracea* var. *botrytis* subvar. *cymosa* Lam. was referred to as broccoli in one study [\(47\)](#page-19-16), whereas the scientific name of broccoli should be *Brassica oleracea* var. *italica*. And the lack of the major broccoli GSL glucoraphanin in *Brassica oleracea* var. *botrytis* subvar. *cymosa* Lam. confirmed its difference from *Brassica oleracea* var. *italica* (Table [4\). To avoid misuse of the plant materials, samples need to be described](#page-5-0) with correct and complete scientific names in addition to the common names.

The number of samples varied considerably based on specific experimental designs. Fifty genotypes of broccoli were included in one study to investigate the variation of GSLs in *Brassica* vegetables [\(48\)](#page-19-20), [whereas in many other studies, only 1 sample was analyzed \(Tables 4–](#page-5-0) 8). Sample handling was also different between different studies. The plant materials were analyzed either as raw or dry forms, with or without cold storage. For the analytical methods, HPLC analysis of desulfated GSL is the predominant analytical method, which was used in 21 of 30 studies. GC or GC-MS were mainly used in the early published articles, and HPLC-MS was used in only 2 recent studies. With the same method, the sample preparation and detailed analytical procedure were also different between different studies. Lastly, the analytical quality control, which ensures that the results of analysis are consistent, accurate, and comparable, was unfortunately not mentioned in any of the 30 studies.

Data variation

In plants, GSLs belong to an extraordinarily diverse group of plant secondary metabolites. They play important roles to help plants cope with continuous environmental challenges [\(49\)](#page-19-32). GSLs are one of the best-known groups of plant secondary metabolites for plant antiherbivore defenses [\(50\)](#page-19-33). In contrast to the primary metabolites, the diversity and variability of plant secondary metabolites are much greater [\(51\)](#page-19-34). It has been shown that the type and concentration(s) of GSLs

TABLE 5 Glucosinolates identified in cabbages in the United States¹

TABLE 5 (Continued)

¹Data reported as dry weight in the literature were converted into fresh weight using the water content in USDA National Nutrient Database for Standard Reference, Legacy Release [\(37\)](#page-19-26), unless they were provided in the literature. FW, fresh weight; GSL, glucosinolate; N/A, not available; Refs, references. 2Estimated from graphic data.

in GSL-containing plants can be greatly affected by the genetic background, agricultural practices, and various environmental factors [\(17\)](#page-19-2). As shown in [Tables 4–8,](#page-5-0) there are substantial variations in the values between different vegetables and different cultivars/genotypes and growing conditions in the same vegetables.

Because more studies were conducted for broccoli than for any other foods summarized in this study, it was selected as an example to discuss the data variation and the major influential factors. First of all, except for 2 studies, which appear to have been conducted by the same group on the same material [\(52,](#page-19-19) [53\)](#page-19-18), the number of GSLs reported in broccoli is different for all other studies (**[Table 10](#page-18-14)**), ranging from 1 [\(44\)](#page-19-21) to 13 [\(48\)](#page-19-20). It should be mentioned that the study that reported only 1 GSL (glucoraphanin) focused on isothiocyanate sulforaphane, and glucoraphanin was quantified as the precursor of sulforaphane. The major GSLs detected in broccoli are glucoraphanin and progoitrin of aliphatic GSLs, and glucobrassicin and neoglucobrassicin of indole GSLs. Yet glucoraphanin is the only GSL that was identified and quantified in all 16

studies. Based on the published data, several key factors are discussed as follows:

The plant's genetic background.

In one study [\(41\)](#page-19-15), 21 cultivars were grown on the same field in the same year. The concentration ranges of the 4 major GSLs were extremely wide: glucoraphanin (1.09–58.94 mg/100 g FW), progoitrin (0.72–19.41 mg/100 g FW), glucobrassicin (1.09–15.66 mg/100 g FW), and neoglucobrassicin (0–22.61 mg/100 g FW), respectively. The variabilities of minor GSLs were even greater.

Growing condition.

