NUTRITION REVIEWS

Lead Article

Sulforaphane: translational research from laboratory bench to clinic

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Cruciferous vegetables are widely acknowledged to provide chemopreventive benefits in humans, but they are not generally consumed at levels that effect significant change in biomarkers of health. Because consumers have embraced the notion that dietary supplements may prevent disease, this review considers whether an appropriately validated sulforaphane-yielding broccoli sprout supplement may deliver clinical benefit. The crucifer-derived bioactive phytochemical sulforaphane is a significant inducer of nuclear factor erythroid 2-related factor 2 (Nrf2), the transcription factor that activates the cell's endogenous defenses via a battery of cytoprotective genes. For a broccoli sprout supplement to demonstrate bioactivity in vivo, it must retain both the sulforaphane-yielding precursor compound, glucoraphanin, and the activity of glucoraphanin's intrinsic myrosinase enzyme. Many broccoli sprout supplements are myrosinase inactive, but current labeling does not reflect this. For the benefit of clinicians and consumers, this review summarizes the findings of in vitro studies and clinical trials, interpreting them in the context of clinical relevance. Standardization of sulforaphane nomenclature and assay protocols will be necessary to remove inconsistency and ambiguity in the labeling of currently available broccoli sprout products.

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INTRODUCTION

The cruciferous vegetable family includes broccoli, cauliflower, cabbage, kale, and others, the consumption of which is associated with reduced cancer risk.[1,2] As foods, crucifers are a significant source of micronutrients, including folate and vitamin C, but perhaps more significant is their nutrigenomic potential.[3] This potential is generated by a family of bioactive compounds, known as isothiocyanates (ITCs). Broccoli-derived ITCs, for example, are not present in the plant cell but are produced when the cell wall is ruptured, resulting in an enzymatic reaction between the myrosinase enzyme and an inert precursor, glucoraphanin. The most well-researched broccoli-derived ITC is sulforaphane (SFN), the compound that appears to be responsible for the larger part of the plant's health-promoting and cancer-preventive properties beyond its nutrient content.[4–6] Whereas many plant-derived nutraceutical supplements contain extracts of the major identified bioactive compound(s), the intrinsic instability[7,8] of the naturally occurring SFN molecule presents a still-unresolved challenge for manufacturing; SFN per se is simply not sufficiently stable to enable production as a supplement. Alternative means of addressing this challenge are discussed herein.

Given the prevalence of diet-related chronic disease and the evidence that many consumers have accepted a role for complementary medicines in their personal health management, there may be a place for a validated, bioactive, plant-derived, SFN-yielding supplement

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capable of modifying biochemical and physiological risk factors for disease. Against this background, the present review investigates the possibility of developing such supplements with significant clinical potential.

EMERGENCE OF NUTRIGENOMICS

Described in 2004[9] as the "next frontier in the post-genomic era," nutritional genomics (more commonly, "nutrigenomics") harnesses multiple disciplines and rests at the interface between the nutritional environment and cellular/genetic processes.[10] Understanding the relationships between phytochemicals and their ability to affect an individual's gene expression underpins the principle of an evolving model of "personalized nutrition."[11] Whereas pharmacogenomics[12] describes the individual effects of pharmaceutical agents on a patient's genome, nutrigenomics describes similar responses to bioactive molecules in foods.[13] Plant biomolecules as components of the human diet take on a new significance as the relevance of their roles in intracellular signaling and nutrigenomic modulation of gene expression continues to unfold.[14]

As a weak pro-oxidant, SFN is a phytochemical with demonstrable nutrigenomic potential that can activate the expression of a battery of cytoprotective genes via the transcription factor nuclear factor erythroid 2-related factor 2 (Nfr2).[15] Nrf2 coordinates the regulation of over 200 genes in humans and animals,[16–18] largely related to mechanisms of endogenous cellular defense and survival.[19] Nrf2 has been variously described as "the master redox switch,"[20] an "activator of cellular defense mechanisms,"[21] and "a guardian of health span and gatekeeper of species longevity."[22] As a mediator for amplification of the mammalian defense system against various stressors, Nrf2 sits at the interface between prior understanding of oxidative stress and the endogenous mechanisms used by cells to respond to oxidative stress. The knowledge that a weak prooxidant, such as SFN, can initiate a sequence of events culminating in increased expression of endogenous antioxidant molecules, such as glutathione, thioredoxin, and hemoxygenase, suggests the value of reexamining the premise that dietary antioxidant supplements are generally beneficial to human health.[23,24]

NUTRIGENETICS

Whereas nutrigenomics investigates the effects of food nutrients and bioactive food-derived compounds on gene expression, nutrigenetics identifies and characterizes gene variants associated with differential responses to nutrients, relating this variation to disease states; both aim to unravel diet-genome relationships.[25] Nutritional epidemiology investigates the association between patterns of food consumption and disease risk, with nutrigenetics asserting that the effects of particular foods or food components may vary with genetic differences that affect the metabolic makeup of the individual.[26] Furthermore, nutrigenetics helps to explain why the findings of epidemiological trials are often inconsistent, since, to express their bioactivity, phytochemicals may need to be metabolized by enzymes with known single-nucleotide polymorphism variants.[27,28]

DIFFERENTIAL RESPONSES DUE TO SINGLE NUCLEOTIDE POLYMORPHISMS IN NRF2-TARGET GENES

Glutathione S-transferases (GSTs) constitute a large multigene family of Phase 2 enzymes involved in detoxification of potentially genotoxic chemicals.[27] The bioactive ITCs of cruciferous vegetables are metabolized via the mercapturic acid pathway to form a glutathione conjugate, which is catalyzed by GST enzymes.[28] Polymorphisms in these enzymes have a significant nutrigenetic impact on overall ITC metabolism, thereby affecting SFN bioavailability, so that cruciferous vegetable intake has sometimes been found to confer greater chemoprevention in those with certain GST polymorphisms; in other studies, the opposite is observed.[29–31] The differing effects of phenotypic expression of *GSTM1* on SFN metabolism are such that *GSTM1*-null subjects excreted almost 100% of ingested SFN.[32] In a later study, Gasper et al.[33] showed that there were apparent thresholds of intracellular SFN concentrations below which gene expression did not occur.[33] This could have implications for dietary recommendations to consume more cruciferous vegetables, since it has been shown that some fresh broccoli purchased from retail stores yielded very low SFN levels, even when consumed raw and ingested frequently.[34] The same study in humans who consumed 300 mL of liquefied commercially available raw broccoli florets found that peak plasma levels of SFN reached just 0.07 μM. A later study that compared the peripheral SFN exposure in volunteers consuming a single serving of lightly cooked fresh broccoli versus a single serving of lightly cooked frozen broccoli showed an approximately 10-fold difference in the plasma concentration, approximately 0.2 μM for the fresh broccoli versus approximately 0.02 μM for the frozen broccoli.[35] Moreover, the lower physiological concentrations observed after ingestion of the frozen vegetable were described as being below the threshold required to cause a range of responses in cellular models.

Wide variations exist in glucosinolate content between varieties of the *Brassica oleracea* seeds[36] from which broccoli sprouts are grown. This would suggest the likelihood of marked variation in the quantities of ITCs

derived from hydrolysis of their glucosinolates. A 27-fold difference between the highest and the lowest levels of glucoraphanin has been observed.[37] As a consequence, for a nutraceutical supplement to demonstrate consistent responses, it will be essential for a myrosinase-active plant powder to contain a standardized content of glucoraphanin, with selection of an appropriate seed cultivar being an important consideration.

GST polymorphisms can be prevalent in the population, with up to 50% of people exhibiting the null genotype for the *GSTM1* isoenzyme.[31] The *GSTM1*-null genotype may confer an advantage because the reduced GST activity could result in slower elimination and longer exposure to ITCs after cruciferous vegetable consumption.[38] The GSTM1-null phenotypes have been associated with reduced risk of breast, prostate, and colon cancers, adding support to SFN's potential as a chemopreventive and cytoprotective agent,[39–42] but not all studies confirm this finding.[43] Human hepatocytes treated with the hepatocarcinogen aflatoxin B_1 after pre-exposure to SFN at doses of 10 μM and 50 μM were investigated for their ability to resist DNA adduct formation; reduction in DNA adduct formation occurred at both doses, with greater reduction observed at the higher concentration.[44] Analyses of gene expression in the SFN-treated hepatocytes demonstrated that SFN greatly decreased cytochrome P450 (CYP3A4) mRNA but did not induce the expression of *GSTM1*, suggesting that induction of GST activity is but one means by which SFN exerts chemopreventive action. The effects of SFN on CYP3A4 are discussed in more depth in a later section.

DIVERSE MODES OF CHEMOPROTECTION OF SULFORAPHANE

A number of cell defects have been observed in cancer cells.[45] Among such defects, mutation of the p53 suppressor gene occurs in about 50% of all cancers.[46] The intrinsic cellular cytoprotective mechanisms are gradually weakened by these accumulated and varied defects.

The most common molecular genetic change in prostate cancer involves the silencing of the expression of the gene that codes for *GSTP1*, a critical enzyme of carcinogen defense.[47] Found in virtually all cases of intraepithelial neoplasia, this change occurs early in prostate carcinogenesis and is a near-universal finding in clinical prostate cancers, regardless of grade or stage.[47] Normal GST function is required to detoxify potential carcinogens; without it, prostate cells are exposed to the damaging effects of endogenous and exogenous toxins.

Fimognari et al.[48] showed that SFN is capable of offsetting the effects of a mutated p53 gene by virtue of its various modes of action, providing evidence that SFN confers chemoprotection even when genetic defects are already apparent, a finding of considerable clinical significance. These findings reaffirm the value of cruciferous vegetables in human diets.

Whether it is possible to develop a clinically efficacious supplement based on the nutrigenomic potential and the pharmacokinetic properties of phytochemicals like SFN is a key question posed by this review.[49,50] A close investigation of numerous phytochemical-derived supplements popular with consumers reveals that, unlike SFN, many exhibit poor bioavailability.[51–53] As a result, it is doubtful that some of the bioactive compounds that demonstrate impressive effects in highly publicized in vitro studies are present in sufficient systemic concentrations to exhibit a clinical effect.[54]

DEVELOPING AN EVIDENCE-BASED SULFORAPHANE SUPPLEMENT

The finding that SFN is both a potent[55] and a bioavailable[53] inducer of Nrf2 places it in a category distinct from most other supplemental phytochemicals, especially the polyphenols.[56] Cell culture studies are a valuable starting point in dose determination, and various human cell types have been investigated as substrates for SFN intervention studies.[57] Of particular value are the studies that reveal the biochemical effect of a specific micromolar concentration of SFN.[57,58] These data can then be reviewed in relation to the SFN yield from a specified oral dose.

Whereas the primary bioactive component of many phytochemicals is extracted and used as the standardized extract in the manufacture of a nutraceutical supplement, the intrinsic instability of the SFN molecule prevents this method of delivery.[8] As such, broccoli-derived foods or supplements require the hydrolysis of the glucoraphanin precursor in the presence of the myrosinase enzyme; typically, this occurs when the fresh plant cell is ruptured or when a dry whole powder becomes moist. In short, SFN must be formed close to the time of ingestion. The challenge for the manufacturer of an SFN-yielding supplement is to retain both the glucoraphanin and the myrosinase enzyme during the processing from fresh whole plant to powder; this aspect is explored in more detail later.

SULFORAPHANE YIELD VERSUS SULFORAPHANE CONTENT

Broccoli sprouts yield more SFN than any other known plant and contain 10–100 times the concentration of glucoraphanin (the major glucosinolate in broccoli) of the mature broccoli vegetable.[55,59] The broccoli seed therefore contains more glucoraphanin per gram than does the sprout or the vegetable of the same seed. The

higher level in broccoli seeds than in young sprouts is apparently attributable to the lack of significant net synthesis of the glucosinolates over the few days following germination.[59,60] As the maturing plant becomes established and is exposed to environmental factors, including the influence of herbivorous predators, additional glucosinolates can be synthesized as part of the plant's defense against predators.[61] Nevertheless, the very young broccoli sprout is typically a far more significant source of SFN on a weight basis compared with the vegetable, and this is why the sprout is chosen as a source of SFN-yielding precursors in many clinical trials.

