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ARTICLE

Feeding Tomato and Broccoli Powders Enriched with Bioactives Improves Bioactivity Markers in Rats

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Many studies have evaluated the cancer -preventive potential of individual bioactives from tomatoes and broccoli, but few have examined them within the context of a whole food. Male Copenhagen rats were fed diets containing 10% standard tomato powder, tomato enriched with lycopene or total carotenoids, standard broccoli floret, broccoli sprouts, or broccoli enriched with indole glucosinolates or selenium for 7 days. All broccoli diets increased the activity of colon quinone reductase (NQO1). Indole glucosinolate-enriched broccoli and selenium-enriched broccoli increased hepatic NQO1 and cytochrome P450 1A activity ($P < 0.05$). Standard broccoli and lycopene-enriched tomato diets down-regulated prostatic glutathione *S*-transferase P1 mRNA expression. Different tomato diets resulted in altered hepatic accumulation of lycopene, phytofluene, and phytoene. These results demonstrate that the bioactive content of vegetables affects both tissue content of bioactives and activity of detoxification enzymes. Enhancing bioactive content of tomatoes and broccoli may enhance efficacy in the prevention of prostate cancer.

KEYWORDS: Tomato; broccoli; lycopene; sulforaphane; indole-3-carbinol; selenium; detoxification enzymes; cancer

INTRODUCTION

The health benefits of functional foods are often associated with their bioactive components, which are believed to deliver a health benefit beyond basic nutrition. For instance, lycopene in tomatoes may help maintain prostate health, and sulforaphane (SF), a hydrolysis product of glucoraphanin from broccoli, may enhance detoxification of carcinogens. Much research has examined the putative health benefits of individual, isolated bioactive compounds. However, in many in vitro studies these bioactive compounds are provided at levels tens to hundreds of times greater than can be physiologically achieved through incorporation of whole foods into the diet. Furthermore, these compounds are typically tested as the aglycone form in cell culture, although they are present as glycosides in plant foods and conjugates in the body following absorption. Therefore, it is also important to consider the impact of chemical form on bioavailability and bioactivity during dietary interventions in vivo. Once the efficacy of functional food components is verified, enhancing the concentrations of health-promoting compounds in foods may be beneficial in preventing and treating chronic diseases such as cancer.

In 1981, Doll and Peto estimated that approximately 35% of human cancer deaths could be prevented through dietary modifications (*1*). This led to an extensive study of fruit and vegetable intake as a modifiable risk factor. Epidemiological studies have linked higher consumption of tomatoes and cruciferous vegetables with a decreased risk of prostate cancer (*2*,*3*). The efficacy of these vegetables is often associated with the anticancer actions of individual bioactives such as lycopene, SF, indole-3-carbinol (I3C, hydrolysis product of the indole glucosinolate glucobrassicin), and selenium. Although these bioactives have been shown to have individual anticancer effects, they may also act additively or synergistically with the many other vitamins, minerals, and bioactive phytochemicals in tomatoes and broccoli to reduce tumor growth.

A few previous studies have examined the effects of increasing the bioactive content of tomatoes and broccoli on the reduction of cancer growth. For reducing the formation of aberrant crypt foci in a chemically induced rat model of colon cancer, selenium-enriched broccoli was shown to be more effective than high doses of selenite alone or a combination of selenite and broccoli sprout powder low in selenium (*4*). "Super broccoli" has been specifically bred to have high glucoraphanin levels (10.6 compared to 4.4 μmol/g of dry weight in store-bought broccoli), and it enhances transforming growth factor β1 signaling, which is important for controlling cell proliferation (*5*). Another study found that an anthocyanin-enriched tomato was more effective than regular red tomato in increasing the survival time of Trp53−/− mice, which predominantly develop lymphomas (*6*). These studies provide encouraging evidence that enrichment of tomatoes and/or broccoli with bioactives may be beneficial in reducing cancer growth.

Dietary interventions may play an important role in delaying prostate cancer growth, especially because it is a slow-growing cancer that takes decades to develop. Optimizing the content of bioactive components within tomatoes and broccoli may provide an important boost to the effectiveness of these vegetables,

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especially considering that less than half of the U.S. population meets recommended intake levels for fruits and vegetables (*7*).

The purpose of this study was to evaluate the effects of preharvest enrichment of tomato and broccoli with different bioactives on tissue accumulation and bioactivity markers in male Copenhagen rats in a 7 day screening trial for a future prostate cancer study. Different tomato and broccoli powders were produced agronomically and profiled for their bioactive content, including levels of carotenoids, glucosinolates, isothiocyanates, and selenium. In vivo exposure to bioactives was assessed by measurement of tissue carotenoid content and urinary SF metabolites. Our primary measure of bioactivity was phase I and phase II detoxification enzyme activity. We chose the phase I enzyme ethoxyresorufin *O*-deethylase (EROD) as reflective of any up-regulation of cytochrome P450 1A and NAD(P)H-quinone oxidoreductase 1 (NQO1) as reflective of any Nrf2-dependent up-regulation of phase II enzymes (*8*). We also measured the phase II enzyme glutathione *S*-transferase P1 (GSTP1), which is associated with the appearance of preneoplastic foci and has previously not always responded in concert with other Nrf2-dependent phase II enzymes (*9*). Markers of androgen metabolism were also assessed because androgens play a key role in the development and progression of prostate cancer.

