

Effects of Dietary Broccoli on Antioxidant Enzymes,
*Ole Vang,^{1,4} Brian F. Rasmussen,² Hilmer Sørensen,³
Jørgen Clausen,¹ and Ole Andersen¹* (¹ Dept. of Life Sci.
and Chem., Roskilde Univ., DK-4000 Roskilde,
Denmark; ² Dept. of Community Health, Odense Univ.,
DK-5000 Odense C, Denmark; ³ Chem. Dept., Royal Vet.
and Agric. Univ., DK-1871 Frederiksberg C, Denmark;
⁴ author for correspondence: fax +45-4675-7721, e-mail
ov@mmf.ruc.dk)

Epidemiological and experimental animal studies indicate that dietary fresh fruit and vegetables decrease the risk of coronary heart disease and cancer (1–3). Besides vitamins and fibers, several groups of compounds are present in diets enriched in plant food. The plant constituents, e.g., carotenoids, polyphenols, flavonoids, and glucosinolates, may have protective potential. Several recent studies focused on the effects of dietary antioxidants on development of cancer and atherosclerosis. In a Dutch study, the reduced risk of coronary heart disease correlated well with the intake of flavonoids (4). Flavonoids, as aglycones, display such biochemical effects as antioxidative action, induction, and (or) inhibition of specific cytochrome P-450 (CYP) isoforms and glutathione S-transferase (GST) isoforms (5) and inhibition of glutathione reductase (GSSG-Red; EC 1.6.4.2) (5), which might play a key role in the protective effects of these compounds.

Indole compounds, derived from glucosinolates and found in relatively high concentrations in cruciferous vegetables (6), repress several types of chemically induced cancer. Indol-3-ylcarbinol, or condensates thereof, modulate several biochemical biomarkers in humans and animals, increasing the activities of several CYP isoenzymes (7) and GST (8). Our report describes studies of the effects of dried broccoli on the activities of several antioxidant enzymes [GSSG-Red, glutathione peroxidase (GSH-Px; EC 1.11.1.9), and superoxide dismutase (SOD; EC 1.15.1.1)] in the liver, kidney, and colon of rats.

Four samples of broccoli from the Danish Institute of Plant and Soil Sciences (*Brassica oleracea italica*, cultivar Shogun) were obtained by growing the broccoli at different fertilizer concentrations (per hectare): S-0, 0 kg of S and 175 kg N; S-100, 100 kg of S and 175 kg of N; N-0, 25 kg of S and 0 kg N; and N-400, 25 kg of S and 400 kg of N. The plant materials were lyophilized (broccoli powder), and aliquots were taken for analysis of the content of glucosinolates as described previously (9). The broccoli powders were then incubated in phosphate-buffered saline (80 mmol/L Na₂HPO₄, 20 mmol/L NaH₂PO₄, 100 mmol/L NaCl, pH 7.5; 4 mL/g broccoli powder) at room temperature for 2 h, during which time the glucosinolates were exposed to endogenous myrosinase, which degrades the glucosinolates. After incubation, the broccoli samples were dried and aliquots again were taken for glucosinolate analysis.

The basic rat diet was a purified powdered semisynthetic diet. The energy contribution was 18% from protein (caseinate), 70% from carbohydrate (sugar, dextrin, corn meal, and potato meal), and 12% from fat [coconut oil, soy oil, Omekol® (fish oil from Nykomed DAK, Copenhagen, Denmark), and olive oil]. The P:S ratio was 0.7. The control diet also contained 70 g/kg nondigestible fibers (cellulose; AG-Frisenette, Aarhus, Denmark). In the broccoli diets, 100 g/kg of the broccoli samples was added to the basic diet by isocaloric exchange, calculated from the

energy composition of the broccoli and the amount of added fiber adjusted to assure that the energy composition and fiber contents of all diets were similar. Assuming that the daily intake of the powdered diet was 15 g per rat, the intake of dried broccoli corresponds to 12 g of fresh broccoli.