In addition, different cultivars of *Brassica oleracea* contain varying levels of glucoraphanin, further establishing the wide variation in glucosinolate content between broccoli sprouts and the broccoli vegetable.[36] Importantly, however, the plant does not contain any SFN; instead, it produces it during the hydrolysis of glucoraphanin in the presence of myrosinase.[62] As a result, broccoli sprouts are correctly described as releasing, generating, or yielding but not containing SFN. The ideal broccoli sprout ingredient contains separately compartmentalized glucoraphanin and myrosinase that react at – or close to – the time of ingestion.[36] The absence of myrosinase in a broccoli sprout food powder or supplement renders the product unable to produce SFN on ingestion.[50]

ROLE OF EPITHIOSPECIFIER PROTEIN

The presence of a pH-sensitive modifier protein, epithiospecifier protein (ESP), affects the amount of SFN produced on hydrolysis.[63] In broccoli, the primary glucosinolate, glucoraphanin, can be converted to two principal products, SFN and SFN nitrile, the latter being several orders of magnitude lower in Nrf2-activating potency than SFN.[64] The importance of this finding is that, whereas SFN has been shown to have anticarcinogenic properties, SFN nitrile has not.[65] Because ESP is temperature sensitive, heating broccoli sprouts to 60°C decreases the formation of SFN nitrile.[66] A challenge for manufacturers of an SFN-yielding supplement lies in deactivating the ESP without simultaneously deactivating the myrosinase enzyme or degrading the glucoraphanin.

PRESENCE OF ERUCIC ACID IN LIPID FRACTIONS OF BROCCOLI SEEDS

Erucic acid is a lipid found in broccoli seeds as well as in other members of the family *Brassicaceae*. Its toxic effects in animals consuming rapeseed (another member of the family *Brassicaceae*) are well known and include myocardial lipidosis, myocardial necrosis, and impaired oxidative phosphorylation.[67] Concerns regarding its possible effects in humans are sufficiently important that erucic acid in rapeseed is regulated in the United States.[68] Broccoli seeds contain about 28% lipid, but broccoli sprouts contain only about 1%. West et al.[67] noted that 1 g of broccoli seeds may contain approximately 120 mg of erucic acid, but 28 g of broccoli sprouts contains 90 mg of erucic acid[67]; this would indicate that the seed contains around 37-fold more erucic acid than the sprout. They calculated that 35 g of broccoli seeds per week could equal the estimated maximum allowable amount of erucic acid, extrapolating from US Food and Drug Administration (FDA) requirements to limit the content of erucic acid in canola oil to a level not exceeding 2% of the component fatty acids. Although it is unlikely that an individual would consume 35 or more grams of broccoli seeds per week, it is not impossible; consumption of supplements derived from broccoli seeds is therefore not recommended.[68]

The production of an aqueous broccoli seed extract provides a way of removing the erucic acid; however, this process also activates the enzymatic conversion of glucoraphanin to SFN, which exhibits relatively high reactivity,[36] making SFN unsuitable as a compound for direct fortification of foods or supplements. A number of myrosinase-inactive broccoli seed extracts are commercially available; these products are a source of glucoraphanin but are devoid of the myrosinase necessary to convert it to SFN on ingestion.[49]

EFFECTS OF SULFORAPHANE ON TARGET GENES

The more than 200 Nrf2 target genes identified can be classified[18] broadly as those that code for a range of cytoprotective proteins, including antioxidants (enzyme and nonenzyme), drug-metabolizing enzymes, drug-efflux pumps, heat-shock proteins, NADPH regenerative enzymes, growth factors, growth factor receptors, heavy-metal-binding proteins, and various transcription factors that include peroxisome proliferator-activator receptor-gamma (PPAR-γ), in addition to genes that code for Nrf2 itself.[69]

An illustrative study investigated the effect of a single oral dose of 200 µmol/L pure SFN in women immediately prior to reduction mammoplasty.[58] This dose can be calculated as equivalent to 35 mg of SFN, given that 5.7 µmol SFN is equal to 1 mg of SFN (molecular weight, 177.29). Two important biomarkers in this study were hemoxygenase-1 (HO-1), a cytoprotective enzyme expressed following Nrf2 induction of the target gene,[70] and NAD(P)H:quinone oxidoreductase (NQO1), an Nrf2-inducible Phase 2 detoxification enzyme[64] that exhibits a range of other cytoprotective properties. The

dose selected was based on an earlier study,[71] which showed that SFN dithiocarbamate metabolites were detected at 15 min in the plasma and peaked at 2.0 ± 0.3 μM 1 h after ingestion, indicating rapid uptake.

A maximal 12-fold induction of NQO1 transcripts was observed in the mammary gland 12 h after dosing, with significant induction detected as early as 2 h. A biphasic pattern of HO-1 transcript induction was observed, with an initial peak at 2 h followed by a subsequent peak at 12 h, indicative of the delayed effect of SFN conversion to dithiocarbamates. The minimal time to statistically significant HO-1 induction was 1 h.

The study of Cornblatt et al.[58] is significant in providing a guide to the expected micromolar concentration for a given quantity of ingested SFN. A dose of 35 mg of SFN (200 μmol) appears to be achievable from a myrosinase-active broccoli sprout powder, provided such a powder is able to yield 1% SFN per gram of powder. As such, a supplement weight of 3.5 g, ingested either as a powder or as several capsules, is within a practical dose range in humans.

RELEVANCE OF IN VITRO STUDIES

Much of the preclinical research examines the effect of SFN in various cell types and at varying micromolar concentrations. It soon becomes clear that different tissues respond differently to a specified dose. Even different cell types in the same organ can respond differently.[72]

The expression of Nrf2 target genes such as NQO1 occurs at lower SFN concentrations, whereas other effects appear to require much higher intracellular concentrations.[73] SFN exhibits effects beyond its ability to activate Nrf2; these effects include HDAC (histone deacetylase) inhibition,[74] apoptosis,[75] antiangiogenesis,[76] and antimetastasis[77] in cancer cells. Induction of NQO1 in the rat colon is threefold higher than it is in the liver for the same dose.[78] Clearly, an effect detected in one tissue cannot be extrapolated to others. This may also suggest that the beneficial effects from both dietary crucifers and supplements may be greater in organs such as the digestive tract and bladder than in organs reached only systemically. A recent study by Veeranki et al.[79] showed that SFN levels measured in the stomach and bladder are higher than those in other organs. It is possible that tissue uptake of SFN is highest in the stomach due to direct exposure, but the levels decline rapidly in the descending gastrointestinal tract, possibly as a result of metabolic conversion.[79,80] Paradoxically, induction of GST and NQO1 are relatively modest in the stomach; the authors state that the reason for high gastric uptake of SFN but low induction of GST and NQO1 remains unexplained. An earlier study by the same researchers showed that the tissue uptake of SFN in the bladder was second only to that in the stomach. There appears to be remarkably high exposure of the bladder epithelium to dietary SFN, with a peak SFN level occurring 6 h after dosing and levels reaching 64–5,509 times higher than those in the plasma.[79] Unlike the relatively low levels of enzyme induction observed in the stomach, significant induction of GST and NQO1 occurred in bladder cells.[79] This may have clinical implications for patients with bladder cancer.[81]

It would seem too that the doses required for disease prevention are far lower than those required to effect change in established disease. A study[82] on men at risk for prostate cancer showed that increasing the intake of cruciferous vegetables from one serving per week to more than three servings per week (presumably cooked) was enough to provide a 41% decreased apparent risk. Data shown in Table 1 review a number of in vitro studies[17,47,72,83–90] that evaluated the effect of SFN in different types of tissues and cells. Notably, but not surprisingly, the micromolar concentrations to effect change are highly variable across cell types, and it has also been shown that the time during which the cells were exposed to SFN markedly altered the response. In cardiomyocytes exposed to hydrogen peroxide (H_2O_2), exposure to a 5 μM SFN dose for 6 h maintained 45% cell viability, but exposure for 24 h and 48 h increased cell viability to 82% and 92%, respectively.[85] How the findings of such cell culture studies translate to the human in vivo situation is not yet known.

The examples shown in Table 1 are a cross-section of the many published in vitro SFN trials of this type. The data show that, in some cell types, the required biochemical changes are readily achievable by supplementation with a myrosinase-active standardized broccoli sprout supplement. It is equally likely that, in some cells and under certain conditions, such supplementation may not provide the required micromolar concentration. For example, inhibition of the transcription factor NF-κB and induction of transcription factor Nrf2 appear to be achievable at micromolar concentrations much lower than those required for the induction of HDAC inhibition, cell cycle arrest, or antiangiogenesis in cancer cell lines.[91,92] As an example, where low SFN doses between 0.1 μM and 3 μM activate Nrf2-related cellular defenses in prostate cells,[47] higher doses of 10 μM for DU145 cells and 30 μM for LNCaP cells are necessary to induce cell cycle arrest and apoptosis.[84]

DATA FROM CLINICAL STUDIES

A summary of 22 published clinical studies using a variety of intervention foods or supplements is shown in Table 2.[32–34,49–51,53,58,74,93–105] These substances include mature broccoli vegetable (raw and cooked), fresh broccoli sprouts, myrosinase-inactive broccoli extract beverage, myrosinase-inactive powdered broccoli sprout

Table 1 **Studies showing the diversity of effects of sulforaphane in different cell types at varying concentrations and incubation times.**

Reference[a]	Type of tissue	SFN concentration (μM)	Cell type and effect evaluated	Findings
Brooks et al. (2001)[47]	Prostate	0.1–15 μM	Six prostate cancer cell lines + normal cells. Effect of SFN, including effect on *GSTP1* silencing	Normal cells: SFN after 48 h→1.35-fold NQO1 induction at 0.1 μM and 2.46-fold NQO1 induction at 1–3 μM LNCaP cells: SFN→4.6-fold induction at 10 μM Modest induction of GST isoforms to 1.7-fold in all cell lines All effects of SFN completely abrogated by 10 μM NAC
Gao & Talalay (2004)[83]	Retinal epithelium	1.25–5 μM	Human adult retinal pigment epithelial cells (ARPE-19) challenged with UV light + all-*trans*-retinaldehyde. Effect on photooxidative damage	Increased cell survival with increasing SFN doses; linear cytoprotective effect with dose Where only 9.4% of cells survived, preincubation with SFN raised survival 3-fold to 27.4% at SFN = 5 μM
Cho et al. (2005)[84]	Prostate cancer	10 μM	Effect of SFN on prostate cancer cell line DU145	Strong cell cycle arrest (G2/M phase) and apoptosis at SFN = 10 μM. No effect at <3 μM LNCaP cells required SFN > 30 μM for arrest Effects of SFN completely abrogated by NAC
Ritz et al. (2007)[72]	Bronchial epithelium	0.3–10 μM	Effect of SFN on 2 types of bronchial cells (cell lines BEAS-2B and NHBEC) exposed to diesel extract	All doses induced GST and NQO1, but to different degree in each cell line SFN at 5 μM→15-fold increase in NQO1 in BEAS-2B cells GST increased 1.9-fold in NHBEC at 1.25 μM SFN→1.43-fold increase in NQO1 in BEAS-2B cells Expression of inflammatory cytokines, IL-8, IL-1β, and GM-CSF were all significantly reduced at SFN = 0.625 μM
Zhu et al. (2008)[17]	Aorta	0.25–5 μM	Cytoprotective effect of SFN in rat aortic smooth muscle cells (A10 cell line)	Significant dose-dependent induction of cytoprotective Nrf2 target genes at SFN as low as 0.25 μM SFN at 0.25 μM→25% increase in GST and 66% increase in NQO1 SFN at 0.5 μM→33% increase in GSH levels SFN protected against toxicity of superoxide, H_2O_2, acrolein, and peroxynitrite
Angeloni et al. (2009)[85]	Cardiac tissue	5 μM	Effect of SFN on cardiomyocyte cell viability in presence of ROS over 6–48 h	Greater effects observed when predosing for 48 h H_2O_2 reduced cell viability to 40% SFN at 5 μM for 6 h→45% viability SFN at 5 μM for 12 h→75% viability SFN at 5 μM for 24 h→82% viability SFN at 5 μM for 48 h→92% viability
Song et al. (2009)[86]	Pancreatic β-cells	0.1–10 μM	Effect of SFN in protecting β-cells (RIN cell line) against oxidative damage, restoring insulin secretion, and preventing type 1 diabetes in STZ-treated mice	~Linear response in Nrf2 target genes to induction by 2.5, 5.0, and 10.0 μM SFN Protection against cytokine-induced β-cell death by SFN Cytokines reduced cell viability to ~54% SFN at 10 μM increased viability to ~96% SFN at 5 μM increased viability to ~92% SFN at 2.5 μM increased viability to ~80%
Kivelä et al. (2010)[87]	Endothelial cells or arteries and veins	HUVEC 0–10 μM. HAEC 0–10 μM	Effect of SFN on ELs and TNF-α expression in HUVEC and HAEC cells	SFN at 10 μM inhibits EL in HUVEC SFN at 2.5 μM inhibits EL in HAEC EL activation occurs through classical (canonical) NF-κB pathway
Mas et al. (2012)[88]	Brain: substantia nigra	0.5–5 μM	Dopaminergic human neuroblastoma cells (SK-N-SH cells). Potential for SFN to protect against oxidative stress induced by antipsychotic drugs	SFN at 2.5 μM and 5 μM→60% and 140% increase in GSH SFN at 2.5 μM and 5 μM→175% and 250% increase in NQO1 activity SFN doses >5 μM reduced cell viability SFN at 2.5 μM and 5 μM maintained normal lipid peroxidation in the presence of haloperidol SFN at these doses protects dopaminergic cells from the oxidative stress induced by antipsychotic drugs, including haloperidol at a dose to reduce cell viability by 80%
Tan et al. (2010)[89]	Bronchial epithelium: normal and cancerous	0.5–2 μM	NHBEC, HBEC and malignant lung adenocarcinoma cells (A549 cells)	NHBE cells: SFN→~4-fold increase in NQO1 at 0.5 μM and 1.0 μM at 24 h and 11.8-fold increase in NQO1 at 6 days HBEC cells: SFN→7.5-fold increase in NQO1 at 2 μM, but only at 48 h A549 cells: SFN→no change at the doses tested No toxicity at 10 μM SFN
Vauzour et al. (2010)[90]	Neural cortex	0.1–1.0 μM	Cortical neurones. Protection against dopamine toxicity	SFN→maximum protection (~30%) at 0.1 μM and ~24% protection at 1.0 μM No toxicity at 10 μM SFN

[a] This list is by no means exhaustive; studies are listed chronologically.