As shown in 1 study [\(54\)](#page-19-23), the same cultivars grown at 3 locations in Ontario, Canada, resulted in a ≤10-fold difference for certain GSLs (e.g., neoglucobrassicin). In another study [\(43\)](#page-19-17), contents of GSLs were found to be significantly different in broccoli grown in the spring and fall. The variation was further complicated by different cultivars.

TABLE 6 Glucosinolates identified in Brussels sprouts in the United States¹

TABLE 6 (Continued)

¹Data reported as dry weight in the literature were converted into fresh weight using the water content in USDA National Nutrient Database for Standard Reference, Legacy Release [\(37\)](#page-19-26), unless they were provided in the literature. FW, fresh weight; GSL, glucosinolate; N/A, not available; Refs, references.

Analytical methods.

Even with the same method, analytical variation is unavoidable between studies due to different sample handling protocols, extraction methods, operation, and instrumentation. Changes in analytical methods, such as from GC to HPLC after desulfation, might lead to very different GSL values. In addition, because of their coexistence with myrosinase in plant tissues, enzyme deactivation is proved to be a critical step to avoid hydrolytic loss and/or de novo synthesis during sample preparation. Different ways of myrosinase deactivation or not deactivating it will inevitably alter the GSL profile and their concentrations [\(55\)](#page-19-35).

Challenges and considerations of developing a valid dietary GSL database

Insufficient data.

Overall, the number of studies on the quantification of GSLs in commonly consumed vegetables is quite limited, with only 30 studies conducted in the United States and Canada across 4 decades. Eleven vegetables were studied in only 1 or 2 articles, which by no means would reflect the variabilities of their GSL concentrations. Not only do we have insufficient data, but some of the data could be outdated. Nearly half of the studies were published >20 y ago. One of the major considerations in developing food composition databases has been providing and maintaining data that reflect the foods that are being currently consumed [\(56\)](#page-20-21). The food composition, including the plant secondary metabolites such as GSLs, changes over time due to the developments of new cultivars, changes in growing conditions and agricultural practices, as well as the advances of new analytical methodology.

Data expression and utilization.

One of the major applications of the food composition databases is to calculate dietary intakes of the food components for clinical and healthrelated research. However, this process is often criticized as too unreliable because food components vary greatly. As discussed in the previous sections, GSLs exhibit extremely high variation. Taking broccoli as an example, variability of GSLs identified in broccoli between different studies [\(Table 10\)](#page-18-14) is almost as great as that in different foods (Table [4\). The values of individual GSLs varied considerably between different](#page-5-0)

studies. As an example, the concentration of glucoraphanin, the major GSL in broccoli, ranged from 1.92 to 217.90 mg/100 g FW [\(Table 4\)](#page-5-0). In several available databases on dietary bioactive compounds, such as the flavonoid databases developed by our group, the data are usually expressed as mean, median, and range [\(23,](#page-19-8) [33\)](#page-19-13). Users of food composition data often do not pay much attention to the information beyond simple mean values, nor are they sufficiently aware of the natural variation in the composition of a food affected by various genetic, environmental, and management factors [\(57\)](#page-20-22). The professionals who calculate dietary intakes based on the dietary database are often unable to decide whether the database entries represent the actual foods eaten by the specific population they are interested in. Better descriptions of the source of data and the genetic background, growing conditions, storage, and processing will minimize the uncertainty but still be unable to completely address the huge variation of the data.

Application of a dietary GSL database in nutritional epidemiological research.

Nutritional research has shifted from addressing nutrient deficiency to the role of diet in preventing chronic and degenerative diseases, as well as in overall well-being and longevity. Nutritional epidemiological research plays a central role in the field of nutritional sciences, through which the diet-disease relations first observed or hypothesized in the laboratory can be examined at the level of free-living populations and clinically defined subgroups [\(58\)](#page-20-23). Well-conducted, large, prospective epidemiological studies have been crucial in updating the dietary guidelines [\(59\)](#page-20-24). However, nutritional epidemiology has recently been criticized for several major shortcomings [\(60\)](#page-20-25), one of which is the inability to accurately measure the diet intake [\(61\)](#page-20-26). In 1 recent study, the intake of GSLs and the risk of type 2 diabetes was assessed in 3 prospective cohorts of US men and women [\(62\)](#page-20-27). The major flaw of this article, argued in a "Letter to the Editor" [\(63\)](#page-20-28), is that the authors adopted the GSL intake data based on a publication [\(18\)](#page-19-3) that only provided mean values but did not consider the effects of processing or the preparation of vegetables [\(63\)](#page-20-28).