MATERIALS AND METHODS

Tomato and Broccoli Powders. Standard tomato powder, lycopene-enriched tomato powder, standard broccoli floret powder (referred to as "standard broccoli"), and broccoli sprout powder were obtained from FutureCeuticals (Momence, IL). Tomato powders were produced by grinding and pureeing the tomatoes, drum drying, and grinding the powder through a USA no. 20 screen (equivalent to particle size of 0.85 mm). To produce the broccoli floret powder, broccoli (*Brassica oleracea* var. Monaco) was cut and diced to produce a floret, blanched, freeze-dried, and ground through a USA no. 20 screen. For the broccoli sprout powder, 3-day-old broccoli sprouts (*B. oleracea* var. Calabrese) were blanched, macerated, and ground, drum dried, and ground through a USA no. 20 screen. In addition, carotenoid-enriched tomatoes, indole glucosinolate-enriched broccoli, and selenium-enriched broccoli were grown by Dr. Gary Bañuelos at the USDA-ARS research facility in Parlier, CA, from March to July 2007. For the California-grown crops, normal agronomic management practices were applied on the test plots including preplant application of fertilizer, insect and animal control, and hand weeding. Broccoli (*B. oleracea* var. Magestic) and tomatoes (*Lycopersicon esculentum* var. APT 410) were started and maintained in a commercial hot house. Six to eight weeks later, tomato and broccoli seedlings were transplanted into experimental beds. To produce indole glucosinolate-enriched broccoli, treatment consisted of the plant hormone methyl jasmonate (1 mg/mL solution) in a 2% ethanol/0.1% Triton X-100 aqueous solution sprayed on individual plants 7 days prior to harvest. To produce selenium-enriched broccoli, selenium was applied as a sodium selenate solution of 10 mg of Se/L to the soil beneath each plant every 2 days beginning at day 45 after transplanting. Both broccoli, harvested 2 in. below the floret, and tomatoes, selected for bright red fruit, were dehydrated at a commercial dehydrator, where they were blanched for 4−5 min, cut, and dried at 110 °C for 24 h to a moisture level of 5%. The product was then shipped to the University of Illinois in dry, opaque plastic bags. Upon receipt, the dried vegetables were stored at 4 °C and then processed into a powder using a hammermill (model 10; C. S. Bell Co., Tiffin, OH).

Glucosinolate Analysis. Intact glucosinolates were analyzed by using modification of the method of Kushad et al. (*10*). Freeze-dried broccoli powder (0.2 g) was placed in an Oak Ridge tube (Nalgene, Rochester, NY) and heated on a heating block at 95 °C for 10 min. To each tube was added 2 mL of boiling 70% methanol and heated for an extra 10 min. After cooling on ice, 0.5 mL of benzylglucosinolate (1 mM) was added, mixed, and centrifuged at 12000*g* for 15 min at 4 °C. The supernatant (extract) was saved, and the pellet was re-extracted with 2 mL of 70% methanol and the extracts were combined. A sample (1 mL) from each pooled extract was transferred into a glass tube. Protein was precipitated with 0.15 mL of a 1:1 mixture of 1 M lead acetate and 1 M barium acetate. Each sample was then loaded onto a DEAE Sephadex A-25 column for desulfation with arylsulfatase for 18 h. Desulfated glucosinolates were eluted from the column with water and injected onto a Lichosphere RP-18 column (Alltech Inc., Springfield, KY). Desulfoglucosinolates were eluted using a linear gradient of 1−40% acetonitrile in water at a flow rate of 1 mL/min over 40 min. Benzylglucosinolate (C2 Bioengineering Co., Denmark) was used as an internal standard.

Isothiocyanate Extraction and Analysis. Broccoli powders were suspended in water (50 mg/mL) for 24 h at room temperature away from light to facilitate glucosinolate hydrolysis. Slurries were centrifuged at 16000*g* for 10 min, and supernatants were filtered through 0.45 μm nylon membranes. Benzyl isothiocyanate (0.1 mg/mL DMSO) or 4-methoxyindole (0.1 mg/mL DMSO) was used as an internal standard to a final concentration of 2 μg/mL for analysis of SF or I3C, respectively. Each supernatant was extracted with an equal volume of dichloromethane. One volume of DMSO was added to 5 volumes of dichloromethane extract, which was removed under a stream of nitrogen gas, leaving the sample in DMSO.

SF and I3C were analyzed by reverse-phase HPLC with UV detection at wavelengths of 254 and 280 nm, respectively (Waters, Milford, MA). Fifty microliters of the extract in DMSO sample was injected onto a Luna reverse-phase C18 column (5 μm, 250 × 4.6 mm, Phenomenex, Torrance, CA). At a flow rate of 1 mL/min, 20% acetonitrile in water was increased linearly to 100%, over 15 min. The mobile phase was then maintained for 5 min and returned to initial conditions, at which the column was re-equilibrated for 10 min. Solvents contained 1% (v/v) glacial acetic acid. Using Empower Pro software (Waters, Milford, MA), quantification was performed by comparison to SF and I3C standards (LKT Laboratories, St. Paul, MN).

Selenium Analysis. Selenium content of vegetable powders was measured by instrumental neutron activation analysis at the University of Missouri-Columbia Research Reactor Center by inducing Se-77m and measuring photon emissions associated with its decay. A standard reference material (SRM 1577, Bovine Liver), certified for selenium at 1.1 ± 0.1 ppm and supplied by the National Institutes for Standards and Technology, was analyzed in replicate as a quality control material.

Carotenoid Extraction and Quantification. Carotenoid extraction and analysis were performed as previously described (*11*). Briefly, 0.1 g of powder or 0.1 g of tissue was combined with 6 mL of a KOH/ethanol solution (1:5) containing 0.1% BHT. Samples were saponified at 60 °C for 30 min. Samples were then placed on ice, and deionized water was added. Powder and tissue carotenoids were extracted three times with the addition of 6 mL of hexane. Hexane extracts were dried in a Speedvac evaporator (model AS160; Savant, Farmingdale, NY), flushed with argon, and stored at −20 °C for ≤48 h prior to HPLC-PDA analysis. All carotenoid extracts were kept on ice and under yellow lights throughout the extraction process.

Carotenoid concentrations in powders and tissues were analyzed by a reverse-phase HPLC-PDA system previously described (*12*). All analyses were performed in duplicate, and the quantification of carotenoid isomers was carried out by comparing the UV spectra and retention times to analytical standards of phytoene, phytofluene, lycopene, and β-carotene (gifts from Hansgeorg Ernst, BASF, Ludwigshafen, Germany).