Abbreviations: EL, endothelial lipase; GM-CSF, granulocyte-macrophage colony-stimulating factor; GSH, glutathione; GST, glutathione S-transferase; HAEC, human arterial endothelial cells; HUVEC, human umbilical vein endothelial cells; IL, interleukin; NAC, *N*-acetyl cysteine; NHBEC, primary normal human bronchial epithelial; HBEC, immortalized human bronchial epithelial cells; NQO1, NAD(P)H (quinone) oxidoreductase; ROS, reactive oxygen species; SFN, sulforaphane; STZ, streptozocin; TNF-α, tumor necrosis factor-alpha; UV, ultraviolet.

Table 2 **Summary of clinical studies investigating sulforaphane.**

Reference	No. of subjects	Intervention	Endpoint	Finding	Relevance of dosage
Conaway et al. (2000)[93]	12	200 g of fresh or steamed broccoli as a single dose	Compare metabolic fate of steamed vs. fresh broccoli	Bioavailability of ITCs from fresh broccoli is approximately 3 times greater than that of ITCs from cooked broccoli	Total ITC plasma metabolites peaked between 1 h and 8 h Total urinary excretion of total ITC equivalents (especially SFN-NAC) occurred at 2–12 h
Galan et al. (2004)[94]	9	14 g, 28 g, or 56 g of fresh broccoli sprouts twice daily for 7 days	Determine whether orally consumed broccoli sprouts can eradicate *Helicobacter pylori* infection	Consumption of oral broccoli sprouts was temporally associated with eradication of *Helicobacter pylori* infection in 3 subjects	No data provided on sprout composition or SFN yield
Murashima et al. (2004)[95]	12	100 g of fresh broccoli sprouts daily for 7 days	Assess markers of oxidative stress and lipid metabolism in healthy males and females	Consumption of broccoli sprouts improved lipid parameters and reduced oxidative stress markers. HDL increased only in females	No data provided on sprout composition or SFN yield
Kensler et al. (2005)[96]	200	Hot water infusions of 3-day-old broccoli sprouts nightly for 2 weeks	Determine whether myrosinase-inactive infusion can alter the disposition of aflatoxin and phenanthrene, predisposing individuals to hepatic carcinoma	Significant interindividual differences in bioavailability – but no overall difference between intervention arms – was observed	Each dose contained 400 μmol SFN Interindividual variability was 1–45% of dose DTC excretion = 49 μmol/12 h
Gasper et al. (2005)[32]	16	Three-phase crossover dietary trial of standard broccoli, super broccoli, and water	Compare SFN metabolism in *GSTM1*-null and *GSTM1*-positive subjects who consumed different types of broccoli	*GSTM1* genotypes have a significant effect on the metabolism of SFN derived from standard or high-glucosinolate broccoli; *GSTM1*-positive persons have greater protection	*GSTM1*-null subjects excreted ~100% of urinary metabolites *GSTM*- positive subjects excreted ~70% SFN
Shapiro et al. (2006)[97]	12 as 3 groups of 4; 3:1 active to placebo	21 doses of glucoraphanin or SFN over 7 days	Evaluate safety, tolerance, and metabolism	No significant or consistent subjective or objective abnormal events (toxicities) associated with any of the sprout extract ingestions were observed	Nontoxic at doses well in excess of any practical dose
Cornblatt et al. (2007)[58]	8	Single dose of oral broccoli sprouts delivering 200 μmol SFN ~1 h presurgery	Evaluate plasma DTC concentration at ~100 min after ingestion	Approx 40-fold increase in urine SFN metabolites and a 90-fold increase in plasma SFN metabolites	200 μmol oral SFN → 2 μM in breast tissue
Rungapamestry et al. (2007)[98]	12	Single meal of broccoli	Determine effect of meal composition (beef or no beef, plus 150 g of lightly cooked or fully cooked broccoli or a broccoli seed extract, with 3 g of mustard) on SFN uptake	SFN yield was 3-fold higher after consumption of lightly cooked than after fully cooked broccoli. No effect of meal matrix, only the manner of cooking	No effect of meal matrix on SFN bioavailability. Complete cooking more destructive than light cooking, which is more destructive than no cooking (raw)
Gasper et al. (2007)[33]	16	Three-phase crossover dietary trial of standard broccoli, 3-fold higher glucosinolate broccoli, and water	Compare gene expression in gastric mucosa 6 h after consuming broccoli of varying glucosinolate content	Only one gene upregulated by more than 1.5-fold in standard broccoli. Several genes upregulated in the high-glucosinolate broccoli	Apparent threshold for doses of SFN influencing gene expression
Traka et al. (2008)[99]	22	Broccoli-rich diet over 6 months (400 g of broccoli per week)	Determine whether broccoli alters expression of *GSTM1* to perturb oncogenic signaling in prostate	Broccoli interacts with *GSTM1* genotype to result in complex changes to signaling pathways associated with inflammation and carcinogenesis in the prostate	Only the broccoli-rich diet influenced expression of genes in the androgen receptor pathway as well as in the insulin signaling, TGF-β, and EGF receptors
Vermeulen et al. (2008)[51]	8	200 g of broccoli, raw and cooked	Determine the bioavailability and kinetics of SFN	Bioavailability is higher with raw broccoli (37%) than with cooked broccoli (3.4%). SFN absorption delayed after consumption of cooked broccoli vs. raw broccoli (peak plasma time = 6 h and 1.6 h, respectively)	10% greater bioavailability from raw vs. cooked broccoli
Myzak et al. (2007)[74]	3	68 g of broccoli sprouts containing approx. 105 mg of SFM	Determine effect of SFN on HDAC activity in peripheral blood mononuclear cells	Strong inhibition of HDAC activity 3–6 h after ingestion. Trend towards increased histone acetylation	68 g of fresh sprouts → 105 mg of SFN Human HDAC activity reduced to ~65% after 3 h – lasted 24–48 h
Riedl et al. (2009)[53]	65	Dose-escalation from 25 g to 200 g of fresh broccoli sprouts	Determine effect of SFN on the expression of *GSTM1*, *GSTP1*, NQO1, and HO-1 in the upper airway of human subjects	Safe and effective induction of mucosal Phase 2 enzyme expression in the upper airway of human subjects, in a dose-dependent manner	125-g dose (64 μmol SFN) → >50% in NQO1 activity 200-g dose → >200% NQO1 activity
Yanaka et al. (2009)[100]	48 *H. pylori*-infected patients	70 g of broccoli sprouts per day for 8 weeks (glucoraphanin 420 μmol/day) vs. alfalfa sprouts	Determine the effect of SFN on gastric *H. pylori* infection	Markers of *H. pylori* diminished in the broccoli sprout group only, remaining low for 8 weeks after cessation	HO-1 increased 2- to 3-fold 24 h after a single 50-g dose of sprouts Three markers of *H. pylori* infection decreased to below cutoff after 8 weeks Levels returned to baseline 8 weeks after end of study

Table 2 **Continued.**

Reference	No. of subjects	Intervention	Endpoint	Finding	Relevance of dosage
Hanlon et al. (2009)[34]	6	Single and repeated intake of raw broccoli (noted lower-than-expected SFN yield in the commercial broccoli)	Pharmacokinetic study of SFN	SFN was rapidly absorbed, with peak plasma levels reached within 1.5 h, characterized by a long terminal elimination phase. No impact of repeated intake or accumulation	Rapid decline in plasma levels to 50% after ~3 h, whether as single or repeated doses Fresh broccoli from retail sources generates very low SFN levels, even when consumed raw
Kumar et al. (2010)[101]	85	Three diet groups to include different dietary glucosinolates over 4 weeks	Develop a method to measure ITC albumin urinary adducts	Hb and albumin adducts are a useful marker of ITC intake	More stable adducts permit easier quantification of ITC
Egner et al. (2011)[102]	50	Two groups over 7 days. Two broccoli-sprout-derived beverages, one glucoraphanin rich (325 µmol glucosinolate/g powder) and the other SFN rich (202 µmol SFN/g powder)	Compare bioavailability and tolerability of SFN from beverages with and without myrosinase	Bioavailability of myrosinase-active SFN-rich beverage = 70% vs. 5% for glucoraphanin-rich myrosinase-inactive beverage	Inter-individual variability in excretion was considerably lower with SFR beverage than with GRR beverage Bioavailability of SFR beverage = 70% vs. 5% for GRR beverage Taste preference significant for GRR beverage 34% null for *GSTM1* genotype 51% null for GSTT1 SFR beverage had better bioavailability and more rapid uptake GRR beverage had more stable but unpredictable and overall poor bioavailability
Clarke et al. (2011)[49]	12	40 g of broccoli sprouts for 4 weeks vs. 6 broccoli sprout myrosinase-inactive pills (equivalent to 3 g of dried sprouts)	Compare glucosinolate metabolites SFN and ERN in myrosinase-active broccoli sprouts and myrosinase-inactive pills	Broccoli supplements devoid of myrosinase activity do not produce equivalent plasma concentrations of bioactive ITC metabolites produced by broccoli sprouts	Plasma level ~2.3 µM with sprouts 7-fold higher peak concentration with sprouts 74% recovery with fresh sprouts vs. 19% with myrosinase-inactive pills Significant interindividual variability SFN and another ITC, ERN, are inconvertible, but effect is variable between subjects
Cramer & Jeffery (2011)[50]	4	42 g of fresh broccoli sprouts (74 µmol SFN); 2 g of myrosinase-inactive broccoli sprout powder (120 µmol SFN)	Determine whether myrosinase-active broccoli sprouts enhance the SFN yield of a glucoraphanin-rich myrosinase-inactive broccoli sprout powder	The 24 h urinary SFN-N-acetyl-cysteine recovery was 65%, 60%, and 24% of the dose ingested from combination, broccoli sprout, and GRP meals, respectively	Enhanced conversion to SFN when myrosinase-inactive powder is combined with myrosinase-active whole sprout powder Synergistic effect of combination Fresh sprouts + myrosinase-inactive powder enabled earlier SFN conversion
Bahadoran et al. (2011)[103]	81 type 2 diabetic patients	10 g and 5 g daily of a myrosinase-active broccoli sprout powder	Determine whether a myrosinase-active broccoli sprout powder can reduce biomarkers of oxidative stress in type 2 diabetes	Significant decrease in MDA, oxidative LDL cholesterol, and oxidative stress index, and significant increase in TAC. No effects were found on total oxidant status. Greater effects found with higher dose	5-g dose → 112 µmol SFN 10-g dose → 225 µmol SFN Higher dose → 9% lower MDA, ~5% lower oxidative LDL, and ~16% higher TAC Lower dose → 4.6% lower MDA, no change in oxidative LDL, and 10.3% higher TAC
Clarke et al. (2011)[104]	24	68 g of broccoli sprouts for 4 weeks vs. 6 broccoli sprout myrosinase-inactive pills (equivalent to 3 g of sprout powder in glucoraphanin content: ~220 µmol)	Compare metabolite levels after consumption of fresh broccoli sprouts vs. myrosinase-inactive supplements. Comparison of HDAC effects	Broccoli sprout supplement resulted in significantly lower excreted ITC metabolites compared with fresh sprouts (5-fold lower excretion of SFN metabolites). Conversion of SFN metabolites to ERN metabolites. HDAC findings inconclusive	Fresh sprouts demonstrate HDAC activity Supplement did not show HDAC activity
Bahadoran et al. (2012)[105]	81 type 2 diabetic patients	5 g and 10 g of myrosinase-active broccoli sprout powder daily for 4 weeks	Assess parameters of cardiovascular risk	At the 10-g dose, significant decrease in serum triglycerides, oxidized LDL:LDL, and atherogenic index of plasma	Daily dose of 10 g required to achieve statistically significant change