Fifty male Wistar rats (age 8 weeks, 250 g; Møllegaard, Ll. Skensved, Denmark) were kept on beechwood bedding in a well-controlled environment (50% ± 5% relative humidity, 20 air changes/h, temperature 21 ± 1 °C, light/dark periods 12/12 h with 0.5-h twilights) with free access to standard rat pellets (Brogaard; Chr. Petersen, Ringsted, Denmark) and drinking water. After 1 week for acclimation, the rats were fed the control diet for 3 weeks and then divided into five groups that received the control diet or the four different broccoli diets for 1 week longer. Each animal was killed by cervical dislocation, and the liver, kidney, and colon were removed to ice. The following steps were performed at 0–4 °C: The colonic mucosa was removed from the underlying tissue by scraping. The liver, kidney, and colon mucosa were homogenized in 154 mmol/L KCl, 10 mmol/L Tris-HCl (pH 7.4), 1 mmol/L EDTA, and 0.25 mmol/L phenylmethylsulfonyl fluoride and centrifuged sequentially (9000g for 30 min and the supernates at 105 000g for 1 h longer). The supernates thus obtained were stored at –80 °C until use.

Biochemical analyses (GSSG-Red, GSH-Px, and SOD) were performed by the Cobas Mira procedure (Roche Diagnostic System). GSSG-Red and total GSH-Px activities were measured according to the method of Wheeler et al. (10), except the pH of the reaction buffer and the temperature were 7.4 and 37 °C, respectively. SOD activity was determined with a commercial kit (Ransod; Randox Labo, Crumlin, UK).

Enzyme activities were compared by ANOVA (Systat, Evanston, IL). If a statistically significant difference was found ($P < 0.05$), activities were further tested as to (a) whether dried broccoli altered the responses and (b) whether differences between S-0 and S-100 broccoli or between N-0 and N-400 broccoli occurred. The correlation between the enzyme activities and the glucosinolate concentrations was analyzed by the Systat data program.

No statistical differences were observed in body, liver, and kidney weights between groups fed the diets for 1 week. The activities of the antioxidant enzymes varied depending on the broccoli diets used (Table 1). One week of feeding on broccoli diet per se induced the activities of the three antioxidant enzymes in all three organs investigated, with two exceptions. The hepatic GSH-Px activity was not substantially increased, and the colonic GSSG-Red activity was increased only in rats fed the N-400 sample diet. The activities of renal GSSG-Red, colonic GSH-Px, and hepatic SOD were induced differentially depending on the broccoli sample used in the diet. With one exception, the activities of the antioxidant enzymes were increased but were less increased in rats eating diets containing broccoli grown without addition of fertilizer, indicating that the concentrations of several compounds in the broccoli vary with the growth conditions.

The activity of hepatic GSSG-Red correlated with the content of glucobrassicin ($r = 0.99$, $P = 0.001$), neoglucobrassicin ($r = 0.91$, $P = 0.03$), and glucoerucin ($r = 0.94$, $P = 0.02$), whereas the hepatic SOD activity correlated with glucoerysolin ($r = 0.98$, $P = 0.002$). Renal GSH-Px

Table 1. Effects of dietary intake of broccoli powder on enzyme activities in rat organs.

	Mean \pm SD activity per mg protein		
	Liver	Kidney	Colon
<i>GSSG-Red</i> ($\mu\text{mol}/\text{min NADPH oxidized}$)			
Control	0.15 \pm 0.022 ^a	0.33 \pm 0.059 ^b	0.49 \pm 0.079
S-0	0.17 \pm 0.009	0.34 \pm 0.064	0.48 \pm 0.049
S-100	0.19 \pm 0.032	0.41 \pm 0.037 ^f	0.52 \pm 0.076
N-0	0.16 \pm 0.016	0.37 \pm 0.020	0.40 \pm 0.089
N-400	0.17 \pm 0.033	0.43 \pm 0.028 ^g	0.66 \pm 0.085 ^h
<i>GSH-Px</i> ($\mu\text{mol}/\text{min NADPH oxidized}$)			
Control	5.7 \pm 0.71	2.5 \pm 0.71 ^c	0.34 \pm 0.052 ^d
S-0	6.2 \pm 0.8	3.1 \pm 0.85	0.21 \pm 0.093
S-100	6.1 \pm 1.7	3.6 \pm 0.88	0.48 \pm 0.098 ⁱ
N-0	5.7 \pm 1.1	3.1 \pm 0.39	0.36 \pm 0.10
N-400	6.9 \pm 1.3	3.5 \pm 0.70	0.67 \pm 0.092 ^j
<i>SOD</i> (U)			
Control	6.7 \pm 1.6 ^e		
S-0	7.4 \pm 1.8		
S-100	9.8 \pm 2.1 ^k		
N-0	9.1 \pm 1.7		
N-400	8.3 \pm 2.0		

^{a-e} Significant difference between controls and broccoli-treated animals: ^a*P* = 0.013, ^b*P* = 0.001, ^c*P* = 0.002, ^d*P* = 0.011, ^e*P* = 0.011.