The observation that only hydrolytic products of GSLs are actual in vivo bioactive compounds posted additional concerns about using the GSL database to assess the intake for nutritional epidemiological

Scientific name Plant material Glucosinolates Concentration range (mg/100 g FW) Refs Brassica oleracea L. botrytis Cauliflower, 5 cultivars Aliphatic GSLs [\(74\)](#page-20-2)
Glucoerucin 0.08–0.55 (74) Glucoerucin 0.08–0.55 Glucoiberin Glucoiberverin 0.65–3.01
Gluconapin 0–0.04 Gluconapin 0–0.04 Glucoraphanin 0–0.74 Sinigrin Indole GSL Glucobrassicin 8.42–46.91 Aromatic GSL Gluconasturtiin 0–0.17 Brassica oleracea L. botrytis Cauliflower, 2 cultivars, 3 y, 2 seasons Aliphatic GSLs [\(43\)](#page-19-17)

Glucoiberin 1.01–10.40 (and the formulation of th Glucoiberin 1.01–10.4
Gluconapin 1.01–10.40 Gluconapin Glucoraphanin 0–1.04 Progoitrin
Sinigrin Sinigrin 1.14–13.10 Indole GSLs Glucobrassicin 9.95–28.78
4-Hydroxyglucobrassicin 0.37–5.15 4-Hydroxyglucobrassicin 0.37–5.15 4-Methoxyglucobrassicin 0.76–2.27 Neoglucobrassicin Aromatic GSL Gluconasturtiin 0–5.03 Brassica oleracea L. botrytis Cauliflower, 3 genotypes genotypes) [\(48\)](#page-19-20) Glucobrassicanapin 0.31 Gluconapin 0.89 Glucoraphanin Napoleiferin 0.64 Progoitrin Sinigrin 26.48 Indole GSLs Glucobrassicin 162
4-Hydroxyalucobrassicin 5.89 4-Hydroxyglucobrassicin 5.89 4-Methoxyglucobrassicin 3.79 Neoglucobrassicin 0.76 Aromatic GSL Gluconasturtiin 1.34
Aliphatic GSLs N/A Cauliflower, 1 cultivar Aliphatic GSLs [\(91\)](#page-20-19) Glucoalyssin 2.11 Glucoerucin 1.09 Glucoiberin 17.60
Gluconapin 17.60 Gluconapin 1.98
Glucoraphanin 1.98 Glucoraphanin 1.98
Progoitrin 1.98 Progoitrin Sinigrin 15.08 Indole GSLs 4-Methoxyglucobrassicin 19.31 Glucobrassicin 68.54
Neoglucobrassicin 11.19 Neoglucobrassicin Brassica oleracea var. acephala Curly kale, 2 cultivars; smooth-leafed kale, 1 cultivar Aliphatic GSLs [\(74\)](#page-20-2)
Glucoiberin 2.70–29.18 Glucoiberin 2.70–29.1
Gluconapin 1990 - 1.53 Gluconapin 0–1.53 Glucoraphanin 0.31–3.3
Progoitrin 0–8.75 Progoitrin
Sinigrin 7.40–10.30 Indole GSL Glucobrassicin 19.80–31.14 Aromatic GSL Gluconasturtiin 0.34–0.93

TABLE 7 Glucosinolates identified in cauliflower and kale grown in the United States¹

TABLE 7 (Continued)

¹Data reported as dry weight in the literature were converted into fresh weight using the water contents in USDA National Nutrient Database for Standard Reference, Legacy Release [\(37\)](#page-19-26), unless they were provided in the literature. FW, fresh weight; GSL, glucosinolate; N/A, not available; Refs, references.

