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ARTICLE

The Impact of Loss of Myrosinase on the Bioactivity of Broccoli Products in F344 Rats

Ning Zhu,[†,∥] Mette Soendergaard,[‡,∥] Elizabeth H. Jeffery,*[,†,§] and Ren-Hau Lai[§]

[†]Department of Food Science and Human Nutrition, University of Illinois, 467 Bevier Hall, 905 South Goodwin Avenue, Urbana, Illinois 61801, [‡]Department of Biochemistry and Natural Product Chemistry, Faculty of Life Sciences, University of Copenhagen, Denmark, and [§]Division of Nutritional Sciences, University of Illinois, Urbana, Illinois. [∥] N.Z. and M.S. contributed equally to this work.

In vitro, animal, and epidemiological studies all show that broccoli products containing sulforaphane, the bioactive hydrolysis product of glucoraphanin (GRP), lower risk for cancer. As a result, GRP-rich extracts are appearing on the market as dietary supplements. However, these products typically have no hydrolyzing enzyme for sulforaphane (SF) formation. We evaluated safety and compared efficacy to other broccoli preparations. Four daily doses of 0.5 mmol GRP/kg BW, given by gavage to adult male F344 rats, caused temporary cecal inflammation that was essentially resolved four days later. A similar dose dispersed in the diet caused no inflammation. To compare efficacy, we fed rats 20% freeze-dried broccoli (heated or unheated), 3.5% broccoli seed meal, or 4.3% semipurified GRP, each balanced within an AIN93G semipurified diet, for 4 days. Diets lacking myrosinase (semipurified GRP and heated broccoli florets) caused upregulation of NAD(P)H-quinone oxidoreductase 1 (NQO1) in colon but not liver. Surprisingly, broccoli seed, rich in myrosinase and GRP, also caused NQO1 upregulation in colon but not liver. In contrast, unheated broccoli florets caused upregulation in both colon and liver. These data suggest that GRP supplements may not exert systemic effects. We hypothesize that within whole broccoli additional components enhanced sulforaphane-dependent upregulation of NQO1 in liver.

KEYWORDS: Glucoraphanin; myrosinase; broccoli; ethoxyresorufin *O*-deethylase; NAD(P)H-quinone oxidoreductase 1

INTRODUCTION

Epidemiological studies suggest, and animal studies confirm, that dietary cruciferous vegetables such as broccoli lower the risk for a number of cancers (*1*). Broccoli (*Brassica oleraceae* L.) and other crucifers belong to the plant family Brassicaceae, the members of which are characterized by their content of a group of secondary metabolites termed glucosinolates (GSL). Broccoli is characterized by its content of the aliphatic GSL, glucoraphanin (GRP, (*R*)-4-methylsulfinylbutyl glucosinolate), which is often the predominant GSL, although the content varies with cultivar (*2*). Glucobrassicin (GB, indol-3-ylmethyl glucosinolate) is the major indole GSL, sometimes found in greater amount than GRP (*3*). Seeds and seedlings tend to have lower GB and higher GRP concentration than mature broccoli (*4*). Brassica plants contain a hydrolyzing enzyme, myrosinase (*β*-thioglucosidase; E.C. 3.2.1.147), which in intact plant tissues is physically separated from its GSL substrates. Following tissue damage, during chopping or chewing of the vegetable, myrosinase comes into contact with the GSL, which are then hydrolyzed to isothiocyanates (ITC), nitriles, epithionitriles, and other products (*5*−*7*).

*To whom correspondence should be addressed. Phone: 217-333-3820. E-mail: ejeffery@illinois.edu.

The anticarcinogenic effects of broccoli are considered to be related to the GSL hydrolysis products, especially sulforaphane (SF, 1-isothiocyanato-4-methylsulfinyl butane). In cell culture and in whole animals, SF and other purified ITC cause upregulation of phase-II detoxification enzymes, such as NAD(P)H-quinone oxidoreductase 1 (NQO1 (*8*, *9*)). In contrast, the hydrolysis product of GB, indole-3-carbinol (I3C), and its further metabolite diindolyl methane, upregulate both the phase I detoxification enzyme CYP1A1 and, to a lesser extent, NQO1 (*10*, *11*). Whereas I3C is sufficiently stable to store and use in dietary studies, SF rapidly reacts with any nucleophile, making incorporation within diet unmanageable. Both SF and I3C given individually have been reported to slow or prevent a number of cancers in animal models, and diets that include 3−5 servings of broccoli a week are found to cause a significant decrease in risk for a number of cancers (*12*).

