Enhancement of Broccoli Indole Glucosinolates by Methyl Jasmonate Treatment and Effects on Prostate Carcinogenesis

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ABSTRACT Broccoli is rich in bioactive components, such as sulforaphane and indole-3-carbinol, which may impact cancer risk. The glucosinolate profile of broccoli can be manipulated through treatment with the plant stress hormone methyl jasmonate (MeJA). Our objective was to produce broccoli with enhanced levels of indole glucosinolates and determine its impact on prostate carcinogenesis. Brassica oleracea var. Green Magic was treated with a 250 $\mu$M MeJA solution 4 days prior to harvest. MeJA-treated broccoli had significantly increased levels of glucobrassicin, neoglucobrassicin, and gluconasturtiin ($P<.05$). Male transgenic adenocarcinoma of mouse prostate (TRAMP) mice ($n=99$) were randomized into three diet groups at 5–7 weeks of age: AIN-93G control, 10% standard broccoli powder, or 10% MeJA broccoli powder. Diets were fed throughout the study until termination at 20 weeks of age. Hepatic CYP1A was induced with MeJA broccoli powder feeding, indicating biological activity of the indole glucosinolates. Following ~15 weeks on diets, neither of the broccoli treatments significantly altered genitourinary tract weight, pathologic score, or metastasis incidence, indicating that broccoli powder at 10% of the diet was ineffective at reducing prostate carcinogenesis in the TRAMP model. Whereas broccoli powder feeding had no effect in this model of prostate cancer, our work demonstrates the feasibility of employing plant stress hormones exogenously to stimulate changes in phytochemical profiles, an approach that may be useful for optimizing bioactive component patterns in foods for chronic-disease-prevention studies.

KEY WORDS: • Brassica oleracea • indole-3-carbinol • indole glucosinolates • neoglucobrassicin • sulforaphane

INTRODUCTION

Epidemiological studies provide moderate support for the hypothesis that consumption of cruciferous vegetables reduces the risk of developing prostate cancer.1 broccoli is the most commonly consumed cruciferous vegetable in the United States with the average American eating 8.6 pounds (3.9 kg) of fresh broccoli and broccoli products each year.2 Broccoli contains many glucosinolates including glucoraphanin, which is hydrolyzed to sulforaphane and glucobrassicin, which is hydrolyzed to indole-3-carbinol (I3C), which both demonstrate anticancer activity in rodent models and in vitro.3,4 However, studying these compounds in isolation may obscure any inhibitory, additive, or synergistic actions or bioactive components that might take place when a whole vegetable is consumed. For example, neoglucobrassican, which is hydrolyzed to N-methoxy-I3C, has not previously been evaluated in an animal model of cancer and is reported to inhibit sulforaphane action in cell culture, but only when both compounds were at concentrations in excess of 5 $\mu$M.5

Glucosinolates are divided into three classes based on the amino acid precursors from which they originate. Aliphatic glucosinolates are derived from methionine, indole glucosinolates are from tryptophan, and aromatic glucosinolates are from phenylalanine. These phytochemicals are constitutively present in cruciferous plant tissues, but they can also be rapidly induced by stresses, such as wounding, pathogens, or herbivore attack. These stress conditions lead to the endogenous biosynthesis of jasmonates, which induce the synthesis of indole glucosinolates.6 External application of methyl jasmonate (MeJA), a volatile methylester of jasmonic acid, has been shown to induce production of indole glucosinolates in Brassica species.7–9 MeJA treatment offers a way of altering the phytochemical profile of broccoli to increase concentrations of potentially anticarcinogenic bioactives.
We were particularly interested in enhancing the indole glucosinolate content of whole broccoli because I3C and 3,3'-diindolylmethane (DIM) have been shown to reduce prostate carcinogenesis. Indole glucosinolates and their metabolites may impact the carcinogenesis process through multiple modes of action, including upregulation of cytochrome P450 1A and associated carcinogen-metabolizing enzymes, inhibition of proliferation, induction of apoptosis, and alterations in hormone metabolism.4 We chose to alter the phytochemical profile of whole broccoli because our lab had previously shown that feeding a diet containing 10% freeze-dried whole tomato or broccoli powder significantly reduced tumor growth in a rat transplantable tumor model of prostate cancer.10