studies. For example, the yield of isothiocyanates from myrosinasecatalyzed hydrolysis of GSLs is influenced by a number of factors. In a study that compared total isothiocyanate yield of 9 commonly consumed raw cruciferous vegetables in the United States [\(64\)](#page-20-29), a wide range (as much as 41-fold difference) of isothiocyanate yield was observed across the vegetables. Striking variation in isothiocyanate yield was also observed within each vegetable. For example, 9 samples of mustard green showed a 345-fold difference in isothiocyanate yield. Cooking is another important factor that can significantly alter the isothiocyanate yields. It has been shown in another study [\(65\)](#page-20-30) that boiling, stewing, and chip-baking reduced isothiocyanate yields, whereas stir-frying, steaming, and microwaving increased isothiocyanate yields from cruciferous vegetables. The extent of change was significant. For instance, a maximum 11-fold increase by stir-frying in broccoli and a 99% reduction by stewing in cabbage. In addition to hydrolysis by myrosinase, GSLs can be hydrolyzed by other "myrosinase-like" enzymes; and nonisothiocyanate breakdown compounds of GSLs are also produced through thermal processing or chemical degradation [\(17\)](#page-19-2). Pathway(s) of GSL hydrolysis/degradation and the conditions determine the type and amount of breakdown compounds being produced. Therefore, the dietary intake of GSLs might not correlate with the concentration of isothiocyanates in the human body. Studies have shown that generally intake of cruciferous vegetables was only weakly correlated with the urinary isothiocyanate concentrations [\(66\)](#page-20-31).

Because intake of GSLs might not correlate with their actual bioactive forms in vivo, nutritional epidemiological studies that attempted

to establish the association between the intake of GSLs and cancer risk can lead to inconsistent or even false results. Alternative methods to quantify the hydrolytic or breakdown compounds of GSLs in the human body could provide more relevant information. One such method is the measurement of urinary isothiocyanates. Isothiocyanates are excreted in urine, and thus provide a sensitive and specific dietary biomarker of GSL intake. Indeed, studies have been carried out to investigate the association between urinary isothiocyanates and cancer risk [\(67–70\)](#page-20-32). Interestingly, in 1 of these studies [\(68\)](#page-20-33), a urinary isothiocyanate biomarker was found to be associated with significantly reduced breast cancer risk in Chinese women, whereas total *Brassica* intake did not show such an association. Nevertheless, measuring urinary isothiocyanate is obviously very much resource dependent. It might not be feasible when the nutritional epidemiological studies are performed with a large human population for multiple years. GSL intake estimated by an FFQ and GSL database/dataset could still be useful. But it is important for the researchers to understand the limitations of using such information when interpreting the data or explaining the discrepancies.

Conclusion

The studies on GSLs in commonly consumed GSL-containing vegetables in the United States are still quite limited, and some of the data could be outdated. The data quality of these studies varies but is generally unsatisfactory because none of them were designed to provide data

TABLE 8 Glucosinolates identified in other foods in the United States¹

(Continued)

TABLE 8 (Continued)

(Continued)

TABLE 8 (Continued)

¹Data reported as dry weight in the literature were converted into fresh weight using the water contents in USDA National Nutrient Database for Standard Reference, Legacy Release [\(37\)](#page-19-26), unless they were provided in the literature. FW, fresh weight; GSL, glucosinolate; N/A, not available; Refs, references.

for the development of a composition database. Enormous variations of GSL data are observed between different foods and between different studies and cultivars in the same foods. Currently available data are not sufficient to develop a valid GSL database in the United States, and more comprehensive studies are needed, especially for the understudied foods. For these reasons, the total GSL concentration in a typical American diet was not calculated in this study to avoid misleading. Another consideration is that GSLs can also be found in various dietary

supplements, and they could contribute significantly to the daily intake. Unfortunately, the information on GSL contents in dietary supplements is largely unavailable.