Animals and Experimental Design. The University of Illinois Animal Care and Use Committee approved the animal protocol. Sixty-four male Copenhagen rats (Cop 2331; Harlan, Indianapolis, IN) were obtained at ∼8 weeks of age and individually housed in wire-bottom cages under controlled conditions (12 h light−dark cycle, 22 °C, 60% humidity). Rats were weighed daily throughout the study. Rats were provided with AIN-93G powdered diet and acclimated for 7 days. On day 7, rats were randomly assigned to eight experimental groups (n = 8). AIN-93G-based experimental diets included AIN-93G control, 10% standard tomato powder, 10% lycopene-enriched tomato powder, 10% carotenoid-enriched tomato powder, 10% standard broccoli floret powder, 10% broccoli sprout powder, 10% IG-enriched broccoli powder, or 10% selenium-enriched broccoli powder. Diets were balanced for protein, fat, energy, and fiber. Diet formulations are shown in **Table 1**. Food intake was measured daily. Animals were pair-fed experimental diets for 7 days.

Urinary Analysis of Sulforaphane Conjugates. For the last 24 h of experimental feeding, urine was collected into 50 mg of ascorbic acid and

Table 1. AIN-93G-Based Diet Formulations

	g/100 g of total diet			
	control	tomato (Std, Lyc, Car)	broccoli (Std, IG, Se)	broccoli (sprout)
cornstarch	39.7	33.2	36.6	38.0
casein	20.0	18.7	16.8	16.9
maltodextrin	13.2	13.2	13.2	13.2
surcose	10.0	10.0	10.0	10.0
fiber[a]	5.0	3.3	1.6	3.4
mineral mix[b]	3.5	3.5	3.5	3.5
vitamin mix[c]	1.0	1.0	1.0	1.0
L-cystine	0.3	0.3	0.3	0.3
choline bitartrate	0.3	0.3	0.3	0.3
soybean oil	7.0	6.5	6.7	5.3
tomato powder		10.0		
broccoli powder			10.0	10.0

[a] Non-nutritive cellulose. [b] AIN93M-MX formulation. [c] AIN93-VX formulation.

Table 2. Glucosinolate, Isothiocyanate, and Selenium Profile of Broccoli Powders[a]

	standard broccoli	broccoli sprout	IG-enriched broccoli	Se-enriched broccoli
			μmol/g of dry weight	
glucoraphanin	3.05 ± 0.36 b	21.60 ± 4.30 a	2.46 ± 0.36 b	3.36 ± 0.55 b
glucobrassicin	2.17 ± 0.06 b	0.05 ± 0.00 d	3.27 ± 0.17 a	0.73 ± 0.05 c
neoglucobrassicin	1.97 ± 0.06 c	0.06 ± 0.00 d	3.74 ± 0.02 b	11.99 ± 0.75 a
sulforaphane	0.98 ± 0.02 b	22.78 ± 1.30 a	1.73 ± 0.11 b	1.75 ± 0.13 b
indole-3-carbinol	0.10 ± 0.01 a	0.04 ± 0.00 c	0.08 ± 0.00 b	0.10 ± 0.01 a
			ppm	
selenium	0.25	0.06	0.06	4.28

[a] Values are means ± SEM, $n = 2-4$ (where $n = 2$, SEM is not reported). Different letters within rows indicate significant differences between treatments ($P < 0.05$).

stored at −80 °C until analyzed. SF and erucin *N*-acetylcysteine conjugates were quantified by HPLC-UV at a wavelength of 254 nm, as previously described (*13*). Intake of glucoraphanin was calculated from broccoli hydrosylate analysis and average food intake (14.5 ± 0.4 g per animal per day).

Preparation of Microsomal and Cytosolic Fractions. Rats were euthanized by CO_2 asphyxiation, and tissues were taken. Livers were perfused via the portal vein with cold 1.15% KCl. Colons were flushed with cold 1.15% KCl, and mucosal scrapings of the proximal 5 cm were collected. Tissues were snap-frozen in liquid nitrogen and stored at −80 °C until use. Liver and colon mucosal samples were thawed on ice, homogenized in 3 and 1 mL of cold 0.05 M Tris-HCl buffer (pH 7.4), respectively, and centrifuged for 20 min at 10000*g* at 4 °C. Colonic mucosal supernatant was stored at −80 °C until use; liver supernatant was further centrifuged for 60 min at 100000*g* at 4 °C. The supernatant (cytosolic fraction) was stored at −80 °C until use, and the pellet (microsomal fraction) was resuspended in 1 mL of cold 0.05 M Tris-HCl buffer (pH 7.4) containing 0.25 M sucrose and then stored at −80 °C until use.

Detoxification Enzyme Activity. The activity of phase I enzyme CYP1A was measured in the microsomal fraction as ethoxyresorufin *O*-deethylase (EROD) activity (*14*) with slight modification (*15*). The activity of cytosolic phase II enzyme NAD(P)H-quinone oxidoreductase 1 (NQO1) was measured according to the method of Prochaska and Santamaria (*16*) with modification (*15*).

Serum Testosterone Measurements. Serum testosterone was quantified using a coated tube radioimmunoassay kit (Diagnostic Systems Laboratory Inc., Webster, TX).

Real-Time Quantitative PCR. Total RNA was prepared from tissues using Trizol (Invitrogen, Carlsbad, CA) per the manufacturer's instructions. cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). mRNA expression of selected genes was measured via real-time PCR using SYBR Green Master Mix (Applied Biosystems). Reactions were monitored by an ABI Prism 7900HT. Primer pairs were designed to measure steroid 5-α-reductase 1 (SRD5A1) (*17*), 5-α-reductase 2 (SRD5A2) (*18*), glutathione *S*-transferase π 1 (GSTP1, NM_012577) forward 5′-CCTATGTGGCTCGCCTCAGT-3′ and reverse 5′-GATGGGACGGTTCAAATGGT-3′, and ribosomal protein L7a (RPL7A, NM_001114391) forward 5′-GAGGCCAAAAAGGTGGTCAATCC-3′ and reverse 5′-CCTGCCCAATGCCGAAGTTCT-3′. A validation experiment was performed on each set of primers (MWG Biotech, Huntsville, AL) to confirm efficiency and product specificity. RPL7A was used as a housekeeping gene (*19*). Relative mRNA abundance was determined using the comparative critical threshold method.

Statistical Analysis. Data were compared among treatments by two-tailed analysis of variance, using SAS Statistical software (version 9.1; SAS Institute, Cary, NC), and values were considered to be different from controls at $P < 0.05$ using Fisher's least significant difference procedure.