Whereas previous studies using the transgenic adenocarcinoma of mouse prostate (TRAMP) model have demonstrated that pharmacologic levels of isolated broccoli bioactive compounds reduce prostate carcinogenesis, it is unknown whether dietary consumption of whole broccoli will alter this process.11-13 Here we examined the effects of standard broccoli and MeJA-treated broccoli on prostate carcinogenesis in TRAMP mice. We hypothesized that both broccoli treatments would reduce prostate carcinogenesis and that MeJA-treated broccoli would further reduce carcinogenesis compared with standard broccoli. To our knowledge, this is only the second study to examine the effects of whole broccoli in an animal model of prostate cancer; the first was our study utilizing the Dunning rat model.10

MATERIALS AND METHODS

Broccoli powder production

Broccoli (Brassica oleracea var. Green Magic) was grown on the University of Illinois campus in Urbana, Illinois, from May through August 2009. Broccoli plants were started and maintained in a commercial hot house for 3 weeks. Before transplanting to experimental fields, broccoli seedlings were hardened off outside for 1 week. To produce indole glucosinolate–enriched broccoli, treatment consisted of the plant hormone MeJA (250 µM) in a 0.1% Triton X-100 aqueous solution sprayed on individual plants 4 days prior to harvest.9 Plant surfaces were fully saturated with MeJA, allowed to dry, and then received an additional application of MeJA. Broccoli heads were collected at harvest maturity with some stem, frozen in liquid nitrogen, stored at −20°C, and then freeze-dried without being allowed to thaw. After freeze-drying, broccoli samples were ground into a fine powder using a commercial coffee grinder and stored at 4°C.

Glucosinolate and sulforaphane analysis

Intact glucosinolates were analyzed by the method described by Kim and Juvik.9 Sulforaphane formation from broccoli powders was determined as previously described.14

Experimental design

The University of Illinois Animal Care and Use Committee approved the animal protocol. Ninety-nine male TRAMP mice on a pure C57BL/6 background [C57BL/6-Tg(TRAMP)8247Ng/J; The Jackson Laboratory, Bar Harbor, ME, USA] were obtained at 5–7 weeks of age and individually housed in shoe-box cages under controlled conditions (12-h light–dark cycle, 22°C, 60% humidity). Mice were weighed weekly throughout the study. Mice were randomly assigned to three experimental groups (n = 33). AIN-93G-based experimental diets included AIN-93G control, 10% control broccoli powder, or 10% MeJA-treated broccoli powder. Diets were balanced for protein, fat, energy, and fiber. Diet formulations are shown in Table 1. Food was replaced and intake was measured every other day. Fresh diets were prepared monthly, tightly sealed in plastic bags, and stored in opaque waterproof plastic bins at 4°C. Mice were sacrificed at 20 weeks of age. At the conclusion of the study, mice were anesthetized with CO₂ and blood was collected by cardiac puncture. Following exsanguination, lungs, liver, and genitourinary tract were removed and weighed. The prostate was microdissected when possible and individual lobes were weighed and preserved in 10% formalin for histology. Lungs and sections of each liver lobe were also preserved in 10% formalin for histology.

Detoxification enzyme activity

Hepatic microsomal and cytosolic fractions were prepared as previously described.14 Activity of the phase I enzyme CYP1A was measured in the microsomal fraction as ethoxyresorufin O-deethylase (EROD) activity15 with slight modification.16 Activity of the cytosolic phase II enzyme NAD(P)H-quinone oxidoreductase 1 (NQO1) was measured according to the method of Prochaska and Santamaria17 with modification.16

Table 1. AIN-93G-Based Diet Formulations

<table>
<thead>
<tr>
<th>g/100 g total diet</th>
<th>Control</th>
<th>Broccoli (Control)</th>
<th>Broccoli (MeJA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornstarch</td>
<td>39.7</td>
<td>36.6</td>
<td>36.6</td>
</tr>
<tr>
<td>Casein</td>
<td>20.0</td>
<td>16.8</td>
<td>16.8</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>13.2</td>
<td>13.2</td>
<td>13.2</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5.0</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>7.0</td>
<td>6.7</td>
<td>6.7</td>
</tr>
<tr>
<td>Control broccoli powder</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>MeJA broccoli powder</td>
<td>10.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Diets were balanced for energy, protein, fat, and fiber.

