Symposium: Relative Bioactivity of Functional Foods and Related Dietary Supplements

Selenium Enrichment of Broccoli: Interactions between Selenium and Secondary Plant Compounds[1–3]

John W. Finley,[4] Anna Sigrid-Keck,* Rebecca J. Robbins,† and Korry J. Hintze**

*USDA, Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, ND; *Department of Food Science and Human Nutrition, University of Illinois, Urbana, IL; †Beltsville Human Nutrition Research Center, ARS, USDA, East Beltsville, MD; and **Children's Hospital Oakland Research Institute, Oakland, CA*

ABSTRACT Multiple components of broccoli, such as sulforaphane (Sf) and phenolic acids, may inhibit cancer. Additionally, broccoli can accumulate selenium (Se), and Se has been demonstrated to reduce the risk of cancer. Studies were conducted to determine whether enhancement of broccoli with Se would produce a plant with superior health benefits. Although increasing the concentration of Se in broccoli from <1.0 to >800 μg/g resulted in inhibition of colon cancer in rats, it also decreased the Sf content by >80% and inhibited production of most phenolic acids. The inclusion of Se-enriched broccoli in the diet of rats induced the activity of the selenoprotein thioredoxin reductase beyond the maximum activity induced by Se alone. These results emphasize the complex interactions of bioactive chemicals in a food; attempts to maximize one component may affect accumulation of another, and consumption of high amounts of multiple bioactive compounds may result in unexpected metabolic interactions within the body. J. Nutr. 135: 1236–1238, 2005.

KEY WORDS: • *selenium* • *broccoli* • *sulforaphane*

Synergism between bioactive components of a plant may result in unexpected metabolic outcomes within the plant and within an animal that consumes it. The success of functional foods in the marketplace has led to intense interest in the discovery and the characterization of plant-based bioactive compounds. Subsequent action often is directed toward maximization of the concentration or bioactivity of the putative health-promoting compound, thus producing a food with inherent health-related "functional" characteristics. Broccoli enriched in selenium (Se) may have functional activity for reduction of cancer, but a series of studies have demonstrated that Se enhancement of broccoli affects the accumulation of the other bioactive components, especially glucosinolates and phenolic acids. Additionally, consumption of Se-enriched broccoli by an animal induces a metabolic interaction that may or may not be beneficial to the animal.

Supplemental Se (200 μg/d) has been demonstrated to reduce cancer risk and mortality in humans (1). Foods that contain Se as a functional ingredient must be enriched naturally, because Se is not on the FDA's GRAS (generally recognized as safe) list and therefore cannot be added to food products. Consequently, dietary supplementation of Se requires enriched food sources, but the Se content of most plants is variable and reflective of the amount available from the soil. Broccoli, however, will actively accumulate Se well beyond the concentration in the soil (up to 10,000 times the concentration in normal broccoli); therefore it may be possible to alter production conditions and to produce a functional broccoli that is a reliable source of supplemental Se. Initial investigations with Se-enriched broccoli produced in the laboratory have demonstrated it to be more effective than selenite, selenate, or broccoli alone for reduction of chemically induced aberrant crypts (an experimental model of colon cancer susceptibility) in rats (2).

Epidemiologic studies indicate that consumption of cruciferous vegetables, including broccoli, is more strongly associated with decreased cancer risk than fruit and vegetable consumption in general. Specifically, broccoli consumption has

[1] Presented as part of the symposium "Relative Bioactivity of Functional Foods and Related Dietary Supplements" given at the 2004 Experimental Biology meeting on April 17, 2004, Washington, DC. The symposium was sponsored by the American Society for Nutritional Sciences and in part by the USDA. The proceedings are published as a supplement to *The Journal of Nutrition*. This supplement is the responsibility of the Guest Editors to whom the Editor of *The Journal of Nutrition* has delegated supervision of both technical conformity to the published regulations of *The Journal of Nutrition* and general oversight of the scientific merit of each article. The opinions expressed in this publication are those of the authors and are not attributable to the sponsors or the publisher, editor, or editorial board of *The Journal of Nutrition*. The Guest Editor for the symposium publication is Elizabeth Jeffery, Department of Food Science and Human Nutrition, University of Illinois at Champaign-Urbana, Urbana, IL.