RESULTS AND DISCUSSION

Powder Characterization. Here we demonstrate that tomato and broccoli powders with differing levels of bioactives can be readily produced by agronomic means and that this leads to altered uptake of bioactives in vivo. We obtained tomato powders with enhanced levels of lycopene or total carotenoids and broccoli powders with enhanced levels of glucosinolates or selenium. Broccoli powders were analyzed for glucosinolate, SF, I3C, and selenium content (**Table 2**). Glucoraphanin levels were similar among all broccoli samples except the broccoli sprout powder, for which levels were 7 times greater. As expected, SF content from dietary glucoraphanin was highest from the broccoli sprouts, being 23 times greater than in standard broccoli. Glucobrassicin was significantly higher in the indole glucosinolate-enriched broccoli, as expected. The indole glucosinolate-enriched broccoli also had increased neoglucobrassicin levels. Surprisingly, neoglucobrassicin levels were dramatically increased in the selenium-enriched broccoli, by approximately 6 times compared to standard broccoli powder. Broccoli sprouts had a low indole

Table 3. Carotenoid Profile of Tomato Powders[a]

	standard tomato	lycopene-enriched tomato	carotenoid-enriched tomato
		nmol/g	
phytoene	102 ± 7 b	92 ± 1 b	347 ± 6 a
phytofluene	74 ± 7 b	71 ± 1 b	224 ± 3 a
β-carotene	13 ± 1 b	13 ± 0 b	50 ± 2 a
cis-lycopene	80 ± 9 b	213 ± 9 a	116 ± 21 b
all-trans- and 5-*cis*-lycopene	92 ± 11 c	233 ± 16 b	490 ± 66 a
total lycopene	172 ± 21 b	446 ± 24 a	606 ± 87 a
total carotenoid[b] (μg/g)	195 ± 19 c	335 ± 14 b	663 ± 52 a

[a] Values are means ± SEM, $n = 3$. Different letters within rows indicate significant differences between treatments ($P < 0.05$). [b] Total carotenoid = phytoene + phytofluene + β-carotene + total lycopene.

glucosinolate content. Following hydrolysis, indole glucosinolate-enriched broccoli contained slightly lower levels of I3C than standard broccoli ($P < 0.05$).

Tomato powders were analyzed for carotenoid content (**Table 3**). Levels of lutein, zeaxanthin, α-carotene, and β-cryptoxanthin were all below 5 nmol/g (data not shown). Lycopene-enriched tomato powder had similar levels of phytoene, phytofluene, and β-carotene compared to standard tomato powder, but it also had approximately 2.6 times more lycopene than the standard tomato powder. The carotenoid-enriched tomato powder contained total carotenoid levels approximately 3.4 times greater than those of the standard tomato powder and 2 times greater than those of the lycopene-enriched tomato powder.

Diet and Weight Gain. The group of rats receiving the selenium-enriched broccoli diet had the lowest food intake. As a result, all other animals were pair-fed to this group. Each day other diet groups received the amount of diet equal to the average amount consumed by the selenium-enriched broccoli group the day before. The average amount of food consumed daily was 14.5 ± 0.4 g. Rats gained weight as expected throughout the study, and there were no significant differences in final body weights between groups. The estimated daily intakes of glucoraphanin from the broccoli powders were 4.4, 31.3, 3.6, and 4.9 μmol for standard broccoli, broccoli sprouts, indole glucosinolate-enriched broccoli, and selenium-enriched broccoli, respectively. The estimated daily intakes of lycopene from the tomato powders were 249, 647, and 879 nmol for standard tomato, lycopene-enriched tomato, and carotenoid-enriched tomato, respectively (**Figure 1**).

Sulforaphane Metabolism. Sulforaphane is metabolized primarily via the mercapturic acid pathway (*20*), and SF-NAC is used as a marker for fractional absorption of this isothiocyanate (*21*). Urinary levels of sulforaphane *N*-acetylcysteine conjugates (SF-NAC) are shown in **Figure 1**. The reduced form, erucin *N*-acetylcysteine, was below the level of detection. Recovery of SF-NAC was significantly greater in the broccoli sprout group. Percent recoveries of SF-NAC from ingested glucoraphanin were 4% for standard broccoli, 49% for broccoli sprouts, 28% for indole glucosinolate-enriched broccoli, and 12% for selenium-enriched broccoli. The enhanced urinary content of SF from sprouts is consistent with individual data for sprouts and standard broccoli from the literature (*13*, *22*). However, early hydrolysis before digestive enzymes destroy broccoli myrosinase cannot explain this, because in the past prehydrolyzing broccoli did not enhance SF-NAC yield (*13*). Blanching of the sprouts may have destroyed the myrosinase cofactor that directs glucoraphanin hydrolysis to nitrile formation in place of SF (*23*). This idea is consistent with the far greater yield of SF from glucoraphanin in sprouts compared to standard broccoli (**Table 2**).

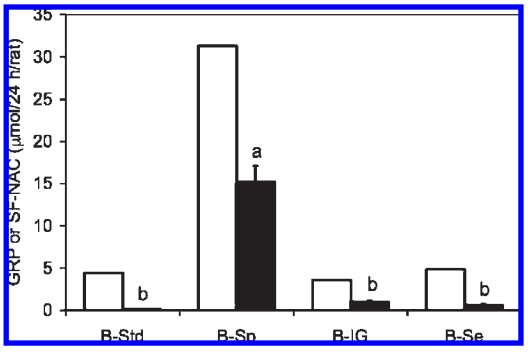

Figure 1. Estimated daily glucoraphanin (GRP) intake (white bars) and 24 h urinary SF-NAC excretion (black bars) on the last day of a 7 day feeding period. Experimental groups: B-Std, standard broccoli; B-Sp, broccoli sprout; B-IG, indole glucosinolate-enriched broccoli; B-Se, selenium-enriched broccoli. Results shown are means ± SEM, $n = 8$. Samples were run in triplicate. Different letters above bars indicate significant differences between treatments ($P < 0.05$).

Hepatic Carotenoid Accumulation. Liver carotenoid content is shown in **Table 4**. Interestingly, hepatic carotenoid accumulation did not strictly follow the patterns seen in the tomato powder carotenoid profiles. The carotenoid-enriched tomato powder had carotenoid levels 2−3 times greater than those of standard tomato powder, but only the hepatic accumulations of phytoene and β-carotene were significantly increased compared to standard tomato powder. The lycopene-enriched tomato group had the expected significantly higher levels of lycopene but also significantly lower levels of phytofluene and phytoene compared to standard and carotenoid-enriched tomato powders.