Because intake of GSLs might not correlate with their actual bioactive forms in vivo, making an association between GSL intake and the disease risk factors can lead to inconsistent or discrepant results in nutritional epidemiological studies. Alternative methods such as the measurement of urinary isothiocyanates as biomarkers of GSL intake could

This table was prepared based on the data presented in Tables 4–8. BR, broccoli; BRM, broccoli microgreens; BRS, broccoli sprouts; BS, Brussels sprouts; CB, cabbage; CF, cauliflower; CL, collard; GSB, green sprout 1This table was prepared based on the data presented in [Tables](#page-5-0) 4–[8.](#page-5-0) BR, broccoli; BRM, broccoli microgreens; BRS, broccoli sprouts; BS, Brussels sprouts; CB, cabbage; CF, cauliflower; CL, collard; GSB, green sprout RD Glucosinolate DR BR BR RB RB RB RB RR KR KR NG DR BRS BRM HR WC RD \times \times Glucoraphanin X X X X X X X X X X X \times Glucobrassicin X X X X X X X X X X X X X X VC \times \times Gluconasturtiin X X X X X X X X X X X X X $\widetilde{\mathbf{f}}$ \times \times \times Sinigrin X X X X X X X X X X **BRM** \times \times \times Glucoerucin X X X X X X X X X Glucoiberin X X X X X X X X X X \times X \times X \times X \times X \times \times \times 4-Hydroxyglucobrassicin X X X X X X X \times X \times X \times X \times X \times X \times X \times **BRS** \times \times \times \times \times \times \times \times \times \times \times X \times Gluconapin X X X X X X X X X X Progoitrin X X X X X X X X X X X \times X X X X X X X X X X X X X X ರ \times \times \times \times \times \times \times ΟÓ \times \times \times \times \times KR \times \times \times \times \times \times \times \leq $\times\times\times\times$ \times \times \times \times \times Glucoberteroin X X Glucobrassicanapin X X X X X X X
Glucocochlearin X \times X \times X \times X \times The left contains a set of \times GSB \times \times \times \times \times \times \times \times \times \times RB \times \times \times \times \times $\times\times$ \times \times \times \times TABLE 9 Glucosinolates identified and quantified in 16 different foods¹ **TABLE9** Glucosinolates identified and quantified in 16 different foods¹ KL \times \times \times \times \times \times $\times\times\times\times$ \times ෪ \times \times \times \times \times \times \times $\times\times\times$ $\times \times \times \times$ \times BS \times \times \times \times \times \times \times \times $\times\times\times$ $\times\times\times\times$ පී \times \times $\times\times\times\times$ \times \times \times \times $\times\times\times\times$ \times \times 4-Hydroxybutyl GSL X Glucoerysolin X Glucotropaeolin X BR \times \times \times $\times\times\times$ $\times \times \times \times$ \times \times \times \times \times 4-Methoxyglucobrassicin 4-Hydroxyglucobrassicin Glucoraphasativusain Glucoraphasativusain Glucobrassicanapin (2R)-Glucobarbarin 4-Hydroxybutyl GSL (2S)-Glucobarbarin (2R)-Glucobarbarin (2S)-Glucobarbarin Neoglucobrassicin Glucoraphasatin Glucotropaeolin Glucocochlearin Glucokohlrabiin Glucoraphasatin Gluconasturtiin Glucoberteroin Glucoiberverin Glucokohlrabiin Glucoraphenin Glucoraphanin Glucobrassicin Glucoraphenin Glucoerysolin Epiprogoitrin Glucoalyssin Glucosinolate Aliphatic GSLs Glucoerucin Gluconapin Napoleiferin Aromatic GSLs Glucoiberin Aromatic GSLs Aliphatic GSLs Sinigrin
Indole GSLs Progoitrin Indole GSLs

broccoli; GSL, glucosinolate; HR, horse radish; KL, kale; KR, kohlrabi; MG, mustard greens; RB, rutabaga; RD, radish; TN, turnip; WC, winter cress.

broccoli; GSL, glucosinolate; HR, horse radish; KL, kale; KR, kohlrabi; MG, mustard greens; RB, rutabaga; RD, radish; TN, turnip; WC, winter cress.

provide more relevant information [\(71\)](#page-20-34). Researchers should be encouraged to include alternative measurements if possible; or use them to verify the data calculated from a dietary composition database/dataset, to avoid bias. Finally, even though the discussion in this study was made specifically for the dietary GSLs, the similar challenges and concerns might also be related to other dietary bioactive compounds regarding the development and applications of the composition databases.

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