[2] Research was partially funded by USDA-IFAFS Grant No. 00–52102-9636.

[3] The USDA, Agricultural Research Service, Northern Plains Area, is an equal opportunity/affirmative action employer and all agency services are available without discrimination. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be suitable.

[4] To whom correspondence should be addressed.
E-mail: jfinley@gfhnrc.ars.usda.gov.

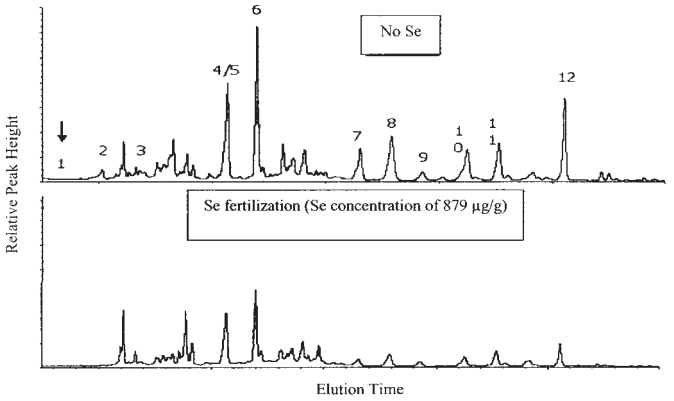

FIGURE 1 Selenium fertilization decreases production of phenolic acids in broccoli as determined by HPLC electrospray mass spectrometry. Peaks 1–3 are chlorogenic acid derivatives; peaks 4–6 are glycosylated flavonoids (quercitin and kaemferol); peaks 7–12 are hydroxycinnamic esters. Data from ref. (5).

been associated with decreased cancer risk in a majority of case-control and cohort studies [for review see (3)]. One mechanism of cancer reduction by broccoli may be because it is a rich dietary source of multiple bioactive secondary plant compounds, such as glucosinolates and phenolic acids. Glucosinolates, precursors of potent phase II enzyme inducers, e.g., sulforaphane (Sf),[5] protect against cancer by inducing proteins that eliminate reactive intermediates and oxidative stressors. Phenolic acids are abundant in broccoli, and their strong antioxidant function may likewise protect against cancer (4).

Interaction of Sf, phenolics, and Se in broccoli

A series of studies examined how Se enhancement of broccoli affects plant physiology and the physiology of the animals that consume broccoli in the diet. Majestic variety broccoli was grown in a manner that allowed accumulation of <1 or >800 μg Se per gram dry tissue. Mature broccoli florets were lyophilized and extracted for phenolic acid analysis by HPLC-electrospray MS or glucosinolate analysis by HPLC. Lyophilized broccoli was included in animal diets, and aqueous extracts of broccoli were used in studies with cultured cells.

Although Se fertilization did not have much effect on the relative distribution of specific phenolic acids, it greatly decreased total phenolic acid production (**Fig 1**). Comparison of individual compounds revealed that 11 of 12 specific phenolic acids were decreased by Se fertilization; quantitative determination of liberated caffeic, ferulic, and sinapic acids confirmed the decrease (5). Se fertilization also resulted in a modest decrease in indole, aliphatic, and total glucosinolates and glucoraphanin (data not shown), but, similar to phenolic acids, Se fertilization greatly depressed Sf production. The Sf content of extracts made from broccoli with a Se concentration of >800 μg/g was only 15% of extracts from broccoli not fertilized with Se (239 ± 2 μmol/L and 35 ± 1 μmol/L for high and low Se broccoli extracts, respectively) (5).

Dietary interaction of Se and Sf

An initial study in which rats were fed broccoli and Sf, but no added Se beyond the dietary requirement, found elevated activity of thioredoxin reductase (TR), an important selenoprotein that reduces thioredoxin and has antioxidant activity (6). This was not a consequence of systemic upregulation of selenoproteins, because activity of glutathione peroxidase, a ubiquitous selenoprotein that reduces hydroperoxides, was decreased by the same treatment. A subsequent experiment in Hepa1c1c7 cells demonstrated that TR activity was synergistically increased by simultaneous addition of Se and Sf to the diet (6).