The lower than expected accumulation level of lycopene and phytofluene from feeding the carotenoid-enriched tomato powder may have been due to differences in processing method. The commercial tomato powders (standard and lycopene-enriched) were more finely ground than powders from fruits grown in California, which were processed using a hammermill (carotenoid-enriched tomato powder), possibly leading to an increase in hepatic accumulation. Alternatively, there is literature indicating that supplementation of a normal diet with β-carotene supplements or β-carotene-rich foods may lead to a decrease in serum lycopene (*24*, *25*). The higher levels of phytoene and β-carotene in the carotenoid-enriched tomato powder could compete with lycopene for absorption and transport. Lycopene-enriched tomato powder feeding resulted in significantly higher hepatic levels of lycopene than standard tomato powder, but it also resulted in significantly reduced uptake of phytoene and phytofluene. These data support the

Table 4. Hepatic Carotenoid Content[a]

	standard tomato	lycopene-enriched tomato	carotenoid-enriched tomato
		μg/g	
phytoene	13.0 ± 0.7 b	8.7 ± 0.6 c	19.4 ± 0.7 a
phytofluene	13.0 ± 0.4 a	9.8 ± 0.7 b	13.4 ± 0.5 a
β-carotene	0.2 ± 0.0 b	0.2 ± 0.0 b	0.3 ± 0.0 a
lycopene	18.4 ± 1.5 b	26.7 ± 2.4 a	19.1 ± 0.9 b
total carotenoid[b]	44.4 ± 2.1 b	45.1 ± 3.1 b	52.0 ± 1.5 a

[a] Values are means ± SEM, n=8. Each sample was run in duplicate. Different letters within a row indicate significant differences between treatments ($P < 0.05$). [b] Total carotenoid = lycopene + phytofluene + phytoene + β-carotene.

possibility that there may be competition between carotenoids for absorption pathways.

Indole Glucosinolate Metabolism. High doses of purified I3C are rapidly degraded in the body. Serum levels of I3C have been reported to fall below detectable levels (0.05 μg/mL measured by HPLC) within an hour after oral administration to mice (250 mg/kg) (*26*). Similarly in humans, I3C was not detectable in serum 1 h after oral administration (400 mg) with a limit of detection of 1.0 ng/mL (measured by HPLC-MS) (*27*). The dose provided here by a 10% indole glucosinolate-enriched broccoli diet is approximately 1000-fold lower than the levels used in the above pharmacokinetic studies and would likely not be detectable, particularly because rats were fed ad libitum rather than a bolus dose of the compound.

Detoxification Enzyme Activity. Broccoli and its glucosinolate hydrolysis products have been shown to induce phase I and phase II drug-metabolizing enzymes (*13*). Detoxification enzymes in the colon and liver play an important role in the metabolism and clearance of carcinogens. Xenobiotics are oxidatively metabolized by phase I enzymes, generating products that are potentially carcinogenic. Phase II conjugating enzymes are then able to add polar molecules (glutathione, glucuronide, sulfate, etc.) to the metabolite, enhancing water solubility. These products are more easily eliminated from the body. Therefore, the balance between activating (phase I) and detoxifying (phase II) reactions is an indication of the risk of ultimate carcinogen available for reaction with cellular DNA. Here we demonstrate that broccoli enriched with bioactive components through agronomic practices enhances both CYP1A and NQO1 activity in liver and colon to a greater extent than standard broccoli.

Tissue enzyme activities of EROD and NQO1 are shown in **Figure 2**. The standard broccoli diet did not alter EROD or NQO1 in liver but did increase NQO1 in the colon. SF-rich broccoli sprouts showed no hepatic EROD induction, but increased NQO1 1.9 times in the liver and 2.3 times in the colon ($P < 0.05$). Indole glucosinolate-enriched broccoli increased hepatic EROD and NQO1 activities 1.4 and 1.3 times, respectively, and colon NQO1 activity 1.6 times ($P < 0.05$). Selenium-enriched broccoli induced hepatic EROD activity 1.8 times; NQO1 activity was elevated 2.2 and 1.8 times in the liver and colon, respectively ($P < 0.05$). No differences in EROD or NQO1 activities were observed between groups receiving the tomato powder diets. Lycopene-enriched tomato and standard broccoli diets decreased glutathione *S*-transferase P1 (GSTP1) mRNA levels by 23.3 and 31.9%, respectively, compared to the control diet ($P < 0.05$) (**Figure 3**).

Glucobrassicin and neoglucobrassicin, as well as their respective hydrolysis products I3C and *N*-methoxyindole-3-carbinol (NI3C), are known to induce CYP1A (*28*−*31*). Although neoglucobrassicin is a weaker inducer than glucobrassicin in vivo, a mixture containing both glucosinolates showed strongest