Even though the public understands that broccoli has health benefits, the average American eats less than one serving of crucifers per week; possibly related to a dislike of the flavor (*13*). Because a dietary supplement or fortified food can overcome this aversion, GRP supplements are appearing on the market, although there is little data to determine whether they are as effective as the whole vegetable. Previous studies from our laboratory have shown that GRP can cause a dose-dependent

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Table 1. Composition of the Balanced AIN 93G Diets[a]

component (%)	control and dGRP	broccoli	broccoli seeds
corn starch	39.8	35.4	39.3
casein	20.0	14.7	19.1
maltodextrin	13.2	11.4	12.9
sucrose	10.0	8.6	9.8
alphacel	5.0	0.1	4.5
mineral mix	3.5	1.9	3.3
vitamin mix	1.0	1.0	1.0
L-cystein	0.3	0.3	0.3
choline bitartrate	0.3	0.3	0.3
soybean oil	7.0	6.3	6.0
broccoli powder	0.0	20.0	0.0
broccoli seeds	0.0	0.0	3.5
total	100.0	100.0	100.0

[a] Modifications are based on nutritional values according to the USDA.

upregulation of NQO1 in rat colon, but not in liver, even when doses were raised to 8-fold that present in effective doses of whole broccoli (*14*). Surprisingly, this very large dose of GRP, administered daily for 4 days by gavage, caused cecal inflammation. It is not known if the inflammation reflects reversible or permanent damage, or even if consumption of similarly large amounts of GRP incorporated into the diet or naturally within broccoli would lead to similar damage.

Broccoli is most often consumed processed (cooked), which at least partly inactivates myrosinase. A GRP supplement typically contains no myrosinase. Unhydrolyzed GSL reaching the lower gut are thought to be hydrolyzed by microbiota in the large intestine, specifically the colon in humans and the cecum in rats (*15*). However, SF conjugate excretion following a meal of cooked broccoli florets is slower and substantially less total SF conjugate is excreted, compared to excretion following an unheated broccoli floret meal (*16*). These data suggest that hydrolysis and/or absorption in the lower gut may be limited, resulting in poor bioavailability and low plasma SF levels. Thus GSL from foods or supplements that lack myrosinase are potentially very low in bioactivity. It is therefore possible that consumption of cooked broccoli and/or a GRP supplement does not have the health benefits associated with ingestion of unheated broccoli.

The present study was carried out in order to: (1) compare the bioactivity of dietary broccoli with GRP that might be used as a supplement, (2) evaluate the safety and efficacy of semipurified GRP, and (3) compare the bioactivity of semipurified GRP (no myrosinase) with GRP within broccoli florets, heated (little or no myrosinase) and unheated (myrosinase present), and broccoli seed (myrosinase, but a very different GSL profile, rich in GRP but lacking indole GSL) (*17*).

MATERIALS AND METHODS

Chemicals. Biochemicals were purchased from Sigma Chemical Co. (St Louis, MO) except where noted. Laboratory chemicals, HPLC-grade solvents, and basal diet components were from Fisher Scientific (Pittsburgh, PA). Semipurified GRP (26.3% pure) was prepared from broccoli seed as described previously (*14*).

Diet Preparations. Broccoli (experiment 1, Peto 7; experiment 2, Marathon) was grown at the University of Illinois, freeze-dried, and finely ground into a powder and stored at −80 °C until use. For heated broccoli samples, freeze-dried broccoli powder was dry heated for 15 min at 80 °C shortly before use. Fresh, dry broccoli seed (Marathon) was finely ground for 30 s and stored at 4 °C until use.

Experimental diets, containing broccoli florets (20%), heated broccoli florets (20%), broccoli seeds (3.5%), or semipurified GRP (4.3%) were balanced to provide similar macronutrient content to the AIN93G diet (**Table 1**) according to the USDA nutrient database (http://www.nal.usda.gov/fnic/foodcomp/search/).