Abbreviations: DTC, dithiocarbamate; EGF, epidermal growth factor; ERN, erucin; GRP, glucoraphanin-rich powder; GRR, glucoraphanin-rich; Hb, hemoglobin; bHHDAC, histone deacetylase; HDL, high-density lipoprotein; HO-1, hemoxygenase-1; ITC, isothiocyanate; LDL, low-density lipoprotein; MDA, malondialdehyde; NAC, N-acetyl-L-cysteine; SFN, sulforaphane; SFN-NAC, N-acetyl-L-cysteine conjugate of sulforaphane; SFR, sulforaphane-rich; TAC, total antioxidant capacity; TGF-β, transforming growth factor-beta.

extract, myrosinase-active broccoli sprout powder, and capsules made from myrosinase-inactive broccoli extract. Several of the studies[49,50,96,102] confirm the necessity for myrosinase in optimizing SFN yield, citing cooking and extraction as the primary processes leading to loss of activity.[51,93,98] The SFN bioavailability[51] from raw broccoli was shown to be 10-fold higher than that from cooked broccoli. Plasma SFN peaked at about 1.6 h after ingestion of raw broccoli, but peaked later, at around 6 h after ingestion of cooked broccoli. It was also apparent that interindividual variability in SFN concentration was considerably greater among subjects who ingested myrosinase-inactive substances.[102]

Other studies[94,100,106,107] demonstrate the bactericidal effect of SFN on *Helicobacter pylori*, associated with gastric reflux and cancer. Forty-eight *H. pylori*-infected subjects were given 70 g of fresh broccoli sprouts daily.[100] Three markers of *H. pylori* infection declined within 8 weeks to below the diagnostic cutoff point. Once the intervention had stopped, however, levels of *H. pylori* returned to baseline levels after 8 weeks.

In evaluating the effect of 25 g to 200 g of a 1:4 fresh broccoli sprout homogenate, a dose-escalation study[53] found a safe and effective induction of Phase 2 enzyme expression in the upper airways in a dose-dependent manner. At the highest intake, there was a 200% increase in NQO1 activity. This study is valuable because it demonstrates the dose required to double the activity of NQO1 in the tissues of the human airway; more importantly, it shows that such doses are achievable through diet.

HDAC inhibition is an epigenetic phenomenon associated with chemoprotection. SFN was shown to act as an HDAC inhibitor[74] in a mechanism unrelated to its activation of Nrf2. In this study, a single dose of 68 g of broccoli sprouts (claimed to yield 105 mg of SFN) "inhibited HDAC activity significantly in peripheral blood mononuclear cells 3 h and 6 h following consumption." The effect lasted 24–48 h.

Clinicians might ask whether the beneficial effects conferred to normal cells by SFN might also protect cancer cells. To ensure that SFN as a therapeutic intervention is both safe and clinically relevant, a recent cell culture study compared the effects of SFN on cancer cells and normal cells.[108] Using four cell types in a prostate model, normal (PrEC), cancer (LNCaP and PC3), and benign prostatic hypertrophic (BPH-1) cells were subjected to 15 μM SFN for varying time periods. The results showed that SFN selectively targets benign hyperplasia cells and cancerous prostate cells while leaving the normal prostate cells unaffected. These findings hold clinical significance, highlighting the potential of SFN as a nontoxic chemopreventive agent readily incorporated into the diet as an uncooked vegetable or taken as a myrosinase-active supplement.

CHARACTERIZATION OF INTERVENTION SUBSTANCES

The intervention substances used in some of the published SFN studies are not well characterized, making it difficult to attribute relevance in some cases. For a food to be considered within the realm of medicine as either a functional food or a nutraceutical, the properties and effects of its bioactive constituents on human cellular function must be well understood. As described earlier in relation to GST polymorphisms, such an understanding should allow for the variable effects in individuals in whom gene variants affecting the relevant pathways demonstrate a different response.[30,32] Development of a plant-derived medicine or supplement requires the knowledge that a certain concentration of the bioactive constituent will result in a measurable change at the cellular level. In the case of studies using broccoli sprouts as the intervention substance, it is essential that the factors responsible for generating bioactive SFN are assayed and quantified. Such characterization should include at least the amount of the precursor – glucoraphanin – present and the quantity of SFN released. Additional assays to quantify the myrosinase enzyme and ESP as a modifier of SFN release may also be performed but are not essential if the yield of SFN and, perhaps, the yield of the other bioactive ITCs in broccoli sprouts, erucin and iberin, are known.[109] In the presence of ESP, variable amounts of glucoraphanin are enzymatically converted to the relatively inactive SFN nitrile,[64] so removal of ESP is a desirable goal in optimizing the SFN yield from a broccoli sprout. If such an ingredient is to be used as a nutraceutical, it must be standardized to one or more of the bioactive constituents in order to provide within-tolerance clinical predictability. In a clinical trial, the outcome is less meaningful if the data do not exist to draw a relationship between the intervention substance and the observable effects.

In a 2004 study, Murashima et al.[95] used 100 g of fresh sprouts in 12 subjects over 7 days and reported positive outcomes in a number of parameters related to lipid metabolism. Sprouts vary widely in their composition[36,110] due to a range of factors, and since the sprouts in this study had not been characterized, the study is of limited value as a basis from which to extrapolate its findings to the development of an efficacious supplement. Subsequent studies using fresh sprouts have more fully characterized the intervention substances. A study that compared fresh sprouts with a myrosinase-inactive sprout powder in tablet form measured the plasma concentration of SFN after a dose of 40 g of fresh sprouts containing 150 μmol glucoraphanin; the SFN yield of the sprouts was not specified.[49] An earlier unrelated study using 68 g of fresh sprouts had shown that this dose released 105 mg of SFN, so that 40 g of fresh sprouts may proportionately yield around 62 mg of SFN.[74]

The effect of a myrosinase-active broccoli sprout powder was investigated in type 2 diabetics.[105] Eighty-one patients were randomized to receive placebo, 5 g, or 10 g of powder daily over 4 weeks. The manufacturer of the powder specified it as yielding 22.5 μg/g, but this was not confirmed by assay as part of the trial. Using these values, subjects received, respectively, 112 μmol and 224 μmol SFN daily. The 10-g dose resulted in a 9% reduction in markers of oxidative stress as measured by plasma levels of malondialdehyde and an approximately 16% increase in total antioxidant capacity. The lower dose resulted in 4.6% lower malondialdehyde values and 10.3% higher total antioxidant capacity. Effects on lipid peroxidation parameters were seen only with the higher dose. Fasting blood glucose dropped by 11.3% at the higher dose and by 4.9% at the lower dose.

In a dose-escalation study[53] investigating the effects of SFN in nasal cells in the upper airways, subjects were given 25–200 g of fresh sprout homogenate daily. Increased Phase 2 enzyme expression in nasal lavage cells occurred in a dose-dependent manner, with maximal enzyme induction observed at the highest dose. The glucosinolate content of the sprouts used in the study was claimed to be 6 μmol/g. The SFN levels of the 175 g and 200 g doses were assayed at 89 μmol and 102 μmol, respectively. At doses yielding >51 μmol SFN (by calculation), a significant dose response in Phase 2 enzyme expression was observed. At the highest dose (102 μmol SFN), there was a 200% increase in NQO1 activity, with a 50% increase observed at the dose that yielded 64 μmol SFN. These findings may imply a protective effect of SFN against diseases of the airways such as asthma and chronic obstructive pulmonary disease as well as against the effects of airborne pollutants.

WHAT MICROMOLAR CONCENTRATIONS ARE POSSIBLE?

The micromolar concentration that may be achievable using a myrosinase-active whole-broccoli-sprout supplement can be estimated on the basis of the available, albeit limited, clinical data. These data are based on the effects of single doses and thus do not adequately reflect the clinical situation or the significant effects of cellular accumulation of SFN and its metabolites.[111]

Ye et al.[71] and Cornblatt et al.[58] showed that 200 μmol SFN was capable of producing a 2 μM concentration in breast cells. A dose of 200 μmol corresponds to approximately 35 mg of SFN. Unpublished tapped density data on a whole sprout powder show that a size 00 capsule is capable of holding 700 mg of powder, with allowance for a small quantity of encapsulation processing aids.[112] Bahadoran et al.,[103] in their 2001 study in type 2 diabetics, showed that 5 g of their powder contained a dose of 112.5 μmol SFN, or 19.7 mg of SFN (3.94 mg per gram, or 0.4% SFN yield). Had they used capsules instead of powder in their study, each subject would have needed more than 12 700-mg capsules daily to supply 35 mg of SFN, a number that is not clinically practical. To provide 35 mg of SFN, 8.9 g of the grade of broccoli sprout powder used by Bahadoran et al.[103] would be required. Therefore, using the powder of Bahadoran et al.,[103] around 13 capsules per day would be required to achieve approximately 2 μM SFN in human breast cells, again impractical. It is likely that other grades of myrosinase-active broccoli sprout powder contain higher levels of glucoraphanin and yield more SFN; with such powders, fewer capsules would be required to achieve the same outcome. If a daily dose of four capsules is practical and a powder with a 1% SFN yield were available, an intracellular concentration of around 2 μM may be achieved.

Clarke et al.[49] used 68 g of fresh sprouts, which they claim contained 105 mg of SFN. With a drying ratio typically of approximately 12:1, the fresh sprouts would be calculated to be equivalent to approximately 5.7 g of powder. For comparison and (unrealistically) assuming no processing losses, the SFN yield of such a powder could be 18.4 mg of SFN per gram (approximately 1.8% SFN yield). The powder used in by Bahadoran et al.[103] yielded 0.4%, so that the fresh sprouts used by Clarke et al.[49] could have provided around fourfold more SFN. There are not sufficient available data to make similar calculations for the other cell types shown in Table 1, but it is clear there can be wide variability in the SFN yield from different sources. Clarke et al.[49] compared their fresh sprout product against a myrosinase-inactive broccoli seed extract capsule to demonstrate the marked contrast in SFN yield between the two.[49] They concluded that their findings have implications for people who purchase supplements devoid of myrosinase and believe incorrectly that they are receiving a dose equivalent to that of fresh broccoli sprouts.

TOXICITY ISSUES

Many of the in vitro studies (Table 1) show that micromolar concentrations of SFN in various cell types needed to activate cellular defenses via Nrf2 range from 0.2 μM to 5 μM.[17,47,72,83,85,88–90] In vitro studies using endothelial cells show that SFN is cytotoxic to these cells at concentrations of 20–40 μM and that such cytotoxicity is mediated through death receptors and apoptotic signaling.[113] These levels are well in excess of anything that can be achieved through diet or indeed via the broccoli sprout supplements currently available.[114] Toxicology studies utilizing 32 different hematology and chemistry

tests in human subjects have supported the safety of myrosinase-active broccoli sprouts.[97]

DETOXIFICATION INTERACTIONS

As phytochemicals have grown in popularity as supplements, the issue of drug-phytochemical interactions must be considered.[115] In particular, phytochemicals that modulate detoxification pathways can have unpredictable adverse effects on patients by either increasing or decreasing the available concentration of prescribed pharmaceutical agents.[116] As an added complication, the patient may fail to inform the attending medical practitioner that one or more supplements are being ingested concomitantly.[115]

The cytochrome P450 (CYP450) family contains drug-metabolizing enzymes involved in the activation and detoxification of a large number of pharmaceutical agents, with the CYP3A subfamily involved in the metabolism of more than 50% of all therapeutic drugs in clinical use.[117] Perhaps surprisingly, naturally occurring chemicals, at dietary levels of intake, can modulate the hepatic and extrahepatic expression of CYP450 levels, resulting in marked changes in the metabolism of pharmaceutical drugs.[116] Once thought to be relatively benign in their effects on drug metabolism, phytochemicals can potentially increase the risk of an adverse drug reaction.