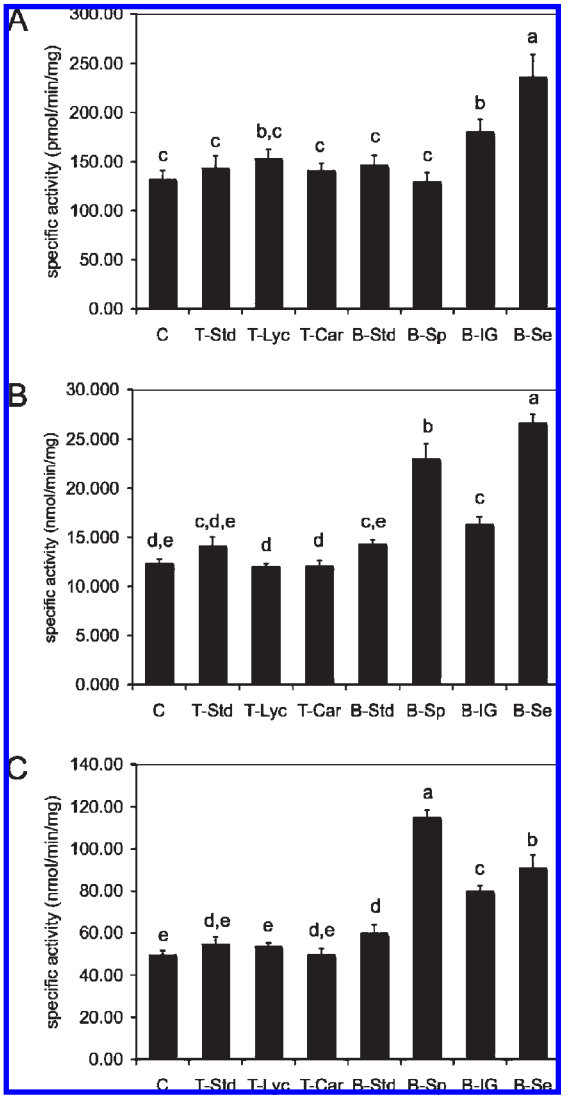

Figure 2. (**A**) Liver EROD, (**B**) liver NQO1, and (**C**) colon NQO1 after a 7 day feeding period. Experimental groups: C, control; T-Std, standard tomato; T-Lyc, lycopene-enriched tomato; T-Car, carotenoid-enriched tomato; B-Std, standard broccoli; B-Sp, broccoli sprout; B-IG, indole glucosinolate-enriched broccoli; B-Se, selenium-enriched broccoli. Results shown are means ± SEM, n = 8. Samples were run in triplicate. For each enzyme, different letters above bars indicate significant differences between treatments ($P < 0.05$).

induction of hepatic CYP1A (*29*). In concordance, indole glucosinolate-enriched broccoli and selenium-enriched broccoli increased hepatic CYP1A-dependent EROD activity. Neither standard broccoli nor broccoli sprouts altered hepatic CYP1A

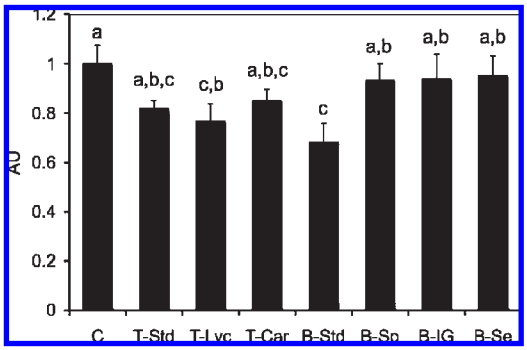

Figure 3. Gene expression of prostatic GSTP1 after a 7 day feeding. AU, arbitrary units. Experimental groups: C, control; T-Std, standard tomato; T-Lyc, lycopene-enriched tomato; T-Car, carotenoid-enriched tomato; B-Std, broccoli; B-Sp, broccoli sprout; B-IG, indole glucosinolate-enriched broccoli; B-Se, selenium-enriched broccoli. Results shown are means ± SEM, $n = 8$. Different letters above bars indicate significant differences between treatments ($P < 0.05$).

activity, which correlates with their low levels of neoglucobrassicin and I3C.

Broccoli sprouts and indole glucosinolate-enriched and selenium-enriched broccoli increased NQO1 in both liver and colon. Standard broccoli effectively up-regulated NQO1 in the colon, but it did not significantly up-regulate enzymes in the liver, possibly because the dose of SF was insufficient. This tissue-specific effect suggests incomplete myrosinase-dependent hydrolysis of glucoraphanin in the upper gut, resulting in hydrolysis in the lower gut and subsequent local increase in NQO1 (*15*).

The relative amounts of SF in standard broccoli, broccoli sprout, and indole glucosinolate-enriched broccoli (1.0, 22.8, and 1.7 μmol of SF/g of dry weight, respectively) elicited increases in NQO1 activity in the liver (20, 90, and 30%, respectively) and colon (20, 130, and 60%, respectively) that were proportional to the SF dose. Selenium-enriched broccoli induced enzyme activity to a greater extent than can be ascribed to its SF level. Broccoli accumulates selenium primarily as Se-methylselenocysteine (*32*), which alone or in synergy with SF may account for the increase in NQO1 activity. These data are in contrast to those of Robbins et al. (*33*), who reported a suppression of SF levels in broccoli treated with a sodium selenate solution (100 or 10000 ppm). This resulted in very high levels of selenium in broccoli (up to 800 ppm). The freeze-dried broccoli used here had 4 ppm Se, which would have been ~0.4 ppm in the fresh plant.

In contrast to feeding standard broccoli, which resulted in greater increases in NQO1 in the colon than in the liver, feeding selenium-treated broccoli resulted in larger increases in NQO1 in the liver than in the colon. One possible cause for this is that hepatic metabolism permitted the formation of a more potent metabolite.

Prostate cancer is characterized by loss of GSTP1 expression, regardless of the grade or stage of the disease (*34*). Unexpectedly, broccoli florets and lycopene-enriched tomato significantly reduced GSTP1 expression by 32 and 23%, respectively. These results appear to contradict previous evidence showing that lycopene partially demethylates the GSTP1 promoter and restores expression in breast cancer cells (*35*) and that oral intake of SF increases GSTP1 expression in nasal mucosa (*36*). The GST family of GST enzymes are not all regulated similarly, and GSTP1 has previously been found to lack the inductive response to dietary crucifers seen for GSTM1 and GSTA1 (*9*).

Androgen Metabolism. Previous studies have suggested that androgen metabolism might be altered by tomato and/or lycopene feeding. Our own laboratory has shown that a 4 day feeding of lycopene or tomato powder resulted in significantly reduced levels of serum testosterone in Fisher 344 rats (*18*). It has also been reported that lycopene feeding decreased prostatic mRNA levels of SRD5A2 (*37*), which is a key enzyme in the conversion of testosterone to the more potent androgen, dihydroxytestosterone. In the current study, no significant alterations were seen in either serum testosterone levels (values ranged from 0.54 to 1.78 ng/mL) or mRNA levels of SRD5A1 or SRD5A2 (data not shown). The lack of significant reduction in serum testosterone level may be due to the longer feeding period (7 vs 4 days) or the strain of rat used. Reduction in expression of SRD5A2 has been seen in Copenhagen rats of a similar age (*37*). However, in that study rats were fed supplemental lycopene for a period of 8 weeks, resulting in a much longer and higher dose lycopene exposure than the present study. As noted above, alterations in serum testosterone were reported for Fisher 344 rats after 4 days of 10% tomato powder feeding (*18*), which is shorter than the 7 day feeding period used in this study, suggesting that the effects of tomato on serum testosterone levels could be a transient effect or that the dose–response varies with strain. None of the broccoli treatments had significant effects on androgen metabolism end points. To our knowledge the effects of broccoli on testosterone metabolism have not been previously investigated, and these data demonstrate that broccoli feeding does not alter serum testosterone levels or expression of two key metabolizing enzymes.