Glucosinolate and Isothiocyanate Analysis. Glucosinolate content was estimated by HPLC of desulfated GSL using benzylglucosinolate (H. Sorensen, University of Copenhagen, Denmark) as internal standard (*15*). Desulfoglucosinolates were detected at 229 nm and reported as μmol/g dry weight (*18*). For hydrolysis and SF analysis, all broccoli florets and seed samples were hydrolyzed by mixing 1:15 (w/v) with water and incubated with/without exogenous myrosinase (0.1 unit white mustard myrosinase) at room temperature in the dark for 1 or 24 h. Benzylisothiocyanate (0.5 mg/mL, LKT Laboratories Inc. St. Paul, MN) was added as internal standard and the sample extracted into dichloromethane (1:2) and subjected to gas chromatographic analysis, as previously described (*6*).

Animals and Housing. Male F344 rats (Harlan Inc., Indianapolis, IN) were housed individually in stainless steel cages with a 12 h light−dark cycle, at 22 °C and 60% humidity and acclimated for three days prior to the study. During acclimation, rats were fed the control diet (AIN93G). Food, provided fresh daily, and tap water was available 24 h per day. Animal use was approved by the Animal Care and Use Committee of the University of Illinois, in accordance with NIH regulations.

Experimental Design. In experiment 1, 30 rats were divided into gavage (experiment 1A) and diet (experiment 1B) groups. In experiment 1A, 12 animals, weighing 163.0 ± 5.7 g (mean ± SD), were divided into three groups ($n = 4$). Animals were given 0.5 mL of saline (control) or 0.5 mmol of GRP/kg BW (gGRP) daily by gavage, for four days; control and four gGRP rats were killed on the fifth day; four other gGRP rats were given no further GRP, but not killed until four days later, on day nine (gGRP+4). In experiment 1B, carried out simultaneously with experiment 1A, 18 animals, weighing 166.9 ± 11.9 g (mean ± SD), were divided into three groups ($n = 6$) and fed either an AIN 93G diet (control) or an AIN 93G diet containing either semipurified GRP (4.3%; dGRP) or 20% freeze-dried, unheated Peto 7 broccoli florets. Animals were pair-fed to the group eating the least.

In experiment 2, 20 animals weighing 170.6 ± 6.2 g (mean ± SD) were divided into four groups ($n = 5$) and fed the AIN 93G diet (control) or AIN 93G containing either 20% unheated Marathon broccoli florets, 20% heated Marathon broccoli florets, or 3.5% Marathon broccoli seed for four days and killed on the fifth day.

Tissue Collection. All rats were anesthetized using ketamine-xylazine (87 mg/mL:13 mg/mL) and killed by cervical dislocation. Livers were immediately perfused with ice-cold 1.15% KCl, patted dry, and weighed. The first five centimeters of the colon were flushed with ice-cold 1.15% KCl, and the mucosa was collected by scraping. Liver and colon samples were snap frozen in liquid nitrogen and stored at −80 °C until use. The ceca were collected into formalin and stained with hematoxylin and eosin for histological inspection.

Preparation of Microsomes and Cytosol. Liver or colonic mucosa was thawed on ice and homogenized in 3 mL of 0.25 M K_2HPO_4/KH_2PO_4-0.15 M KCl buffer (pH 7.25) or 1 mL 0.1 M Na_2HPO_4/NaH_2PO_4 buffer (pH 7.40), respectively, and centrifuged at 10000*g* for 20 min at 4 °C. The supernatant was recentrifuged at 100000*g* for 60 min at 4 °C. This second supernatant (cytosol) was collected, and the pellet (microsomes) was resuspended in 1 mL of 0.1 M phosphate/0.25 M sucrose buffer (pH 7.4). Cytosol and microsomes were each snap frozen in liquid nitrogen and stored at −80 °C until use.

NAD(P)H-Quinone Oxidoreductase 1(NQO1) and Ethoxyresorufin *O*-Deethylase (EROD) Activities. Activity of NQO1 was measured according to the method of Prochaska and Santamaria (1988), with modification (*14*). Activity of EROD was measured according to the method of Paolini (*19*) with modifications (*14*). Protein was determined by the Bradford assay (Bio-Rad Laboratories, Hercules, CA).

Urinary Analysis. Urine samples were collected directly into 50 mg of L-ascorbic acid. Sulforaphane *N*-acetylcyteine conjugate (SF-NAC) was quantified by HPLC-UV as described previously, using synthesized standards to quantify the conjugates (*20*).