Initial studies of the mechanism of cancer reduction by Se and Sf focused on different events. Favored theories for the mode of action of Se included inducement of cell-cycle arrest, increased apoptosis, and inhibition of angiogenesis [for review see (7)]. Se availability controls selenoprotein production (8); but, because activity is maximized at dietary Se concentrations below those needed for a reduction in carcinogenesis, selenoproteins have not been thought to have a major role in cancer reduction. Conversely, Sf-mediated cancer reduction may be through increased (phase II) enzyme activity (3).

The Se-mediated regulation of selenoprotein production is through a translational mechanism (8), but emerging information suggests that Sf regulates phase II protein production by transcriptional control. Sf induces the release of the transcription factor nrf2, which translocates to the nucleus and binds to a regulatory element [the antioxidant responsive element (ARE)] in the promoter region of many phase II genes (9). Examination of the TR-promoter sequence (accession number AF 247671) revealed two possible ARE on the coding and noncoding strand. The putative coding strand ARE, 5′-TGACAAAGC-3′ contained the consensus sequence (5′-TGACnnnGC-3′) and the core sequence (5′-TGAC-3′) most important for transcription factor binding (10). To further investigate the TR ARE, a luciferase reporter gene construct was made from the entire promoter region of TR and transfected into Hep-G2 cells. Sf resulted in a dose-dependent induction of constructs, with maximal induction of ~3-fold basal activity, whereas Se and Sf synergistically upregulated TR activity (**Fig. 2**). Mutation of this putative ARE ablated Sf-mediated induction (10).

Implications of synergistic interactions of Se, Sf, and phenolic acids

The analysis of Se, Sf, and phenolic acids in broccoli demonstrate that it may not be advantageous or even possible to maximize multiple bioactive components of a single plant. There is a growing awareness that a plethora of genetic and

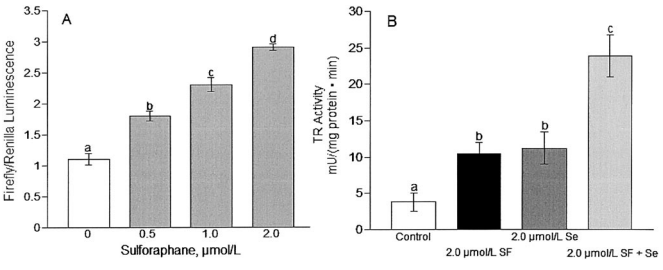

FIGURE 2 Sulforaphane (Sf) upregulates reporter gene constructs of human thioredoxin reductase promoter/firefly luciferase in HepG2 cells (*A*), and Se and Sf synergistically upregulate TR activity in Hep-G2 cells (*B*). Data are means ± SD; $n = 3$ culture dishes per treatment for each experiment (each experiment was replicated twice). Significant effect of Sf for luminescence reporter gene constructs ($P = 0.0001$); data from, and significant interaction of Sf and Se ($P = 0.036$) for activity; means with different letters differ ($P < 0.05$). Data from ref. (6).

[5] Abbreviations used: ARE, antioxidant responsive element; Sf, sulforaphane; TR, thioredoxin reductase.

environmental fluctuations alter the distribution, speciation, and concentration of bioactive components in a plant [for review see (11)]. These data further demonstrate that previously uncharacterized interactions may cause one component to interfere with the production of the other. The interference with phenolic acid production is unreported, and unexpected, especially given that phenolic acids have been reported to be induced by stress (12). Because Se and sulfur follow similar metabolic routes, the negative interaction between Sf and Se may be related to interference with sulfur assimilation pathways.

The dietary interactions of Se and Sf on TR were unexpected but are explained by the discovery of the ARE. Se control of selenoprotein expression by a translational mechanism (8) is consistent with no observed effect of Se on TR-promoter reporter gene constructs. However, Sf acts transcriptionally, as demonstrated by the upregulation of promoter constructs. The combination of Se and Sf allows simultaneous activation of transcriptional and translational controls, resulting in the observed synergistic upregulation of TR activity.

The functional consequences of the Se and Sf dietary interaction are presently unknown. Initial studies indicate that TR activity is strongly associated with protection against DNA strand breaks (Finley, unpublished results), implying that synergism may lead to enhanced protection against oxidative stress. However, there is no consensus as to whether minimizing oxidative stress is always physiologically beneficial to the cell. Also, there is conflicting evidence regarding the role of TR in carcinogenesis, because some studies have indicated that increased TR activity is associated with greater cancer risk (13). Efforts to maximize the activity of TR should proceed with caution until these questions are answered.