A well-studied example of such drug-phytochemical interactions is *Hypericum perforatum* (commonly known as St. John's wort), which can adversely affect the immunosuppressive effect of the drug cyclosporine, thus potentially placing the well-being of the transplanted patient at risk.[115] Similarly, grapefruit and its bioactive component, naringenin, have been known for over 20 years to inhibit CYP3A4, and patients are often advised to avoid its consumption when taking pharmaceutical agents.[118,119] To illustrate the uncertainty associated with trying to classify these phytochemical-drug responses, a series of reviewed clinical studies was unable to verify green tea's known potent in vitro inhibition of CYP3A4.[120]

If the metabolism of a pharmaceutical agent is significantly inhibited, higher-than-intended levels of the pharmaceutical can accumulate, thereby providing a greater-than-expected dose. Phytochemicals have the potential to both elevate and suppress CYP450 activity, so that both over- and underdosing effects can be observed.[115] Coupled with CYP3A4 inhibition, there are other documented effects of phytochemical-drug reactions; the effects on the rate or amount of absorption and the interaction with ATP-dependent transporters can modify the clinical response. Moreover, genetic polymorphisms and environmental factors have been shown to alter CYP450 activities, resulting in interindividual differences in drug effects and thereby adding to the complexity of phytochemical-drug reactions.[121] If the degree of inhibition is significant, the clinical implications may be relevant for the many patients chronically ingesting commonly prescribed pharmaceutical agents such as statins, benzodiazepines, antihypertensives, antimicrobial agents, and numerous others.[118]

While SFN can be an inhibitor of the Phase 1 enzyme CYP3A4, it is also a potent inducer of the Phase 2 detoxification enzymes, including GST and NQO1.[122] SFN typically does not induce Phase 1 enzymes but does induce Phase 2; this is thought to retard the activation of a presenting toxin to its more toxic intermediate, enabling metabolic conversion to excretable nontoxic compounds via the Phase 2 enzymes.[42]

There are limited data on the extent to which SFN inhibits CYP3A4, but a series of experiments that investigated both broccoli extract and SFN evaluated CYP3A4 protein levels in human hepatocytes. There was a reduction of CYP3A4 protein to 33 ± 5% and 37 ± 8% with the 10 μM and 25 μM SFN concentrations, respectively.[44] The authors considered whether it is possible for SFN to reach a concentration in the liver sufficient to inhibit CYP3A4 expression following dietary exposure, given that a single dose of 200 μmol (35 mg of SFN) has been shown to be necessary to achieve a plasma level of 2 μM.[71] They comment that substantially higher peak concentrations are likely to be seen in the liver because of extensive first-pass clearance of SFN by the liver.[44] SFN in this study was shown to reduce DNA adduct formation following exposure to aflatoxin B_1. Although suppression of genes involved in CYP3A4 gene activation can lead to undesirable interactions with pharmaceutical agents, it can also have beneficial effects, such as, in this case, reduction in aflatoxin B_1 bioactivation with subsequent reduction in DNA damage. In a tissue culture study using Caco-2 cells, Lubelska et al.[123] recently described the variable effects of different concentrations of SFN in combination with several pharmaceutical drugs; SFN was shown to alter the metabolism and transport of the drug. How this might translate to the in vivo situation is not known.

Clearly, this complex area requires much more investigation, and clinicians should be aware that when any phytochemical supplement is ingested in conjunction with a pharmaceutical agent, the patient's response should be monitored with a view to modifying dosages if necessary.

ANTICOAGULANTS RELIANT ON VITAMIN K_1 INHIBITION

The pharmaceutical drug Coumadin® (warfarin) is an anticoagulant drug with a narrow therapeutic index. As such, too much or too little of the drug can lead to either hemorrhage or formation of vascular blood clots. Its principal mechanism is reliant on inhibition of endogenous synthesis of vitamin-K-dependent clotting factors.

Because vitamin K is found abundantly in plant foods, especially green leafy vegetables, including crucifers, patients taking this drug are typically advised to avoid consuming these vegetables and to ensure that such intake is consistent.[124] In considering whether to recommend a broccoli sprout supplement to a patient prescribed coumadin, the effect of such a supplement on the drug's vitamin-K-related metabolism is of clinical relevance.

A standard 80-g (half-cup) serving of broccoli vegetable contains about 110 μg of vitamin K,[125] while a 28-g (half-cup) serving of fresh broccoli sprouts contains approximately 38 μg.[126] No direct data on the vitamin K content of dried whole broccoli sprouts are available, but hypothetically – assuming no loss of vitamin K during the drying process – an estimate could be made as follows: A half cup of fresh sprouts is equivalent to about 2.3 g of powdered broccoli sprouts, assuming a typical 12:1 drying ratio. In most instances, a daily dose of the powder would be less than 2.3 g, providing less than one-third of the vitamin K found in a single serving of broccoli vegetable.

A dose-response study examined the effect of broccoli vegetable on the stability of oral anticoagulant treatment in healthy adults.[127] The relative bioavailability of vitamin K from broccoli was 29% compared with that of vitamin K supplements, illustrating the unlikelihood that vegetables consumed in dietary quantities would adversely affect coagulation.[127] Furthermore, it was shown that the threshold dose causing a statistically significant lowering of the international normalized ratio (INR) occurred at a dose of 150 μg daily.

It is not known how the bioavailability of dried whole broccoli sprouts compares with that of fresh sprouts or fresh mature broccoli vegetable. Even though it might appear that an amount of less than 38 μg of vitamin K obtained from less than 2.3 g of broccoli sprout powder would be unlikely to destabilize the activity of the drug, supporting evidence does not exist, and further investigation is needed.

As a separate issue, any biochemical agent that induces the expression of CYP2C9 can increase warfarin clearance, thereby reducing the antithrombotic response to a given warfarin dose. Whereas watercress-derived phenyl-ethyl isothiocyanate can inhibit CYP2C9, it is not clear if SFN does the same.[128] Given the growing popularity of broccoli sprouts as functional foods and supplements, knowledge of the relationship between regular consumption of dried sprouts and possible interactions with pharmaceutical agents, including warfarin, is of clinical importance.

THYROID DYSFUNCTION

A toxic effect of certain glucosinolates on livestock has been known for decades,[129,130] and animals consuming rapeseed meal as a major food source have been especially susceptible to the adverse physiological effects of various degradative products of glucosinolates. In humans, such products can block iodine uptake by the thyroid gland and retard synthesis of T3 and T4 hormones in this gland, sometimes resulting in hypothyroid goiter.[131] Just how significant this is in humans who regularly consume broccoli or other crucifers is not clear. A study on thyroid-cancer-prone Melanesian women concluded that a relatively high crucifer intake of approximately 70 g/day increased the risk of thyroid cancer only in those women who were moderately iodine deficient (daily iodine intake <96 μg/day).[132] The same effect was not demonstrated in European women.

Clinicians tend to be mindful of the need for caution when counseling patients with thyroid conditions about the consumption of cruciferous vegetables[131]; however, little is known about the threshold levels requiring such caution. It may be that at-risk individuals unnecessarily avoid cruciferous vegetables, which could provide more benefits than risk.[133] In the absence of more definitive guidelines, it would seem prudent to consider the possibility of iodine deficiency.

In a mechanistic study, it was recently shown that administration of thyroid hormone T3 to rats triggers a redox-mediated translocation of Nrf2 from the cytosol to the nucleus in rat liver.[134] This may represent a cytoprotective mechanism of T3 to counter electrophile toxicity. The induction of Nrf2 in this rat model was blocked by pretreatment with *N*-acetyl cysteine (NAC). This has clinical implications because NAC is being consumed with increasing frequency as a dietary supplement promoted as providing a precursor compound for glutathione synthesis. Such negative implications of NAC are relevant not just to thyroid function but also to the numerous cytoprotective compounds generated in response to Nrf2 activation.

CLASSICAL ANTIOXIDANT SUPPLEMENTS

It may not be intuitive to consider that vitamins A, C, and E could counter the effects of Nrf2-activating phytochemicals. However, several lines of evidence converge on the notion that modulation of the intracellular redox environment by use of supraphysiological doses of "antioxidant" vitamins significantly interferes with Nrf2 signaling mechanisms.[135–138] It is well known that the results of numerous clinical trials using "antioxidant" vitamins have been disappointing.[139] The mechanism to explain this failure is not yet certain, but it would seem reasonable to hypothesize that interference with Nrf2 signaling may offer one explanation.[135] It could be hypothesized that SFN-initiated gene expression of cytoprotective genes via activation of Nrf2 can be

inhibited by an altered redox state due to the presence of directly acting antioxidants. How this translates to the clinical environment is not known, but it may signify the need for caution when combining supplements. In the context of an SFN-yielding broccoli sprout supplement, concomitant ingestion with supraphysiological doses of redox-modulating supplements such as vitamins A, C, E, and NAC might negate the potential benefits of the broccoli sprout supplement.

CRITERIA FOR A SULFORAPHANE-RELEASING SUPPLEMENT: AIMING FOR BOTH PREVENTIVE AND THERAPEUTIC EFFECTS

For a broccoli sprout supplement to be suitable as a preventive and/or therapeutic agent, it must meet a number of criteria. These criteria include demonstration of retention or presence of both glucoraphanin and myrosinase in quantifiable amounts, elimination of factors that favor SFN nitrile formation over SFN, standardization of a specified glucoraphanin content and/or SFN yield, a potency capable of significantly activating Nrf2, low residue of particulate seed matter and oil, compliance with specifications for microbiological species and concentrations for supplements, and stability for a period of at least 24 months.

CURRENT STATUS OF BROCCOLI SPROUT SUPPLEMENTS

A number of broccoli-derived supplements are already commercially available and can be classified into several categories, each with its own properties.

Broccoli sprout extracts or broccoli seed extracts delivered as powders

An extract is a common delivery form for phytochemical supplements. To produce a powder, typically only the fibrous plant material and water are removed, with or without added solvent(s). Although extraction is a time-honored method of concentrating bioactive compounds, in the case of cruciferous plants, extraction is not appropriate. Because the reaction between the precursor glucoraphanin and the enzyme myrosinase is initiated in the presence of moisture, the conversion of glucoraphanin to SFN may be complete before the extraction process is finished. Although glucoraphanin is stable over a long period of time, SFN is not.[7] Consequently, the final extract is not likely to contain the bioactive components.

To avoid total loss of glucoraphanin content in producing an extract, myrosinase in the broccoli sprout material must be completely deactivated prior to extraction.[140] The product of this process is a water-soluble glucoraphanin-rich powder devoid of myrosinase and, as such, has no intrinsic ability to generate SFN on ingestion. Such products abound in the market, and the labels typically specify a quantity of "sulforaphane glucosinolate." However, "sulforaphane glucosinolate" is not a scientific term, and consumers and clinicians alike understandably but erroneously assume that the quantity of the material shown on the label refers to SFN, when in fact it refers to glucoraphanin.[141] Any reference to "sulforaphane" is inappropriate for extracts, especially since no SFN is present in any broccoli sprout powder; SFN is generated only upon the addition of water, and then only in a myrosinase-active powder. Myrosinase-inactive products, such as extracts, claim to rely on the uncertain composition of the colonic microflora to produce limited quantities of SFN.[36,142] It is often claimed that extracts have the advantages of being water soluble and having a milder flavor than whole-sprout powders.[141]

Broccoli seed powders or minimally sprouted powders

Some products labeled as "broccoli sprout powder" are derived from minimally sprouted seed (less than 3 days of sprouting). These powders are yellowish brown in color, coarse in texture, and contain a high proportion of particulate matter, including visible seed husks. They tend to contain a high proportion of oil, making the finely milled sprout powder difficult to handle if it is to be used for manufacturing ingestible capsules. Because this oil may also be a source of potentially toxic erucic acid, such seed powders are unsuitable for use in encapsulated supplements. Seed powders are sometimes marketed as food supplements, but bioactive substances are not named or quantified. These powders typically contain both glucoraphanin and myrosinase, although amounts are typically not assayed.

Whole-broccoli-sprout powders

Although whole-broccoli-sprout products have been developed with the aim of optimizing SFN yield, there are other bioactive substances in the broccoli plant with similar and supporting activities.[143] The value of a whole-food supplement has been highlighted by studies that compare the SFN yield of a whole sprout with that of an extract. The recent work by Clarke et al.[49] comparing the in vivo effects of SFN ITCs in whole sprouts versus those in a myrosinase-inactive supplement in humans not only reaffirms that erucin and SFN are capable of interconversion but also provides further evidence that the bioavailability of SFN and erucin is dramatically lower when subjects consume myrosinase-inactive broccoli supplements versus fresh broccoli sprouts.