Statistical Analysis. Statistical analysis was performed using the Statistical Analysis System (SAS, Inc., Cary, NC). Treatment effects were compared by one-way analysis of variance (ANOVA). For significant effects ($p \leq 0.05$), Tukey's studentized test was used to determine differences between groups.

Table 2. Major Glucosinolate Content of Broccoli Tissue[a]

glucosinolates (μmol per g dry weight)	Peto 7 floret	Marathon floret	heated Marathon floret	Marathon broccoli seeds
glucoraphanin	6.7 ± 0.5 a	1.1 ± 0.2 b	1.2 ± 0.1 b	14.2 ± 1.3 c
glucobrassicin	4.4 ± 0.2 a	3.5 ± 0.3 b	3.4 ± 0.1 b	0.0 ± 0.0
gluconasturtiin	2.7 ± 0.2 a	1.8 ± 0.2 b	1.8 ± 0.1 b	0.3 ± 0.1 c
neoglucobrassicin	1.8 ± 0.1 a	2.3 ± 0.1 b	2.3 ± 0.1 b	0.2 ± 0.0 c

[a] Values are mean ± SE, $n = 3$. Different letters indicate significant differences across rows: i.e., across broccoli preparations for a single glucosinolate ($p \leq 0.05$).

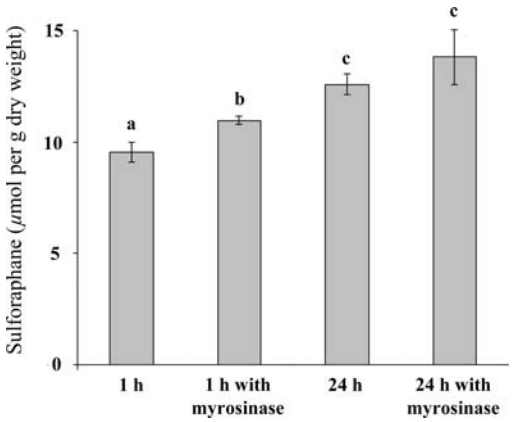

Figure 1. Sulforaphane formation in Marathon broccoli seeds after 1 and 24 h incubation with/without exogenous myrosinase (0.1 unit/mL). Values are mean ± SE, $n = 3$. Different letters indicate significant differences ($p \leq 0.05$).

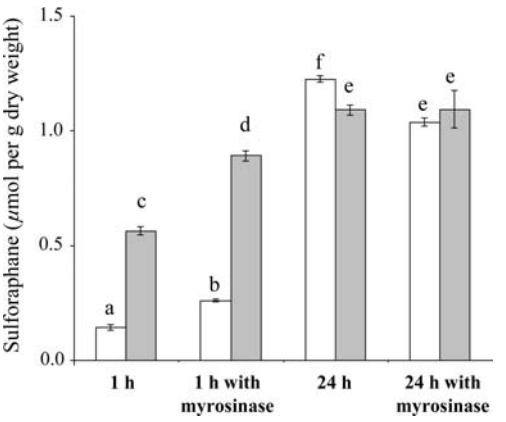

Figure 2. Sulforaphane formation in unheated (closed bar) and heated (open bar) Marathon broccoli florets after 1 and 24 h incubation with/without exogenous myrosinase (0.1 unit/mL). Values are mean ± SE, $n = 3$. Different letters indicate significant differences ($p \leq 0.05$).