In summary, we have demonstrated that levels of bioactive components in tomatoes and broccoli can be altered through agronomic means. This increase in bioactives in the vegetable can translate to increased tissue content and increased bioactivity in vivo. These encouraging findings suggest that bioactive-enriched vegetables should be tested for cancer prevention.

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LITERATURE CITED

(1) Doll, R. The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. *J. Natl. Cancer Inst.* **1981**, *66*, 1191.

(2) Etminan, M.; Takkouche, B.; Caamaño-Isorna, F. The role of tomato products and lycopene in the prevention of prostate cancer: a meta-analysis of observational studies. *Cancer Epidemiol. Biomarkers Prev.* **2004**, *13*, 340–5.

(3) Kristal, A. R.; Lampe, J. W. Brassica vegetables and prostate cancer risk: a review of the epidemiological evidence. *Nutr. Cancer* **2002**, *42*, 1–9.

(4) Finley, J.; Ip, C.; Lisk, D.; Davis, C.; Hintze, K.; Whanger, P. Cancer-protective properties of high-selenium broccoli. *J. Agric. Food Chem.* **2001**, *49*, 2679–83.

(5) Traka, M.; Gasper, A. V.; Melchini, A.; Bacon, J. R.; Needs, P. W.; Frost, V.; Chantry, A.; Jones, A. M.; Ortori, C. A.; Barrett, D. A.; Ball, R. Y.; Mills, R. D.; Mithen, R. F. Broccoli consumption interacts with GSTM1 to perturb oncogenic signalling pathways in the prostate. *PLoS ONE* **2008**, *3*, e2568.

(6) Butelli, E.; Titta, L.; Giorgio, M.; Mock, H.; Matros, A.; Peterek, S.; Schijlen, E.; Hall, R.; Bovy, A.; Luo, J.; Martin, C. Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors. *Nat. Biotechnol.* **2008**, *26*, 1301–8.

(7) Guenther, P. M.; Dodd, K. W.; Reedy, J.; Krebs-Smith, S. M. Most Americans eat much less than recommended amounts of fruits and vegetables. *J. Am. Diet. Assoc.* **2006**, *106*, 1371–1379.