Table 3 **Summary of effects of increasing sensitivity to sulforaphane in different tissues or cell types.**

Tissue or cell type	Summary of effect	Lowest effective SFN dose (μM)
Prostate cells: normal and cancer cell lines[47]	Increases activity of *GSTP1*, which is silenced in prostate cancer cells. Also increases other phase 2 enzymes in normal cells	0.1
Neural cortical cells[90]	Protects against dopamine toxicity	0.1
Aortic cells[17]	Significantly dose-dependently induces Nrf2 target cytoprotective genes	0.25
Bronchial epithelial cells[72]	Upregulates phase 2 enzymes and downregulates inflammatory cytokines	0.3
Brain: substantia nigra cells[88]	Significantly increases GSH and NQO1 at both doses	0.5
Bronchial epithelial cells[89]	Significantly increases Nrf2 targets in normal cells; no effects on cancerous cells	0.5
Retinal epithelial cells[83]	Linear cytoprotective effects with increasing SFN doses	1.25
Pancreatic β-cells[86]	Protective against cytokine-induced cell death	2.5
Endothelial cells: arteries and veins[87]	Protective against endothelial lipase at lower dose	2.5 μM (arterial) 10 μM (venous)
Cardiomyocytes[85]	Greatest effects with SFN exposure up to 48 h	5 μM
Prostate cancer cells[84]	Strong cell-cycle arrest in prostate cancer	10 μM (no effect at <3 μM)

Abbreviations: GSH, glutathione; NQO1, NAD(P)H (quinone) oxidoreductase; SFN, sulforaphane.

Earlier in the same year, another group had combined a glucoraphanin-rich but myrosinase-inactive supplement with fresh myrosinase-containing broccoli sprouts to demonstrate that the combination enhanced the conversion to SFN of the glucoraphanin in the myrosinase-inactive supplement.[50]

Although there are some whole-broccoli-sprout powders that retain both glucoraphanin and myrosinase, they are not as widely available as the extracts. Their appearance is typically more olive green than yellowish brown, and they may or may not be finely milled. Most are available as unspecified food powders, although some manufacturers produce higher-grade finely milled material, optimized for SFN bioactivity. These ingredients are much better suited to medicinal applications. The dilemma for the clinician is in identifying those commercially available supplements capable of delivering the desired clinical outcomes.

What can a clinician expect of a myrosinase-active supplement?

Clinicians know there are many factors that can prevent a patient from adopting changes in eating behavior; failure is common. If a patient simply does not consume the recommended diet, is there a case for appropriate supplementation, not just of nutrients but also of phytochemicals such as SFN, known to enhance cellular defenses? At this incomplete stage of SFN research, clinical trial data regarding therapeutic interventions are limited. Nevertheless, a large body of epidemiological evidence shows that even several servings per week of (presumably cooked) broccoli vegetable may confer measureable cytoprotection.[82] Therefore, even small amounts of a myrosinase-active broccoli sprout supplement may offer at least the same degree of protection. As a preventive strategy, especially in patients unresponsive to clinical advice to increase vegetable intake, appropriate SFN supplementation may be indicated. However, it is difficult to determine a dose relevant for therapeutic purposes. The in vitro data shown in Table 1 and the in vivo data shown in Table 2, clearly show there is wide variability in the response to SFN in different tissue types. For example, the neural cortex is protected against dopamine toxicity at intracellular SFN concentrations as low as 0.1 μM.[90] Pancreatic β-cells respond to slightly higher doses, with SFN at 2.5 μM increasing cell viability to approximately 80% under conditions of oxidative stress.[86] Aortic levels of glutathione and NQO1 levels increased 25% and 66%, respectively, at just 0.25 μM.[17] Cardiomyocytes, however, required an SFN level of 5 μM to retain 92% cell viability over a 48-h period.[85] Table 3[17,47,72,83–90] summarizes the effects of increasing sensitivity to SFN in different tissues or cell types.

Doses required for HDAC inhibition and direct cancer cell kill appear to be much higher. Tan et al.[89] showed that malignant lung adenocarcinoma cells were unresponsive to doses up to 2 μM, even though normal lung cells showed up to a 7.5-fold increase in cytoprotective NQO1 at this dose. Inhibition of HDAC activity required 15 μM in prostate epithelial cells.[92]

Clinically relevant doses

The earlier estimate indicated that approximately 0.6 μM SFN could be achieved in breast cells with ingestion

of four capsules daily of the powder used in the trial of Bahadoran et al.[103] This may be sufficient for cytoprotection in pancreatic β-cells, neurons, aortic cells, and other cell types.

Table 1 shows that induction of cytoprotective genes coding for NQO1 and GST can occur at low intracellular concentrations in a range of tissues. This should be readily achievable with a modest intake of a whole-sprout powder supplement. However, SFN has been shown to accumulate in cells and to conjugate with glutathione, so that both SFN and its metabolites are bioactive. Accumulation can lead to millimolar concentrations.[144] Ye and Zhang[80] show that area under the curve calculations based on multiple doses over an extended period may better correlate with inducer capacity than that derived from a single dose. In this way, multiple dosing within a fixed time period may enhance induction by some ITCs with initially low activity. This is clinically relevant because multiple dosing has a different effect than the single doses used in many in vitro studies.

Future research

Development of a pharmaceutical product is a multifaceted, complex, protracted, and expensive process. Many of the same steps are essential in the development of a functional food or nutraceutical supplement, but since the FDA does not formally approve supplements prior their market release,[145] a consumer or a clinician cannot be assured of either the quality or the potential efficacy of supplements.

There is a popular perception[146] that dietary supplements are inherently safer than pharmaceutical agents, and in 1994 the U.S. Congress enacted the Dietary Supplement and Health Education Act (DSHEA)[147] to authorize the regulation of such supplements. The implementation of DSHEA, however, has led to considerable confusion for manufacturers and marketers. Aware of both the growing consumer interest in supplements and the burgeoning costs of delivering healthcare to the community, the FDA recently released proposed guidelines that, if implemented, will significantly affect the supplement industry.[148] The guidelines specify that new ingredients in supplements must be proven to be safe, a requirement likely to result in considerable extra manufacturing costs.[145] However, if inexpensive dietary supplements are found to be safe and effective, the requirement for additional research could yield significant cost savings as well as health benefits for the community.[149]

The number of published clinical trials on SFN is comparatively small, although 25 planned or in-process studies were listed with the U.S. National Institutes of Health (www.clinicaltrials.gov) at the time of manuscript preparation, when the search term "broccoli sprouts" was applied. Consideration of the existing clinical trial data on SFN against a background of epidemiological and in vitro data for cruciferous vegetables and for SFN itself would seem to indicate that an appropriately produced myrosinase-active broccoli sprout supplement is safe and could have preventive and clinical value. In order to give clinicians the confidence they need to recommend such supplements, standardization of both nomenclature and assay protocols is essential.

Not only is labeling of the currently available broccoli sprout supplements inconsistent, but there also is no reliable way for a clinician or consumer to know if the product will yield SFN on ingestion. The ability to influence cell function is due entirely to the yield of SFN and other ITCs, so that reporting other parameters is largely irrelevant.

CONCLUSION

Nutrition professionals and public health authorities encourage individuals to consume a diet with the appropriate proportion of plant foods; however, clinician experience and large-scale survey data continue to show that adherence to such recommendations is generally poor. Although no supplement can replace the value of a balanced and varied diet, an SFN-yielding supplement capable of nutrigenomically inducing the expression of cytoprotective genes may provide a health benefit not achievable with the more popular antioxidant vitamin supplements. A myrosinase-active, SFN-yielding, standardized whole-broccoli-sprout supplement may allow the health-promoting benefits of the unpopular cruciferous vegetable to become more widely and conveniently available.

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REFERENCES

1. Verhoeven DT, Goldbohm RA, van Poppel G, et al. Epidemiological studies on brassica vegetables and cancer risk. Cancer Epidemiol Biomarkers Prev. 1996;5:733–748.
2. Traka MH, Mithen RF. Plant science and human nutrition: challenges in assessing health-promoting properties of phytochemicals. Plant Cell. 2011;23:2483–2497.
3. Lee SA, Fowke JH, Lu W, et al. Cruciferous vegetables, the *GSTP*$_1$ *Ile*^{105}*Val* genetic polymorphism, and breast cancer risk. Am J Clin Nutr. 2008;87:753–760.
4. Bao YP. Bioactivities of dietary isothiocyanates. Agro Food Ind Hi-Tech. 2005;16:24–26.
5. Nestle M. Broccoli sprouts in cancer prevention. Nutr Rev. 1998;56:127–130.
6. Vasanthi HR, Mukherjee S, Das DK. Potential health benefits of broccoli – a chemico-biological overview. Mini Rev Med Chem. 2009;9:749–759.