RESULTS AND DISCUSSION

Characterization of Plant Materials. Florets of the broccoli cultivar Peto 7 had significantly greater GSL content than florets of the broccoli cultivar Marathon, due mostly to a 6-fold increase in GRP, indicative of the broad range of GSL levels across common commercial varieties of broccoli (*2*) (**Table 2**). As expected, the GSL profile differed between seed and floret, with indole GSL being extremely low in seed and thus GRP a far greater proportion of total GSL (**Table 2**. It is this profile change that provides the advantage in using broccoli seed as a starting material for GRP extraction and purification. Heating Marathon broccoli florets had no effect on any individual GSL (**Table 2**), confirming that GSL are relatively stable to heat, and therefore any difference in bioactivity between cooked and uncooked broccoli is unlikely to be due to a change in GSL profile. When Marathon seed was hydrolyzed using endogenous myrosinase, by homogenization in water, SF production was rapid, being ~90% complete within the first hour (**Figure 1**). Addition of excess exogenous myrosinase had little impact on SF formation, reflecting the large amount of myrosinase naturally present in seed. In contrast, unheated Marathon broccoli florets only converted ~50% GSL within the first hour, reflecting the lower level of myrosinase in florets. When excess exogenous myrosinase was added, conversion within the first hour only rose to 80% (**Figure 2**). These data show that even excess exogenous myrosinase requires more than an hour to complete hydrolysis within floret tissue, suggesting that some GRP was not immediately exposed to the exogenous myrosinase. Hydrolysis was complete by 24 h, and the addition of exogenous myrosinase had no impact on SF formation at 24 h. Heating broccoli destroyed some, but not all endogenous myrosinase, so that ~10% GSL was hydrolyzed in the first hour, which was approximately doubled by the addition of exogenous myrosinase. This poor activity of excess exogenous myrosinase again suggests poor accessibility. Again, hydrolysis was complete at 24 h without addition of exogenous myrosinase. Incomplete hydrolysis during the first hour clearly shows the limitation of endogenous myrosinase-dependent hydrolysis of GRP that may occur during ingestion of broccoli, even if unheated. Typically, maceration, swallowing, and passage through the stomach is complete in less than an hour and thus even uncooked broccoli can be expected to be incompletely hydrolyzed prior to digestive destruction of myrosinase. This idea is supported by the finding that prolonged chewing of broccoli sprouts enhanced SF bioavailability (*21*). These data suggest that unheated, crushed seed could be an excellent dietary source of SF, having ~90% hydrolysis within the first hour. However, as little as 5 g seed/day reaches the human safety limit for erucic acid (*22*).

Intake, Absorption, and Excretion. In experiments 1A and 1B, there were no differences in body weight gain between groups (data not shown). Rats fed Peto 7 broccoli florets in experiment 1B ate significantly less on day one, and thereafter all animals in experiment 1 were pair-fed to the group eating the least (~12 g/rat). Although the gGRP rats received exactly 500 μmol/kg BW/day, given by gavage, other treatment group doses were subject to dietary intake, so the dGRP rats only received 437 μmol/kg BW/day and the rats given Peto 7 broccoli received 82 μmol/kg BW/day. During day four, all animals in experiment 1 were placed in metabolic cages and 24 h urines were collected to evaluate excretion of SF-NAC. The fractional SF-NAC excretion was similar in rats given GRP by gavage (27.6%) and in the diet (23.8%; **Table 3**). Although the fractional SF-NAC recovery in rats fed Peto 7 broccoli florets was significantly greater (62.5%), in absolute terms it remained far below the SF-NAC excreted from rats receiving either of the GRP diets: 50.9, compared to 138.1 and 103.8 μmol/kg BW for rats in the gGRP and dGRP groups, respectively (**Table 3**).

Safety. Four out of six rats receiving the GRP by gavage (gGRP) suffered acute inflammation, the data of which are

Table 3. Glucoraphanin Intake, Excretion, and Recovery

group	GRP intake (μmol/kg BW)	SF conjugate excretion (μmol/kg BW)[a]	% recovery
gGRP	500	138.1 ± 25.5 a	27.6
dGRP	436.7 ± 12.9	103.8 ± 18.1 a	23.8
20% Peto 7 broccoli florets	81.5 ± 2.1	50.9 ± 6.82 b	62.5

[a] Values are mean ± SE, $n = 5$ for day four 24 h urines. Different letters indicate significant differences ($p \leq 0.05$).

Table 4. Effect of Glucoraphanin on Cecum in F344 Rats[a]

group[b]	normal	mild inflammation	severe inflammation	total
control (gavage)	4	0	0	4
gGRP	0	2	2	4
gGRP+4	3	1	0	4
control (dietary)	6	0	0	6
dGRP	6	0	0	6
broccoli	6	0	0	6

[a] Stages of cecal inflammation were categorized as follows: normal (non-inflamed), mildly inflamed, or severely inflamed. Mild inflammation included an expanded submucosa (edema) and decreased mucin in mucosal goblet cells; severe inflammation included a greatly expanded submucosa, infiltration of submucosa by numerous neutrophils, and undetectable mucin in mucosal goblet cells, as well as severe engorgement of mucosal capillaries. [b] gGRP: gavaged with GRP; gGRP+4: gavaged with GRP followed by 4 days no GRP; dGRP: GRP mixed within the diet.