Implications for future research

These studies with Se-enhanced broccoli demonstrate important pitfalls and opportunities for development of functional foods. The studies of accumulation of Se, glucosinolates, and phenolic acids in broccoli demonstrate that it may not be possible to maximize the concentration of multiple bioactive ingredients in a single food; selection for one compound may induce a concomitant decrease in other seemingly unrelated compounds. These studies also illustrate the need for careful experimentation to determine whether positive or negative interactions occur. The dietary studies with Sf and Se show that synergism between bioactive components may result in unintended biological interactions in the animal that consumes the food. Also, it is important to understand the functional consequences of altered metabolic processes; simplistic assumptions, e.g., "decreased oxidative stress is always beneficial," may not be true and may, in some situations, actually result in harm. Ignoring possible synergistic interactions in foods may result in missed opportunities for targeting health outcomes in some cases and may result in potential harm to the individual in other cases.

ACKNOWLEDGMENTS

The author would like to thank Brian Gregoire for laboratory analyses, Denice Schafer for animal care, Mary Briske-Anderson for cell-culture assistance, and LuAnn Johnson for statistical assistance.

LITERATURE CITED

1. Clark, J., Combs, G., Turnbull, B., Slate, E., Chalker, D., Chow, J., Davis, L., Glover, R., Graham, G. et al. (1996) Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. J. Am. Med. Assoc. 276: 1957–1963.

2. Finley, J. W., Davis, C. & Feng, Y. (2000) Selenium from high-selenium broccoli protects rats from colon cancer. J. Nutr. 130: 2384–2389.

3. Keck, A. S. & Finley, J. W. (2004) Cruciferous vegetables: cancer protective mechanisms of glucosinolate hydrolysis products and selenium. Integr. Cancer Ther. 3: 5–12.

4. Proteggente, A. R., Pannala, A. S., Paganga, G., Van Buren, L., Wagner, E., Wiseman, S., Van De, P. F., Dacombe, C. & Rice-Evans, C. A. (2002) The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. Free Radic. Res. 36: 217–233.

5. Robbins, R., Keck, A. S., Banuelos, G. & Finley, J. (2004) Cultivation conditions and selenium fertilization alter the phenolic profile glucosinolate and sulforaphane content of broccoli. J. Med. Foods (in press).

6. Hintze, K. J., Keck, A. S., Finley, J. W. & Jeffery, E. H. (2003) Induction of hepatic thioredoxin reductase activity by sulforaphane, both in Hepa1c1c7 cells and in male Fisher 344 rats. J. Nutr. Biochem. 14: 173–179.

7. Combs, G. F., Jr. (1999) Chemopreventive mechanisms of selenium. Med. Klin. (Munich) 94 (suppl. 3): 18–24.

8. Driscoll, D. M. & Copeland, P. R. (2003) Mechanism and regulation of selenoprotein synthesis. Annu. Rev. Nutr. 23: 17–40.

9. Finley, J. W. (2003) The antioxidant responsive element (ARE) may explain the protective effects of cruciferous vegetables on cancer. Nutr. Rev. 61: 250–254.

10. Hintze, K. J., Wald, K. A., Zeng, H., Jeffery, E. H. & Finley, J. W. (2003) Thioredoxin reductase in human hepatoma cells is transcriptionally regulated by sulforaphane and other electrophiles via an antioxidant response element. J. Nutr. 133: 2721–2727.

11. Dekker, M. & Verkerk, R. (2003) Dealing with variability in food production chains: a tool to enhance the sensitivity of epidemiological studies on phytochemicals. Eur. J. Nutr. 42: 67–72.

12. Newman, M. A., Roepenack-Lahaye, E., Parr, A., Daniels, M. J. & Dow, J. M. (2001) Induction of hydroxycinnamoyl-tyramine conjugates in pepper by *Xanthomonas campestris,* a plant defense response activated by hrp gene-dependent and hrp gene-independent mechanisms. Mol. Plant Microbe Interact. 14: 785–792.

13. Mustacich, D. & Powis, G. (2000) Thioredoxin reductase. Biochem. J. 346: 1–8.