*AIN93M-MX formulation.

*AIN93-VX formulation.

Ten grams of broccoli powder provides 3.4 g total dietary fiber, 3.1 g carbohydrate, 3.2 g protein, and 3.0 g fat (estimated from the USDA National Nutrient Database for Standard Reference).

MeJA, methyl jasmonate.
Tissue histology and immunohistochemistry

A section of each prostate lobe was preserved in 10% formalin for 24 h and then transferred to 70% ethanol. Formalin-fixed tissues were embedded in paraffin, sectioned, and transferred to slides. Four-micrometer sections for histological evaluation were stained with hematoxylin/eosin.

Pathologic evaluation was performed by a veterinary pathologist as previously described. Each of the four prostate lobes (dorsal, ventral, lateral, and anterior) of C57BL/6 TRAMP mice were assessed individually and assigned two grades. The first grade represented the most severe lesion within that lobe, and the second grade identified the most common lesion in the lobe. The distributions of each of these lesions within the individual lobe were determined and described as focal, multifocal, or diffuse. Adjusting the lesion grades (0–7) to include an indication of distribution (focal, multifocal, or diffuse) provided adjusted scores, ranging from 0 (normal) to 21 [diffuse, poorly differentiated (PD) carcinoma]. The adjusted lesion scores are as follows: 0, normal prostate; 1–3, low-grade prostatic intraepithelial neoplasia (PIN); 4–6, moderate-grade PIN; 7–9, high-grade PIN; 10–12, phyllodes-like tumor; 13–15, well-differentiated (WD) carcinoma; 16–18, moderately differentiated carcinoma; and 19–21, poorly differentiated carcinoma.

Four-micrometer sections were stained for proliferating cell nuclear antigen (PCNA) (Abcam, Cambridge, MA, USA) and cleaved caspase-3 (Cell Signaling Technology, Danvers, MA, USA). Images were captured and quantified as previously described with the researchers blinded to treatments. Two representative images without necrosis or artifacts were captured for the dorsal and lateral lobes of the prostate. For PCNA, a proliferative index percentage, (PCNA positive/total nuclei counted) × 100, was calculated. For cleaved caspase-3 the number of apoptotic nuclei was counted at 400× magnification.

Statistical analysis

Data were compared among treatments by two-tailed analysis of variance, using the Mixed Models procedure of SAS Statistical Software (version 9.2; SAS Institute, Cary, NC, USA). Values were considered different from controls at P < .05 using Tukey’s procedure.

RESULTS

Broccoli powder characterization

Broccoli powders were analyzed for glucosinolate content (Table 2). MeJA treatment significantly increased levels of indole glucosinolates, glucobrassicin, and neoglucobrassicin, and the aromatic glucosinolate gluconasturtiin (P < .05). Strikingly, neoglucobrassicin levels were over seven times higher in the MeJA broccoli (39.77 ± 0.67 μmol/g) than in the control broccoli (5.23 ± 0.67 μmol/g). This induction was approximately two times greater than levels previously observed with the same variety of broccoli. Levels of aliphatic glucosinolates, including glucoraphanin, were similar in both broccoli powders.

<table>
<thead>
<tr>
<th>Glucosinolate</th>
<th>Standard broccoli</th>
<th>MeJA broccoli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucobrassicin</td>
<td>2.61 ± 0.07</td>
<td>3.06 ± 0.07*</td>
</tr>
<tr>
<td>Neoglucobrassicin</td>
<td>5.23 ± 0.67</td>
<td>39.77 ± 0.67*</td>
</tr>
<tr>
<td>Gluconasturtiin</td>
<td>0.87 ± 0.19</td>
<td>3.79 ± 0.19*</td>
</tr>
<tr>
<td>Glucoraphanin</td>
<td>2.49 ± 0.59</td>
<td>3.64 ± 0.59</td>
</tr>
<tr>
<td>Glucopinan</td>
<td>0.50 ± 0.03</td>
<td>0.41 ± 0.03</td>
</tr>
<tr>
<td>Glucoiberin</td>
<td>0.32 ± 0.08</td>
<td>0.49 ± 0.08</td>
</tr>
<tr>
<td>Progoitrin</td>
<td>0.46 ± 0.07</td>
<td>0.49 ± 0.07</td>
</tr>
<tr>
<td>Sinigrin</td>
<td>0.24 ± 0.02</td>
<td>0.22 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE, n = 3. *Within rows it indicates significant differences between treatments (P < .05).