7. Jin Y, Wang M, Rosen RT, et al. Thermal degradation of sulforaphane in aqueous solution. J Agric Food Chem. 1999;47:3121–3123.
8. Wu H, Liang H, Yuan Q, et al. Preparation and stability investigation of the inclusion complex of sulforaphane with hydroxypropyl-cyclodextrin. Carbohydr Polym. 2010;82:613–617.
9. Kaput J, Rodriguez RL. Nutritional genomics: the next frontier in the postgenomic era. Physiol Genomics. 2004;16:166–177.
10. Fenech M, El-Sohemy A, Cahill L, et al. Nutrigenetics and nutrigenomics: viewpoints on the current status and applications in nutrition research and practice. J Nutrigenet Nutrigenomics. 2011;4:69–89.
11. Joost HG, Gibney MJ, Cashman KD, et al. Personalised nutrition: status and perspectives. Br J Nutr. 2007;98:26–31.
12. Mancinelli L, Cronin M, Sadée W. Pharmacogenomics: the promise of personalized medicine. AAPS PharmSci. 2000;2:E4.
13. Costa V, Casamassimi A, Ciccodicola A. Nutritional genomics era: opportunities toward a genome-tailored nutritional regimen. J Nutr Biochem. 2010;21: 457–467.
14. Surh Y-J, Dong Z, Cadenas E, Packer L (eds). *Dietary Modulation of Cell Signaling Pathways*. Boca Raton, FL: Taylor & Francis; 2008.
15. Klaassen CD, Reisman SA. Nrf2 to the rescue: effects of the antioxidative/electrophilic response on the liver. Toxicol Appl Pharmacol. 2010;244: 57–65.
16. Hu R, Xu C, Shen G, et al. Gene expression profiles induced by cancer chemopreventive isothiocyanate sulforaphane in the liver of C57BL/6J mice and C57BL/6J/Nrf2 (–/–) mice. Cancer Lett. 2006;243:170–192.
17. Zhu H, Jia Z, Strobl JS, et al. Potent induction of total cellular and mitochondrial antioxidants and phase 2 enzymes by cruciferous sulforaphane in rat aortic smooth muscle cells: cytoprotection against oxidative and electrophilic stress. Cardiovasc Toxicol. 2008;8:115–125.
18. Hayes JD, McMahon M. *NRF2* and *KEAP1* mutations: permanent activation of an adaptive response in cancer. Trends Biochem Sci. 2009;34:176–188.
19. Niture SK, Kaspar JW, Shen J, et al. Nrf2 signaling and cell survival. Toxicol Appl Pharmacol. 2010;244:37–42.
20. Surh YJ, Kundu JK, Na HK. Nrf2 as a master redox switch in turning on the cellular signaling involved in the induction of cytoprotective genes by some chemopreventive phytochemicals. Planta Med. 2008;74:1526–1539.
21. Lee JM, Johnson JA. An important role of Nrf2-ARE pathway in the cellular defense mechanism. J Biochem Mol Biol. 2004;37:139–143.
22. Lewis KN, Mele J, Hayes JD, et al. Nrf2, a guardian of healthspan and gatekeeper of species longevity. Integr Comp Biol. 2010;50:829–843.
23. Hart C, Cohen R, Norwood M, et al. The emerging harm of antioxidants in carcinogenesis. Future Oncol. 2012;8:535–548.
24. Zhang Q, Pi J, Woods CG, et al. A systems biology perspective on Nrf2-mediated antioxidant response. Toxicol Appl Pharmacol. 2010;244:84–97.
25. Mutch DM, Wahli W, Williamson G. Nutrigenomics and nutrigenetics: the emerging faces of nutrition. FASEB J. 2005;19:1602–1616.
26. Ambrosone CB, Tang L. Cruciferous vegetable intake and cancer prevention: role of nutrigenetics. Cancer Prev Res (Phila). 2009;2:298–300.
27. Kim MK, Park JH. Cruciferous vegetable intake and the risk of human cancer: epidemiological evidence. Proc Nutr Soc. 2009;68:103–110.
28. Joseph MA, Moysich KB, Freudenheim JL, et al. Cruciferous vegetables, genetic polymorphisms in glutathione S-transferases M1 and T1, and prostate cancer risk. Nutr Cancer. 2004;50:206–213.
29. Obeidat M, Wain LV, Shrine L, et al. A comprehensive evaluation of potential lung function associated genes in the SpiroMeta general population sample. PLoS ONE. 2011;6:e19382.
30. Reszka E, Wasowicz W. Significance of genetic polymorphisms in glutathione S-transferase multigene family and lung cancer risk. Int J Occup Med Environ Health. 2001;14:99–113.
31. Clarke JD, Dashwood RH, Ho E. Multi-targeted prevention of cancer by sulforaphane. Cancer Lett. 2008;269:291–304.
32. Gasper AV, Al-Janobi A, Smith JA, et al. Glutathione S-transferase M1 polymorphism and metabolism of sulforaphane from standard and high-glucosinolate broccoli. Am J Clin Nutr. 2005;82:1283–1291.
33. Gasper AV, Traka M, Bacon JR, et al. Consuming broccoli does not induce genes associated with xenobiotic metabolism and cell cycle control in human gastric mucosa. J Nutr. 2007;137:1718–1724.
34. Hanlon N, Coldham N, Gielbert A, et al. Repeated intake of broccoli does not lead to higher plasma levels of sulforaphane in human volunteers. Cancer Lett. 2009;284:15–20.
35. Saha S, Hollands W, Teucher B, et al. Isothiocyanate concentrations and interconversion of sulforaphane to erucin in human subjects after consumption of commercial frozen broccoli compared to fresh broccoli. Mol Nutr Food Res. 2012;56:1906–1916.
36. West LG, Meyer KA, Balch BA, et al. Glucoraphanin and 4-hydroxyglucobrassicin contents in seeds of 59 cultivars of broccoli, raab, kohlrabi, radish, cauliflower, brussels sprouts, kale, and cabbage. J Agric Food Chem. 2004;52:916–926.
37. Kushad MM, Brown AF, Kurilich AC, et al. Variation of glucosinolates in vegetable crops of *Brassica oleracea*. J Agric Food Chem. 1999;47:1541–1548.
38. Ketterer B. Dietary isothiocyanates as confounding factors in the molecular epidemiology of colon cancer. Cancer Epidemiol Biomarkers Prev. 1998;7: 645–646.
39. Seow A, Vainio H, Yu MC. Effect of glutathione-S-transferase polymorphisms on the cancer preventive potential of isothiocyanates: an epidemiological perspective. Mutat Res. 2005;592:58–67.
40. Lin HJ, Probst-Hensch NM, Louie AD, et al. Glutathione transferase null genotype, broccoli, and lower prevalence of colorectal adenomas. Cancer Epidemiol Biomarkers Prev. 1998;7:647–652.
41. Lampe JW, Peterson S. Brassica, biotransformation and cancer risk: genetic polymorphisms alter the preventive effects of cruciferous vegetables. J Nutr. 2002;132:2991–2994.
42. Juge N, Mithen RF, Traka M. Molecular basis for chemoprevention by sulforaphane: a comprehensive review. Cell Mol Life Sci. 2007;64:1105–1127.
43. Fruth K, Best N, Amro M, et al. No evidence for a correlation of glutathione S-tranferase polymorphisms and chronic rhinosinusitis. Rhinology. 2011;49:180–184.
44. Gross-Steinmeyer K, Stapleton PL, Tracy JH, et al. Sulforaphane- and phenethyl isothiocyanate-induced inhibition of aflatoxin B-mediated genotoxicity in human hepatocytes: role of GSTM1 genotype and CYP3A4 gene expression. Toxicol Sci. 2010;116:422–432.
45. Reinhardt HC, Schumacher B. The p53 network: cellular and systemic DNA damage responses in aging and cancer. Trends Genet. 2012;28:128–136.
46. Bourdon JC. p53 family isoforms. Curr Pharm Biotechnol. 2007;8:332–336.
47. Brooks JD, Paton VG, Vidanes G. Potent induction of phase 2 enzymes in human prostate cells by sulforaphane. Cancer Epidemiol Biomarkers Prev. 2001;10:949–954.
48. Fimognari C, Sangiorgi L, Capponcelli S, et al. A mutated p53 status did not prevent the induction of apoptosis by sulforaphane, a promising anti-cancer drug. Invest New Drugs. 2005;23:195–203.
49. Clarke JD, Hsu A, Riedl K, et al. Bioavailability and inter-conversion of sulforaphane and erucin in human subjects consuming broccoli sprouts or broccoli supplement in a cross-over study design. Pharmacol Res. 2011;64: 456–463.
50. Cramer JM, Jeffery EH. Sulforaphane absorption and excretion following ingestion of a semi-purified broccoli powder rich in glucoraphanin and broccoli sprouts in healthy men. Nutr Cancer. 2011;63:196–201.
51. Vermeulen M, Klopping-Ketelaars IW, van den Berg R, et al. Bioavailability and kinetics of sulforaphane in humans after consumption of cooked versus raw broccoli. J Agric Food Chem. 2008;56:10505–10509.
52. Anand P, Kunnumakkara AB, Newman RA, et al. Bioavailability of curcumin: problems and promises. Mol Pharm. 2007;4:807–818.
53. Riedl MA, Saxon A, Diaz-Sanchez D. Oral sulforaphane increases Phase II antioxidant enzymes in the human upper airway. Clin Immunol. 2009;130: 244–251.
54. Johanningsmeier SD, Harris GK. Pomegranate as a functional food and nutraceutical source. Annu Rev Food Sci Technol. 2011;2:181–201.
55. Fahey JW, Kensler TW. Role of dietary supplements/nutraceuticals in chemoprevention through induction of cytoprotective enzymes. Chem Res Toxicol. 2007;20:572–576.
56. D'Archivio M, Filesi C, Vari R, et al. Bioavailability of the polyphenols: status and controversies. Int J Mol Sci. 2010;11:1321–1342.
57. Jeffery EH, Keck AS. Translating knowledge generated by epidemiological and in vitro studies into dietary cancer prevention. Mol Nutr Food Res. 2008;52(Suppl 1):S7–S17.
58. Cornblatt BS, Ye L, Dinkova-Kostova AT, et al. Preclinical and clinical evaluation of sulforaphane for chemoprevention in the breast. Carcinogenesis. 2007;28:1485–1490.
59. Fahey JW, Zhang Y, Talalay P. Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. Proc Natl Acad Sci U S A. 1997;94:10367–10372.
60. Gorrisen A, Kraut NU, de Visser R, et al. No *de novo* sulforaphane biosynthesis in broccoli seedlings. Food Chem. 2011;127:192–196.
61. Bekaert M, Edger PP, Hudson CM, et al. Metabolic and evolutionary costs of herbivory defense: systems biology of glucosinolate synthesis. New Phytol. 2012;196:596–605.
62. Zhang Y, Talalay P, Cho CG, et al. A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure. Proc Natl Acad Sci U S A. 1992;89:2399–2403.
63. Basten GP, Bao Y, Williamson G. Sulforaphane and its glutathione conjugate but not sulforaphane nitrile induce UDP-glucuronosyl transferase (UGT1A1) and glutathione transferase (GSTA1) in cultured cells. Carcinogenesis. 2002;23:1399–1404.
64. Jeffery EH, Stewart KE. Upregulation of quinone reductase by glucosinolate hydrolysis products from dietary broccoli. Methods Enzymol. 2004;382: 457–469.
65. Matusheski NV, Swarup R, Juvik JA, et al. Epithiospecifier protein from broccoli (*Brassica oleracea* L. ssp. *italica*) inhibits formation of the anticancer agent sulforaphane. J Agric Food Chem. 2006;54:2069–2076.