similar to results previously published (*14*) (**Table 4**). However, rats given a similar dose of GRP, incorporated into the diet rather than as a bolus by gavage, showed no inflammation, probably reflecting their ingestion over several hours rather than as a bolus, leading to far lower concentrations in the cecum. Furthermore, when gavaged rats were allowed to rest for four days without receiving any further GRP, there was significant improvement in the health of the cecum (**Table 4**). The number of rats exhibiting severe inflammation decreased from two to zero, and the number exhibiting mild inflammation decreased from two to one. No section of the gut other than cecum showed any inflammation, either immediately following four daily doses of GRP or after resting for four days (data not shown). Our previous study included a dose−response, showing a slight inflammation following four daily doses of 120 mg (275 μmol) GRP/kg BW and no inflammation following 60 mg/kg BW (137 μmol (*14*)), considerably more than the GRP dose from the 20% Peto 7 diet, 82 μmol/kg BW. In that earlier study, we also reported that a single dose caused no inflammation. Here we show that the inflammation is only temporary, with no sign of chronic inflammation as a result of this very high daily dose for four days (**Table 4**). Whereas these data confirm the safety of even large doses of GRP, they are also useful in considering the mechanism of hydrolysis in the absence of myrosinase. Because ITC are known irritants at high concentration, the appearance of inflammation in the cecum confirms the commonly held view that the GRP can be hydrolyzed by cecal microflora (*23*, *24*). The lack of any inflammation following dGRP is consistent with the idea that when GRP is incorporated into the diet, it does not reach the cecum as a bolus and therefore is at a far lower concentration. Yet GRP had a similar inducing effect on colonic NQO1 whether it was given by gavage or incorporated into the diet (see below).

Bioactivity. In experiment 1, a similar increase in colonic NQO1 was seen for rats receiving the Peto 7 broccoli floret diet, the GRP diet (dGRP), and the GRP by gavage (gGRP), (**Figure 3**). This was despite the fact that the GRP content of the Peto 7 broccoli floret diet was ~20% or less GRP than the purified GRP dose provided to the gGRP and dGRP groups.

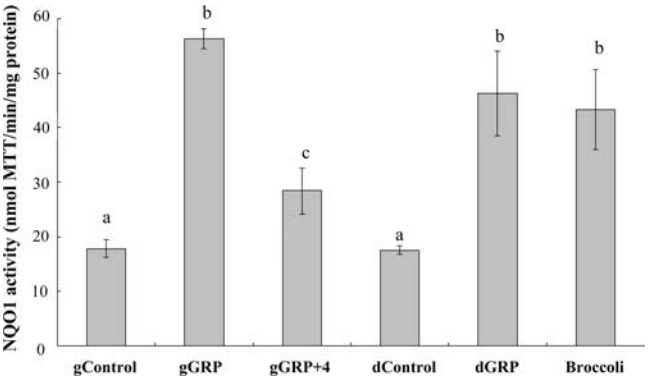

Figure 3. Effect of glucoraphanin, administered by gavage for four days (gGRP) or for four days followed by control diet for four days (gGRP+4) or in the diet (dGRP), or a Peto 7 broccoli floret diet on colonic mucosal NAD(P)H-quinone oxidoreductase 1 activities of rats. Bars represent mean ± SE, $n = 4$ (groups treated by gavage), $n = 6$ (groups treated by diet). Different letters indicate significant differences ($p \leq 0.05$).

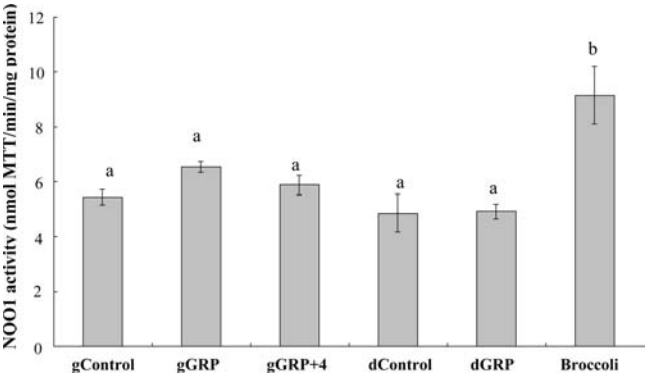

Figure 4. Effect of glucoraphanin, administered by gavage for four days-(gGRP) or for four days followed by control diet for four days (gGRP+4) or in the diet (dGRP) or a Peto 7 broccoli floret diet on hepatic NAD(P)H-quinone oxidoreductase 1 activity of rats. Bars represent mean ± SE, $n = 4$ (groups treated by gavage), $n = 6$ (groups treated by diet). Different letters indicate significant differences ($p \leq 0.05$).