Diet and weight gain

Diets were well tolerated and there were no significant differences among groups in food intake or body weight. Two animals had to be put down prior to completion of the study for health reasons unrelated to cancer development. On average, mice consumed 4.9 ± 0.1 g per day. The estimated daily intakes of glucoraphanin, glucobrassicin, and neoglucobrassicin per mouse were 1.2, 1.3, and 2.6 μmol for standard broccoli, and 1.8, 1.5, and 19.9 μmol for MeJA broccoli, respectively.

Detoxification enzyme activity

Broccoli and its glucosinolate hydrolysis products have been shown to induce phase I and phase II detoxification enzymes. Glucobrassicin and neoglucobrassicin, as well as their respective hydrolysis products I3C and N-methoxy-I3C, are known to induce CYP1A. As expected, we observed a dose-dependent increase in CYP1A activity with increasing levels of indole glucosinolates in broccoli (Fig. 1A). MeJA broccoli feeding resulted in hepatic EROD activity 2.5 times greater than the control group (P < .05). Standard broccoli also significantly increased hepatic EROD activity but to a lesser magnitude, 1.5 times greater than control (P < .05). The induction of EROD activity with MeJA broccoli feeding indicates biological activity of indole glucosinolates in the liver. Hepatic NQO1 activity did not change with either of the broccoli treatments (Fig. 1B).

Tissue weights

Liver and genitourinary tract weights were not different among groups. Mean genitourinary tract weights were 1.00 ± 0.06, 1.25 ± 0.12, and 1.25 ± 0.12 g for control, standard broccoli, and MeJA broccoli groups, respectively.

Proliferation and apoptosis

There were no significant differences among groups in prostatic proliferation or apoptosis as measured via PCNA and cleaved caspase-3 (data not shown). Our values are within the normal range for proliferative index and apoptotic index values observed in TRAMP mice.
Histology

Prostate cancer progression varied by lobe as expected. The lateral prostate had mostly diffuse WD carcinomas while the dorsal prostate had predominantly diffuse high-grade PIN. Within each prostate lobe, no significant differences in adjusted lesion score were observed for either the most severe lesion present or the most common lesion present, indicating that broccoli feeding did not impact prostate cancer progression (Tables 3 and 4).

Within each prostate lobe, no significant differences in adjusted lesion score were observed for either the most severe lesion present or the most common lesion present, indicating that broccoli feeding did not impact prostate cancer progression (Tables 3 and 4). As expected for TRAMP mice of this age, few animals developed metastases. Three mice (3% of all mice) developed liver metastases, one from each diet group. Fourteen mice (14% of all mice) developed microscopic lung metastases: 3 control, 7 standard broccoli, and 4 MeJA broccoli.

DISCUSSION

We succeeded in altering the phytochemical profile of broccoli through MeJA treatment, which produced broccoli with substantially higher levels of indole glucosinolates, modestly increased levels of aromatic glucosinolates, and with relatively unchanged levels of aliphatic glucosinolates. This phytochemical profile differs both from regular broccoli and from broccoli sprouts, which have very high levels of glucoraphanin and minor amounts of other glucosinolates.

We observed a significant increase in hepatic CYP1A activity, indicating biological activity of indole glucosinolates. Surprisingly, neither of the broccoli treatments increased NQO1 activity. Sulforaphane is known to induce NQO1 activity in short-term studies, including work by our group with diets containing 10% or 20% broccoli powder fed for 5–7 days. The lack of induction observed in the present study may indicate an adaptation to chronic feeding. Previous work from our lab found no alterations in hepatic NQO1 activity after male Copenhagen rats were fed a 10% broccoli powder diet for 22 weeks (unpublished data), suggesting that NQO1 may not remain upregulated during long-term feeding.