66. Matusheski NV, Juvik JA, Jeffery EH. Heating decreases epithiospecifier protein activity and increases sulforaphane formation in broccoli. Phytochemistry. 2004;65:1273–1281.
67. West L, Tsui I, Balch B, et al. Determination and health implication of the erucic acid content of broccoli florets, sprouts, and seeds. J Food Sci. 2002;67: 2641–2643.
68. US Food and Drug Administration. Code of Federal Regulations Title 21. Subchapter B. Food for Human Consumption. Part 184 – Direct Food Substances Affirmed as Generally Recognized as Safe (GRAS). Published 2012; http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr =184.1555. Accessed October 5, 2013.
69. Kwak MK, Itoh K, Yamamoto M, et al. Enhanced expression of the transcription factor Nrf2 by cancer chemopreventive agents: role of antioxidant response element-like sequences in the *nrf2* promoter. Mol Cell Biol. 2002;22:2883–2892.
70. Prawan A, Kundu JK, Surh YJ. Molecular basis of heme oxygenase-1 induction: implications for chemoprevention and chemoprotection. Antioxid Redox Signal. 2005;7:1688–1703.
71. Ye L, Dinkova-Kostova AT, Wade KL, et al. Quantitative determination of dithiocarbamates in human plasma, serum, erythrocytes and urine: pharmacokinetics of broccoli sprout isothiocyanates in humans. Clin Chim Acta. 2002;316:43–53.
72. Ritz SA, Wan J, Diaz-Sanchez D. Sulforaphane-stimulated phase II enzyme induction inhibits cytokine production by airway epithelial cells stimulated with diesel extract. Am J Physiol Lung Cell Mol Physiol. 2007;292:L33–L39.
73. Juge N, Mithen RF, Traka M. Molecular basis for chemoprevention by sulforaphane: a comprehensive review. Cell Mol Life Sci. 2007;64: 1105–1127.
74. Myzak MC, Tong P, Dashwood W-M, et al. Sulforaphane retards the growth of human PC-3 xenografts and inhibits HDAC activity in human subjects. Exp Biol Med (Maywood) 2007;232:227–234.
75. Dashwood RH, Myzak MC, Ho E. Dietary HDAC inhibitors: time to rethink weak ligands in cancer chemoprevention? Carcinogenesis. 2006;27:344–349.
76. Bertl E, Bartsch H, Gerhauser C. Inhibition of angiogenesis and endothelial cell functions are novel sulforaphane-mediated mechanisms in chemoprevention. Mol Cancer Ther. 2006;5:575–585.
77. Chu WF, Wu DM, Liu W, et al. Sulforaphane induces G2-M arrest and apoptosis in high metastasis cell line of salivary gland adenoid cystic carcinoma. Oral Oncol. 2009;45:998–1004.
78. Keck AS, Qiao Q, Jeffery EH. Food matrix effects on bioactivity of broccoli-derived sulforaphane in liver and colon of F344 rats. J Agric Food Chem. 2003;51:3320–3327.
79. Veeranki OL, Bhattacharya A, Marshall JR, et al. Organ-specific exposure and response to sulforaphane, a key chemopreventive ingredient in broccoli: implications for cancer prevention. Br J Nutr. 2013;109:25–32.
80. Ye L, Zhang Y. Total intracellular accumulation levels of dietary isothiocyanates determine their activity in elevation of cellular glutathione and induction of Phase 2 detoxification enzymes. Carcinogenesis. 2001;22:1987–1992.
81. Munday R, Mhawech-Fauceglia P, Munday CM, et al. Inhibition of urinary bladder carcinogenesis by broccoli sprouts. Cancer Res. 2008;68:1593–1600.
82. Cohen JH, Kristal AR, Stanford JL. Fruit and vegetable intakes and prostate cancer risk. J Natl Cancer Inst. 2000;92:61–68.
83. Gao X, Talalay P. Induction of phase 2 genes by sulforaphane protects retinal pigment epithelial cells against photooxidative damage. Proc Natl Acad Sci U S A. 2004;101:10446–10451.
84. Cho SD, Li G, Hu H, et al. Involvement of c-Jun N-terminal kinase in G_2/M arrest and caspase-mediated apoptosis induced by sulforaphane in DU145 prostate cancer cells. Nutr Cancer. 2005;52:213–224.
85. Angeloni C, Leoncini E, Malaguti M, et al. Modulation of phase II enzymes by sulforaphane: implications for its cardioprotective potential. J Agric Food Chem. 2009;57:5615–5622.
86. Song M-Y, Kim E-K, Moon W-S, et al. Sulforaphane protects against cytokine- and streptozotocin-induced beta-cell damage by suppressing the NF-κB pathway. Toxicol Appl Pharmacol. 2009;235:57–67.
87. Kivelä AM, Mäkinen PI, Jyrkkänen HK, et al. Sulforaphane inhibits endothelial lipase expression through NF-κB in endothelial cells. Atherosclerosis. 2010;213:122–128.
88. Mas S, Gassó P, Trias G, et al. Sulforaphane protects SK-N-SH cells against antipsychotic-induced oxidative stress. Fundam Clin Pharmacol. 2012;26: 712–721.
89. Tan XL, Shi M, Tang H, et al. Candidate dietary phytochemicals modulate expression of phase II enzymes GSTP1 and NQO1 in human lung cells. J Nutr. 2010;140:1404–1410.
90. Vauzour D, Buonfiglio M, Corona G, et al. Sulforaphane protects cortical neurons against 5-S-cysteinyl-dopamine-induced toxicity through the activation of ERK1/2, Nrf-2 and the upregulation of detoxification enzymes. Mol Nutr Food Res. 2010;54:532–542.
91. Shan Y, Wu K, Wang W, et al. Sulforaphane down-regulates COX-2 expression by activating p38 and inhibiting NF-κB-DNA-binding activity in human bladder T24 cells. Int J Oncol. 2009;34:1129–1134.
92. Myzak MC, Hardin K, Wang R, et al. Sulforaphane inhibits histone deacetylase activity in BPH-1, LnCaP and PC-3 prostate epithelial cells. Carcinogenesis. 2006;27:811–819.
93. Conaway CC, Getahun SM, Liebes LL, et al. Disposition of glucosinolates and sulforaphane in humans after ingestion of steamed and fresh broccoli. Nutr Cancer. 2000;38:168–178.
94. Galan MV, Kishan AA, Silverman AL. Oral broccoli sprouts for the treatment of *Helicobacter pylori* infection: a preliminary report. Dig Dis Sci. 2004;49: 1088–1090.
95. Murashima M, Watanabe S, Zhuo XG, et al. Phase 1 study of multiple biomarkers for metabolism and oxidative stress after one-week intake of broccoli sprouts. Biofactors. 2004;22:271–275.
96. Kensler TW, Chen JG, Egner PA, et al. Effects of glucosinolate-rich broccoli sprouts on urinary levels of aflatoxin-DNA adducts and phenanthrene tetraols in a randomized clinical trial in He Zuo township, Qidong, People's Republic of China. Cancer Epidemiol Biomarkers Prev. 2005;14:2605–2613.
97. Shapiro TA, Fahey JW, Dinkova-Kostova AT, et al. Safety, tolerance, and metabolism of broccoli sprout glucosinolates and isothiocyanates: a clinical phase I study. Nutr Cancer. 2006;55:53–62.
98. Rungapamestry V, Duncan AJ, Fuller Z, et al. Effect of meal composition and cooking duration on the fate of sulforaphane following consumption of broccoli by healthy human subjects. Br J Nutr. 2007;97:644–652.
99. Traka M, Gasper AV, Melchini A, et al. Broccoli consumption interacts with *GSTM1* to perturb oncogenic signalling pathways in the prostate. PLoS ONE. 2008;3:e2568.
100. Yanaka A, Fahey JW, Fukomota A, et al. Dietary sulforaphane-rich broccoli sprouts reduce colonization and attenuate gastritis in *Helicobacter pylori*-infected mice and humans. Cancer Prev Res (Phila). 2009;2:353–360.
101. Kumar A, Vineis P, Sacerdote C, et al. Determination of new biomarkers to monitor the dietary consumption of isothiocyanates. Biomarkers. 2010;15: 739–745.
102. Egner PA, Chen JG, Wang JB, et al. Bioavailability of sulforaphane from two broccoli sprout beverages: results of a short-term, cross-over clinical trial in Qidong, China. Cancer Prev Res (Phila). 2011;4:384–395.
103. Bahadoran Z, Mirmirian P, Hosseinpanah F, et al. Broccoli sprouts reduce oxidative stress in type 2 diabetes: a randomized double-blind clinical trial. Eur J Clin Nutr. 2011;65:972–977.
104. Clarke JD, Riedl K, Bella D, et al. Comparison of isothiocyanate metabolite levels and histone deacetylase activity in human subjects consuming broccoli sprouts or broccoli supplement. J Agric Food Chem. 2011;59:10955–10963.
105. Bahadoran Z, Mirmirian P, Hosseinpanah F, et al. Broccoli sprouts powder could improve serum triglyceride and oxidized LDL/LDL-cholesterol ratio in type 2 diabetic patients: a randomized double-blind placebo-controlled clinical trial. Diabetes Res Clin Pract. 2012;96:348–354.
106. Yanaka A. Sulforaphane enhances protection and repair of gastric mucosa against oxidative stress in vitro, and demonstrates anti-inflammatory effects on *Helicobacter pylori*-infected gastric mucosae in mice and human subjects. Curr Pharm Des. 2011;17:1532–1540.
107. Yanaka A, Tauchi M, Yamamoto M. Effects of sulforaphane-rich broccoli sprouts on *H. pylori*-infected gastric mucosa [in Japanese]. Nihon Rinsho. 2005;63(Suppl 11):582–586.
108. Clarke JD, Hsu A, Yu Z, et al. Differential effects of sulforaphane on histone deacetylases, cell cycle arrest and apoptosis in normal prostate cells versus hyperplastic and cancerous prostate cells. Mol Nutr Food Res. 2011;55: 999–1009.
109. Munday R, Munday CM. Induction of phase II detoxification enzymes in rats by plant-derived isothiocyanates: comparison of allyl isothiocyanate with sulforaphane and related compounds. J Agric Food Chem. 2004;52:1867–1871.
110. Robbins RJ, Keck AS, Banuelos G, et al. Cultivation conditions and selenium fertilization alter the phenolic profile, glucosinolate, and sulforaphane content of broccoli. J Med Food. 2005;8:204–214.
111. Zhang Y, Callaway EC. High cellular accumulation of sulphoraphane, a dietary anticarcinogen, is followed by rapid transporter-mediated export as a glutathione conjugate. Biochem J. 2002;364:301–307.
112. Newton JM, Bader F. The prediction of the bulk densities of powder mixtures, and its relationship to the filling of hard gelatin capsules. J Pharm Pharmacol. 1981;33:621–626.
113. Xue M, Qian Q, Adaikalakoteswari A, et al. Activation of NF-E2-related factor-2 reverses biochemical dysfunction of endothelial cells induced by hyperglycemia linked to vascular disease. Diabetes. 2008;57:2809–2817.
114. Ioannides C, Hanlon N, Konsue N. Isothiocyanates: a chemical class of potential nutraceuticals. Open Nutraceuticals J. 2010;3:55–62.
115. Ioannides C. Drug-phytochemical interactions. Inflammopharmacology. 2003;11:7–42.
116. Ioannides C. Pharmacokinetic interactions between herbal remedies and medicinal drugs. Xenobiotica. 2002;32:451–478.
117. Williams JA, Ring BJ, Cantrell VE, et al. Comparative metabolic capabilities of CYP3A4, CYP3A5, and CYP3A7. Drug Metab Dispos. 2002;30:883–891.

118. Hanley MJ, Cancalon P, Widmer WW, et al. The effect of grapefruit juice on drug disposition. Expert Opin Drug Metab Toxicol. 2011;7:267–286.
119. Imboden M, Downs SH, Senn O, et al. Glutathione S-transferase genotypes modify lung function decline in the general population: SAPALDIA cohort study. Respir Res. 2007;8:2.
120. Engdal S, Nilsen OG. In vitro inhibition of CYP3A4 by herbal remedies frequently used by cancer patients. Phytother Res. 2009;23:906–912.
121. Rodríguez-Antona C, Leskelä S, Zajac M, et al. Expression of CYP3A4 as a predictor of response to chemotherapy in peripheral T-cell lymphomas. Blood. 2007;110:3345–3351.
122. Mahéo K, Morel F, Langouët S, et al. Inhibition of cytochromes P-450 and induction of glutathione S-transferases by sulforaphane in primary human and rat hepatocytes. Cancer Res. 1997;57:3649–3652.
123. Lubelska K, Milczarek M, Modzelewska K, et al. Interactions between drugs and sulforaphane modulate the drug metabolism enzymatic system. Pharmacol Rep. 2012;64:1243–1252.
124. Booth SL, Centurelli MA. Vitamin K: a practical guide to the dietary management of patients on warfarin. Nutr Rev. 1999;57:288–296.
125. Bolton-Smith C, Price RJ, Fenton ST, Harrington DJ, Shearer MJ. Compilation of a provisional UK database for the phylloquinone (vitamin K1) content of foods. Br J Nutr 2000;83:389–399.
126. Brassica Protection Products LLC. *Brassica.com FAQs*. 2010; Available at: http://www.sgs-broccoli.com/emerging-science/faqs. Accessed October 5, 2012.
127. Schurgers LJ, Shearer MJ, Hamulyak K, et al. Effect of vitamin K intake on the stability of oral anticoagulant treatment: dose-response relationships in healthy subjects. Blood. 2004;104:2682–2689.
128. Cheung KL, Kong AN. Molecular targets of dietary phenethyl isothiocyanate and sulforaphane for cancer chemoprevention. AAPS J. 2010;12:87–97.
129. Mithen RF, Dekker M, Verkerk R, et al. The nutritional significance, biosynthesis and bioavailability of glucosinolates in human foods. J Sci Food Agric. 2000;80:967–984.
130. Verkerk R, Schreiner M, Krumbein A, et al. Glucosinolates in *Brassica* vegetables: the influence of the food supply chain on intake, bioavailability and human health. Mol Nutr Food Res. 2009;53(Suppl 2):S219.
131. McMillan M, Spinks EA, Fenwick GR. Preliminary observations on the effect of dietary brussels sprouts on thyroid function. Hum Toxicol. 1986;5:15–19.
132. Truong T, Baron-Dubourdieu D, Rougier Y, et al. Role of dietary iodine and cruciferous vegetables in thyroid cancer: a countrywide case-control study in New Caledonia. Cancer Causes Control. 2010;21:1183–1192.
133. Assayed ME, Abd El-Aty AM. Cruciferous plants: phytochemical toxicity versus cancer chemoprotection. Mini Rev Med Chem. 2009;9:1470–1478.
134. Romanque P, Cornejo P, Valdes S, et al. Thyroid hormone administration induces rat liver Nrf2 activation: suppression by *N*-acetylcysteine pretreatment. Thyroid. 2011;21:655–662.
135. Wagner AE, Boesch-Saadatmandi C, Breckwoldt D, et al. Ascorbic acid partly antagonizes resveratrol mediated heme oxygenase-1 but not paraoxonase-1 induction in cultured hepatocytes – role of the redox-regulated transcription factor Nrf2. BMC Complement Altern Med. 2011;11:1.
136. Halliwell B. Vitamin C: antioxidant or pro-oxidant in vivo? Free Radic Res. 1996;25:439–454.
137. Peternelj TT, Coombes JS. Antioxidant supplementation during exercise training: beneficial or detrimental? Sports Med. 2011;41:1043–1069.
138. Bjelakovic G, Gluud C. Vitamin and mineral supplement use in relation to all-cause mortality in the Iowa Women's Health Study. Arch Intern Med. 2011;171:1633–1634.
139. Pashkow FJ. Oxidative stress and inflammation in heart disease: do antioxidants have a role in treatment and/or prevention? Int J Inflamm. 2011;2011:514623.
140. Pustateri DJ, Chiang WC, Letiz REA, Sperrazzo PM, inventors. Amway Corporation, assignee. Brassica vegetable supplement and process for manufacture. US patent 5,882,646. March 16, 1999.
141. [No authors listed]. Sulforaphane glucosinolate. Monograph. Altern Med Rev. 2010;15:352–360.
142. Rungapamestry V, Rabot S, Fuller Z, et al. Influence of cooking duration of cabbage and presence of colonic microbiota on the excretion of N-acetylcysteine conjugates of allyl isothiocyanate and bioactivity of phase 2 enzymes in F344 rats. Br J Nutr. 2008;99:773–781.
143. Wang W, Wang S, Howie AF, et al. Sulforaphane, erucin, and iberin up-regulate thioredoxin reductase 1 expression in human MCF-7 cells. J Agric Food Chem. 2005;53:1417–1421.
144. Zhang Y, Talalay P. Mechanism of differential potencies of isothiocyanates as inducers of anticarcinogenic phase 2 enzymes. Cancer Res. 1998;58:4632–4639.
145. US Food and Drug Administration. *Draft Guidance for Industry: Dietary Supplements: New Dietary Ingredient Notifications and Related Issues*. Published July 2011; Available at: http://www.fda.gov/food/guidanceregulation/guidancedocumentsregulatoryinformation/dietarysupplements/ucm257563.htm. Accessed October 7, 2013.
146. Egan B, Hodgkins C, Shepherd R, et al. An overview of consumer attitudes and beliefs about plant food supplements. Food Funct. 2011;2:747–752.
147. US Food and Drug Adminstration. Dietary Supplement and Health Education Act. http://www.fda.gov/RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCAct/SignificantAmendmentstotheFDCAct/ucm148003.htm. Published 1994; Accessed January 18, 2012.
148. Bailey RL, Gahche JJ, Lentino CV, et al. Dietary supplement use in the United States, 2003–2006. J Nutr. 2011;141:261–266.
149. Umhau JC, Garg K, Woodward AM. Dietary supplements and their future in health care: commentary on draft guidelines proposed by the Food and Drug Administration. Antioxid Redox Signal. 2012;16:461–462.