This could reflect a far greater fractional SF formation and/or absorption when endogenous myrosinase is present. To test this idea, we re-examined total SF-NAC conjugate excreted (**Table 3**). Excreted SF-NAC from broccoli-fed rats was ~50% or less of the conjugate excreted from GRP-fed rats. Therefore, absorption of SF alone is insufficient to explain why Peto 7 broccoli florets were as effective as the larger doses of semipurified GRP in colonic NQO1 induction (**Figure 3**). An alternative explanation might be that other components, such as idole GSL, present in Peto 7 broccoli florets, also increase colonic NQO1 activity. Also shown in **Figure 3** is the colonic NQO1 activity of rats receiving GRP by gavage for four days and then no GSL (a control diet) for four days (gGRP+4). It is interesting to note that although greatly diminished, there is still significant activity four days after exposure to GSL had ceased.

In evaluating the systemic effect of these diets, we found that neither of the semipurified GRP diets caused hepatic NQO1 induction (**Figure 4**). In contrast, the Peto 7 broccoli floret diet caused a significant increase in hepatic NQO1. It is possible that in cecum there may be limited absorption of SF such that for diets where SF formation occurred in the cecum, plasma SF levels did not reach threshold for induction of hepatic NQO1. To this end, in a second experiment a low-GRP broccoli (Marathon) was fed

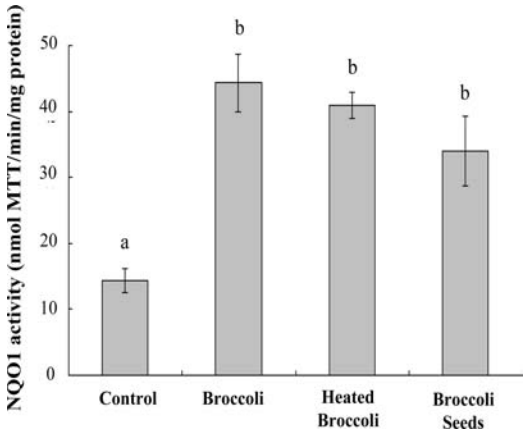

Figure 5. Effect of diets containing low-GRP Marathon broccoli florets, heated or unheated, or Marathon seed on colonic mucosal NAD(P)H-quinone oxidoreductase 1 activity of rats. Bars represent mean ± SE, $n = 5$. Different letters indicate significant differences ($p \leq 0.05$).

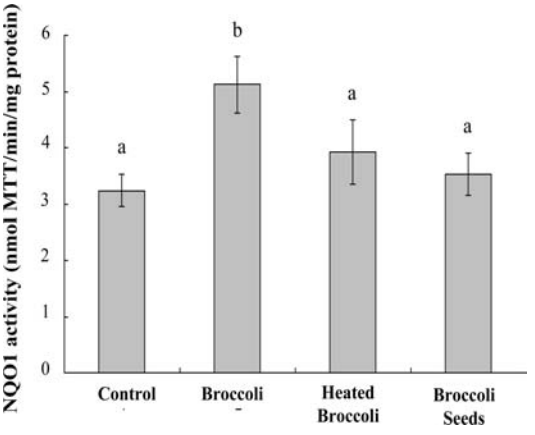

Figure 6. Effect of diets containing low-GRP Marathon broccoli florets, heated or unheated, or Marathon seed on hepatic NAD(P)H-quinone oxidoreductase 1 activities of rats. Bars represent mean ± SE, $n = 5$. Different letters indicate significant differences ($p \leq 0.05$).