An alternate possibility is that high levels of neoglucobrassicin may actually inhibit Nrf2 action and suppress induction of phase II enzymes. Work by Haack et al. demonstrated that 7.5 μM of neoglucobrassicin hydrolyzed in situ inhibited sulforaphane’s induction of Nrf2 target genes NQO1 and glutathione peroxidase 2 in HepG2 cells. It is possible that while MeJA treatment enhanced levels of total glucosinolates, the specific increase in neoglucobrassin levels may have abrogated sulforaphanes’s effects. However, to date this inhibitory effect has not been evaluated in vivo.

Our study was limited in that we did not measure serum or tissue levels of indole glucosinolate metabolites such as I3C. However, previous pharmacokinetic studies suggest that these metabolites would likely not be detectable in our study samples since I3C is rapidly degraded in the body and falls below detectable levels within 1 h after oral administration. The dose provided here by a 10% MeJA broccoli diet is ~1000-fold lower than the bolus dose given in pharmacokinetic studies and would likely not be detectable.

**Table 3. Adjusted Lesion Scores of the Most Severe Lesion Present in Prostate Lobes**

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Ventral</th>
<th>Lateral</th>
<th>Dorsal</th>
<th>Anterior</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.76±1.04</td>
<td>14.56±0.45</td>
<td>9.18±0.66</td>
<td>8.22±0.31</td>
</tr>
<tr>
<td>Standard broccoli</td>
<td>8.70±1.04</td>
<td>15.73±0.44</td>
<td>11.45±0.66</td>
<td>8.54±0.30</td>
</tr>
<tr>
<td>MeJA broccoli</td>
<td>7.29±1.07</td>
<td>15.00±0.45</td>
<td>10.69±0.67</td>
<td>7.75±0.31</td>
</tr>
</tbody>
</table>

Values are means±SE, n=31–33. Scale ranges from 0 (normal prostate tissue) to 21 (diffuse PD carcinoma).
particular since mice were fed *ad libitum* rather than given a bolus gavage of pure compound.

Our results indicate that feeding diets containing 10% broccoli powder did not alter tumor growth or progression of prostate carcinogenesis, which was surprising given that several articles have shown that individual phytochemicals from broccoli reduced prostate carcinogenesis.  

Consumption of MeJA broccoli resulted in potent induction of CYP1A in the liver; however, consumption of both standard broccoli and MeJA-treated broccoli failed to reduce prostate carcinogenesis. This is in contrast to previous studies that demonstrate that I3C and DIM (both breakdown products of the indole glucosinolate glucobrassicin and known CYP1A inducers) can reduce prostate carcinogenesis \(^{11,13,23}\) (Table 5). For example, a diet containing 1% I3C significantly reduced incidence of WD carcinoma, indicating a slowing of lymph node metastases. Our work demonstrates the feasibility of consuming MeJA in the diet, but the quantity or profile of indole glucosinolates was much higher in other studies showing reduction of prostate carcinogenesis \(^{12,23,24}\) (Table 5). Clearly more work is needed to determine the optimal dose and profile of broccoli bioactives that are necessary for prostate cancer prevention. These studies should also evaluate dose response, phytochemical bioavailability, and tissue distribution of metabolites. Our work demonstrates the feasibility of using MeJA as a bolus gavage of pure compound.

Additionally several other studies that treated TRAMP mice with individual phytochemicals found in broccoli or broccoli sprouts have also observed reductions in prostate carcinogenesis \(^{12,23,24}\) (Table 5 and personal communication with Dr. Emily Ho, Oregon State University, Corvallis, OR, USA, April 16, 2011). Reduction in the incidence of PIN and WD carcinomas was observed when TRAMP mice were gavaged with 6 μmol sulforaphane every other day. Further, there was a decreased incidence of PD cancers when TRAMP mice received ~15 μmol of phenethyl isothiocyanate per day in their diet. These effective outcomes, compared with the present study, suggest that the effects of a complex mixture of glucosinolates within whole broccoli may be quite different from the effects of pharmacological doses of a pure compound such as I3C.