(8) Jeffery, E. H.; Araya, M. Physiological effects of broccoli. *Phytochemical Rev*. **2009**, *8*, 283–298.
(9) McWalter, G. K.; Higgins, L. G.; McLellan, L. I.; Henderson, C. J.; Song, L.; Thornalley, P. J.; Itoh, K.; Yamamoto, M.; Hayes, J. D. Transcription factor Nrf2 is essential for induction of NAD(P)H: quinone oxidoreductase 1, glutathione S-transferases, and glutamate cysteine ligase by broccoli seeds and isothiocyanates. *J. Nutr*. **2004**, *134*, 3499S–3506S.
(10) Kushad, M. M.; Brown, A. F.; Kurilich, A. C.; Juvik, J. A.; Klein, B. P.; Wallig, M. A.; Jeffery, E. H. Variation of glucosinolates in vegetable crops of *Brassica oleracea*. *J. Agric. Food Chem*. **1999**, *47*, 1541–1548.
(11) Zaripheh, S.; Boileau, T. W.; Lila, M. A.; Erdman, J. W. Jr. [^{14}C]-lycopene and [^{14}C]-labeled polar products are differentially distributed in tissues of F344 rats prefed lycopene. *J. Nutr*. **2003**, *133*, 4189–4195.
(12) Lu, C. H.; Engelmann, N. J.; Lila, M. A.; Erdman, J. W. Jr. Optimization of lycopene extraction from tomato cell suspension culture by response surface methodology. *J. Agric. Food Chem*. **2008**, *56*, 7710–7714.
(13) Keck, A. S.; Qiao, Q.; Jeffery, E. H. Food matrix effects on bioactivity of broccoli-derived sulforaphane in liver and colon of F344 rats. *J. Agric. Food Chem*. **2003**, *51*, 3320–3327.
(14) Paolini, M.; Perocco, P.; Canistro, D.; Valgimigli, L.; Pedulli, G. F.; Iori, R.; Croce, C. D.; Cantelli-Forti, G.; Legator, M. S.; Abdel-Rahman, S. Z. Induction of cytochrome P450, generation of oxidative stress and in vitro cell-transforming and DNA-damaging activities by glucoraphanin, the bioprecursor of the chemopreventive agent sulforaphane found in broccoli. *Carcinogenesis* **2004**, *25*, 61–67.
(15) Lai, R. H.; Keck, A. S.; Wallig, M. A.; West, L. G.; Jeffery, E. H. Evaluation of the safety and bioactivity of purified and semi-purified glucoraphanin. *Food Chem. Toxicol*. **2008**, *46*, 195–202.
(16) Prochaska, H. J.; Santamaria, A. B. Direct measurement of NAD(P)H:quinone reductase from cells cultured in microtiter wells: a screening assay for anticarcinogenic enzyme inducers. *Anal. Biochem*. **1988**, *169*, 328–336.
(17) Killian, J.; Pratis, K.; Clifton, R. J.; Stanton, P. G.; Robertson, D. M.; O'Donnell, L. 5-α-reductase isoenzymes 1 and 2 in the rat testis during postnatal development. *Biol. Reprod*. **2003**, *68*, 1711–1718.
(18) Campbell, J. K.; Stroud, C. K.; Nakamura, M. T.; Lila, M. A.; Erdman, J. W. Jr. Serum testosterone is reduced following short-term phytofluene, lycopene, or tomato powder consumption in F344 rats. *J. Nutr*. **2006**, *136*, 2813–2819.
(19) Chen, H.; Pan, Y. X.; Dudenhausen, E. E.; Kilberg, M. S. Amino acid deprivation induces the transcription rate of the human asparagine synthetase gene through a timed program of expression and promoter binding of nutrient-responsive basic region/leucine zipper transcription factors as well as localized histone acetylation. *J. Biol. Chem*. **2004**, *279*, 50829–50839.
(20) Kassahun, K.; Davis, M.; Hu, P.; Martin, B.; Baillie, T. Biotransformation of the naturally occurring isothiocyanate sulforaphane in the rat: identification of phase I metabolites and glutathione conjugates. *Chem. Res. Toxicol*. **1997**, *10*, 1228–1233.
(21) Vermeulen, M.; van den Berg, R.; Freidig, A. P.; van Bladeren, P. J.; Vaes, W. H. Association between consumption of cruciferous vegetables and condiments and excretion in urine of isothiocyanate mercapturic acids. *J. Agric. Food Chem*. **2006**, *54*, 5350–5358.
(22) Munday, R.; Mhawech-Fauceglia, P.; Munday, C. M.; Paonessa, J. D.; Tang, L.; Munday, J. S.; Lister, C.; Wilson, P.; Fahey, J. W.; Davis, W.; Zhang, Y. Inhibition of urinary bladder carcinogenesis by broccoli sprouts. *Cancer Res*. **2008**, *68*, 1593–1600.
(23) Matusheski, N. V.; Jeffery, E. H. Comparison of the bioactivity of two glucoraphanin hydrolysis products found in broccoli, sulforaphane and sulforaphane nitrile. *J. Agric. Food Chem*. **2001**, *49*, 5743–5749.
(24) Castenmiller, J. J.; West, C. E.; Linssen, J. P.; van het Hof, K. H.; Voragen, A. G. The food matrix of spinach is a limiting factor in determining the bioavailability of β-carotene and to a lesser extent of lutein in humans. *J. Nutr*. **1999**, *129*, 349–355.
(25) van het Hof, K. H.; Brouwer, I. A.; West, C. E.; Haddeman, E.; Steegers-Theunissen, R. P.; van Dusseldorp, M.; Weststrate, J. A.; Eskes, T. K.; Hautvast, J. G. Bioavailability of lutein from vegetables is 5 times higher than that of β-carotene. *Am. J. Clin. Nutr*. **1999**, *70*, 261–268.
(26) Anderton, M. J.; Manson, M. M.; Verschoyle, R. D.; Gescher, A.; Lamb, J. H.; Farmer, P. B.; Steward, W. P.; Williams, M. L. Pharmacokinetics and tissue disposition of indole-3-carbinol and its acid condensation products after oral administration to mice. *Clin. Cancer Res*. **2004**, *10*, 5233–5241.
(27) Reed, G. A.; Arneson, D. W.; Putnam, W. C.; Smith, H. J.; Gray, J. C.; Sullivan, D. K.; Mayo, M. S.; Crowell, J. A.; Hurwitz, A. Single-dose and multiple-dose administration of indole-3-carbinol to women: pharmacokinetics based on 3,3′-diindolylmethane. *Cancer Epidemiol. Biomarkers Prev*. **2006**, *15*, 2477–2481.
(28) Vang, O.; Jensen, M. B.; Autrup, H. Induction of cytochrome P450IA1 in rat colon and liver by indole-3-carbinol and 5,6-benzoflavone. *Carcinogenesis* **1990**, *11*, 1259–1263.
(29) Bonnesen, C.; Stephensen, P. U.; Andersen, O.; Sorensen, H.; Vang, O. Modulation of cytochrome P-450 and glutathione S-transferase isoform expression in vivo by intact and degraded indolyl glucosinolates. *Nutr. Cancer* **1999**, *33*, 178–187.
(30) He, Y. H.; Friesen, M. D.; Ruch, R. J.; Schut, H. A. Indole-3-carbinol as a chemopreventive agent in 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) carcinogenesis: inhibition of PhIP-DNA adduct formation, acceleration of PhIP metabolism, and induction of cytochrome P450 in female F344 rats. *Food Chem. Toxicol*. **2000**, *38*, 15–23.
(31) Stephensen, P. U.; Bonnesen, C.; Schaldach, C.; Andersen, O.; Bjeldanes, L. F.; Vang, O. *N*-Methoxyindole-3-carbinol is a more efficient inducer of cytochrome P-450 1A1 in cultured cells than indol-3-carbinol. *Nutr. Cancer* **2000**, *36*, 112–121.
(32) Cai, X. J.; Block, E.; Uden, P. C.; Zhang, X.; Quimby, B. D.; Sullivan, J. J. *Allium* chemistry—identification of selenoamino acids in ordinary and selenium-enriched garlic, onion, and broccoli using gas-chromotography with atomic-emission detection. *J. Agric. Food Chem*. **1995**, *43*, 1754–1757.
(33) Robbins, R. J.; Keck, A. S.; Banuelos, G.; Finley, J. W. Cultivation conditions and selenium fertilization alter the phenolic profile, glucosinolate, and sulforaphane content of broccoli. *J. Med. Food* **2005**, *8*, 204–214.
(34) Brooks, J. D.; Goldberg, M. F.; Nelson, L. A.; Wu, D.; Nelson, W. G. Identification of potential prostate cancer preventive agents through induction of quinone reductase in vitro. *Cancer Epidemiol. Biomarkers Prev*. **2002**, *11*, 868–875.
(35) King-Batoon, A.; Leszczynska, J. M.; Klein, C. B. Modulation of gene methylation by genistein or lycopene in breast cancer cells. *Environ. Mol. Mutagen*. **2008**, *49*, 36–45.
(36) Riedl, M. A.; Saxon, A.; Diaz-Sanchez, D. Oral sulforaphane increases phase II antioxidant enzymes in the human upper airway. *Clin. Immunol*. **2009**, *130*, 244–251.
(37) Herzog, A.; Siler, U.; Spitzer, V.; Seifert, N.; Denelavas, A.; Hunziker, P. B.; Hunziker, W.; Goralczyk, R.; Wertz, K. Lycopene reduced gene expression of steroid targets and inflammatory markers in normal rat prostate. *FASEB J*. **2005**, *19*, 272–274.

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