to rats, as heated or unheated broccoli florets, in an effort to focus the difference between diets on the presence or absence of myrosinase. In addition, rats were fed crushed broccoli seeds, to test two hypotheses: (1) when GRP hydrolysis is rapid and thus occurs early in the digestive process, systemic bioactivity is improved, (2) the difference in bioactivity between broccoli florets and semipurified GRP seen in the first experiment is partly due to the presence of indole GSL in broccoli florets diet (seed has little or no indole GSL). Heated and unheated Marathon broccoli floret diets provided 16.5 and 14.5 μmol of GRP/kg BW/day, respectively; seed provided 32.7 μmol GRP/kg BW/day. These two diets and the broccoli seed diet all caused a similar, significant increase in NQO1 activity in colon (**Figure 5**) even though myrosinase activity and early hydrolysis was very different across diets (**Figures 1**, **2**). In evaluating the impact of these diets on hepatic NQO1, even though Marathon broccoli florets had much lower GSL content than the Peto 7 used in experiment 1, still the unheated Marathon diet caused a significant increase in hepatic NQO1 (**Figure 6**). The bioactivity of heated Marathon broccoli florets and of broccoli seed diets in the colon was not reflected in the liver. These data confirm our earlier finding that purified GRP did not upregulate hepatic NQO1 (*14*) and extend this to include heated broccoli and broccoli seed. Further studies are needed to determine the cause(s) for this lack of systemic activity.

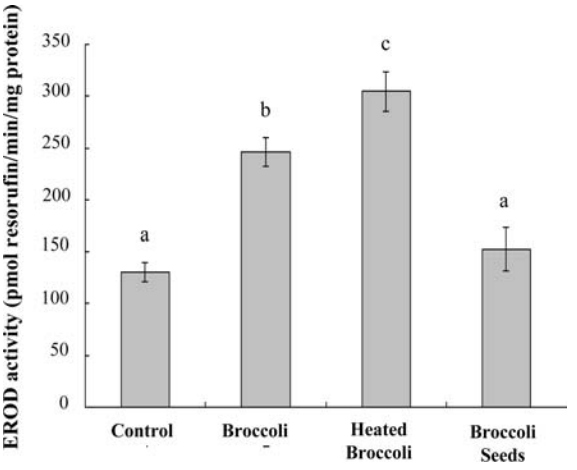

Figure 7. Effect of diets containing Marathon broccoli florets, heated or unheated, or Marathon broccoli seed on hepatic ethoxyresorufin *O*-deethylase activity in rats. Bars represent mean ± SE, $n = 5$. Different letters indicate significant differences ($p \leq 0.05$).

One possible cause is that, if absorption from the lower gut is limiting, plasma levels might not reach threshold and thus lack of activity might be overcome with larger doses. The seed meal contained substantial myrosinase and GRP; one possible cause for the lack of activity from seeds, compared to unheated broccoli, may be that only the broccoli florets contained indole GSL, which may reflect a role for indole hydrolysis products in synergizing NQO1 induction systemically (*25*).

Ethoxyresorufin *O*-deethylase activity is considered representative of CYP1A activity and classically associated with indole GSL hydrolysis products as well as many polycyclic precarcinogens (*26*). It was not surprising to us therefore that purified GRP, given by gavage (*14*) or in the diet (data not shown), caused no increase in EROD. In contrast, 20% Marathon broccoli florets, but not broccoli seed, caused a significant increase in hepatic EROD, reflecting the presence of indole GSL hydrolysis products in the broccoli floret diet (**Figure 7**). Furthermore, when heated and unheated broccoli diets were compared, unlike the response of NQO1, the EROD response was greatest for the heated diet (**Figure 7**). It is possible that the reason for this is the presence of pyrolysis products formed during heating that are able to upregulate CYP1A. Protein pyrolysis products have been reported to upregulate CYP1A1 (*27*).

In conclusion, this study shows that when myrosinase activity is very low or absent, either by heating or purification, GRP exerts no systemic effect. In the presence of myrosinase and the broccoli matrix, even diets containing very small amounts of GRP upregulated hepatic NQO1. However, when these small doses were in the seed matrix with high myrosinase but very low levels of indole GSL, no effect was seen in liver. Further studies are needed to determine the reason for the greater efficacy exerted by the broccoli matrix and whether synergy by indoles plays a role.

ABBREVIATIONS USED

ANOVA, analysis of variance; BW, body weight; dGRP, group treated with dietary GRP; EROD, ethoxyresorufin *O*-deethylase; GB, glucobrassicin; gGRP, group gavaged with GRP; gGRP+4, group gavaged with GRP and 4 days rest; GRP, glucoraphanin; GSL, glucosinolates; I3C, indole-3-carbinol; ITC, isothiocyanate; NQO1, NAD(P)H-quinone oxidoreductase 1; SAS, Statistical Analysis System; SF, sulforaphane; SF-NAC, sulforaphane *N*-acetylcyteine conjugate.

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