Our study was limited in that we did not have a positive control such as pure sulforaphane or I3C that might have demonstrated efficacy of the model. However, the TRAMP model is known to be a robust model of prostate cancer that has been successfully used for several dietary intervention studies. The progression of cancer observed in our control mice mirrored the established progression course that has been well documented in this strain. We have no reason to believe that the model was ineffective and conclude that there was no effect of broccoli treatment on this model of prostate carcinogenesis.

In summary, neither of the broccoli treatments significantly reduced prostate carcinogenesis in TRAMP mice. Apparently, the quantity or profile of indole glucosinolates contributed by the 10% broccoli powders in this study was not sufficient to reduce prostate carcinogenesis in the TRAMP model. Quantities of broccoli bioactive compounds were much higher in other studies showing reduction of carcinogenesis in this model (Table 5). Clearly more work is necessary to determine the optimal dose and profile of broccoli bioactives that are necessary for prostate cancer prevention. These studies should also evaluate dose response, phytochemical bioavailability, and tissue distribution of metabolites. Our work demonstrates the feasibility of using broccoli bioactives as a bolus gavage of pure compound. 

### Table 5. Summary of Studies with Broccoli or Broccoli Compounds in the Transgenic Adenocarcinoma of Mouse Prostate Model

<table>
<thead>
<tr>
<th>Study</th>
<th>Compound–delivery route</th>
<th>Estimated cumulative bioactive exposure (^{a})</th>
<th>Background strain</th>
<th>Estimated age at sacrifice</th>
<th>Serum biomarker</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liu (current study)</td>
<td>Broccoli: 10% powder in diet</td>
<td>80 μmol (estimated daily intake)</td>
<td>C57BL/6</td>
<td>13–15 weeks</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Keum et al. (^{24})</td>
<td>Broccoli sprout: 8% powder in diet</td>
<td>210 μmol I3C/day</td>
<td>C57BL/6</td>
<td>16 weeks</td>
<td>No effect</td>
<td>Decreased GU tract weight</td>
</tr>
<tr>
<td>Ho (unpublished)</td>
<td>Broccoli sprout: 15% powder in diet</td>
<td>633 μmol DIM</td>
<td>C57BL/6</td>
<td>12 and 26 weeks</td>
<td>No effect</td>
<td>Increased PIN at 28 weeks</td>
</tr>
<tr>
<td>Wu et al. (^{13,23})</td>
<td>Pure compound</td>
<td>38.6 μmol/IC (estimated daily dose)</td>
<td>C57BL/6</td>
<td>24 weeks</td>
<td>C57BL/6 (11.3 μmol I3C/day)</td>
<td>Decreased WD carcinoma, increased PIN</td>
</tr>
<tr>
<td>Wu et al. (^{13,23})</td>
<td>Pure compound</td>
<td>38.6 μmol/IC (estimated daily dose)</td>
<td>C57BL/6</td>
<td>24 weeks</td>
<td>C57BL/6 (11.3 μmol I3C/day)</td>
<td>Decreased WD carcinoma, increased pulmonary metastases</td>
</tr>
<tr>
<td>Singh et al. (^{13})</td>
<td>Sulforaphane: 6 μmol gavage every other day</td>
<td>306–324 μmol SF/day</td>
<td>C57BL/6</td>
<td>17–19 weeks</td>
<td>C57BL/6 (11.3 μmol I3C/day)</td>
<td>Decreased PIN, WD carcinoma and pulmonary metastases</td>
</tr>
</tbody>
</table>

\(^{a}\) If applicable, based on estimated average food intake of 4.9 g to yield 0.03 μmol/mouse/day.  

\(^{b}\) Based on daily dose \(
\times\) number of days treated per week \(
\times\) number of weeks that mice received treatment.
employing plant stress hormones exogenously to stimulate changes in phytochemical profiles, an approach that may be useful for optimizing bioactive component patterns in foods for chronic-disease-prevention studies.

**ACKNOWLEDGMENTS**

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**AUTHOR DISCLOSURE STATEMENT**

No competing financial interests exist.

**REFERENCES**