^{f-k} Significant difference between S-0 and S-100 or between N-0 and N-400: ^f*P* = 0.001, ^g*P* = 0.009, ^h*P* = 0.000, ⁱ*P* = 0.000, ^j*P* = 0.000, ^k*P* = 0.01.

activity correlated with the content of glucoerucin ($r = 0.92$, $P = 0.03$) and glucobrassicin ($r = 0.89$, $P = 0.04$).

A dietary intake of cruciferous vegetables induces several phase I and phase II enzymes: different CYP isoforms in rats (11) and humans (12), as well as different GST isoforms in rats (13) and humans (14). Our study also demonstrates that activities of important antioxidant enzymes are modulated by broccoli constituents or their products. The increased hepatic SOD activity counteracts the potential toxic effects of superoxide by catalyzing its conversion to hydrogen peroxide. Therefore, dietary broccoli conceivably would decrease the superoxide concentration.

Additionally, GSH-Px catalyzes the conversion of peroxides to alcohols. Dried broccoli increased the GSH-Px activities in kidney and colon. The renal GSSG-Red activity is also increased by some of the broccoli samples. An increased GSH-Px activity may decrease the potential toxic effect of peroxides. Chen et al. (15) found that the glutathione concentration of different segments of intestinal mucosa was increased by dietary cruciferous vegetables. Because increased GSSG-Red activities would increase the available concentration of glutathione, our results agree with those of Chen et al.

Broccoli contains several substances that may upregulate the activities of these antioxidant enzymes (6). The contents of different glucosinolates, e.g., glucoerysolin, varied up to 10-fold (O. Vang et al., manuscript in preparation) in the different broccoli batches used in our study. The concentrations of glucobrassicin and neoglucobrassicin, from which indol-3-ylcarbinol and *N*-methoxyindol-3-ylcarbinol, respectively, are generated, correlated with the activity of hepatic GSSG-Red. Indol-3-ylcarbinol has been found to increase rat hepatic GSSG-Red activity (16).

In conclusion, broccoli upregulates GSH-Px, GSSG-

Red, and SOD in liver, kidney, and colon. The effect varies with the batch of broccoli used in relation to the amounts of active compounds present in the broccoli, especially glucosinolates and derived products. The observed effects of dried broccoli are probably caused by the combined effects of several broccoli constituents or their products. Data from our study support the hypothesis that increased consumption of cruciferous vegetables may increase the activities of antioxidant enzymes, which in turn may decrease the risk of oxidative stress.

References

- Steinmetz KA, Potter JD. Vegetables, fruit, and cancer: I. Epidemiology. *Cancer Causes Control* 1991;2:325-57.
- Hubbard RW, Mejia A, Horning M. The potential of diet to alter disease processes. *Nutr Res* 1994;14:1853-95.
- Izzotti A, D'Agostini F, Bagnasco M, Scatolini L, Rovida A, Balansky RM, et al. Chemoprevention of carcinogen-DNA adducts and chronic degenerative diseases. *Cancer Res* 1994;54:1994s-8s.
- Hertog MGL, Feskens EJM, Hollman PCH, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet* 1993;342:1007-11.
- Iio M, Kawaguchi H, Sakota Y, Otonari J, Nitahara H. Effects of polyphenols, including flavonoids, on glutathione *S*-transferase and glutathione reductase. *Biosci Biotechnol Biochem* 1993;57:1678-80.
- Hansen M, Møller P, Sørensen H. Glucosinolates in broccoli stored under controlled atmosphere. *J Am Hort Sci* (in press).
- Helzlsouer KJ. Epidemiology, early detection, and prevention of breast cancer. *Curr Opin Oncol* 1993;5:955-9.
- Stresser DM, Williams DE, McLellan LI, Harris TM, Bailey GS. Indole-3-carbinol induces a rat liver glutathione transferase subunit (Yc2) with high activity toward aflatoxin B-1 exo-epoxide: association with reduced levels of hepatic aflatoxin-DNA adducts in vivo. *Drug Metab Dispos* 1994;22:392-9.
- Michaelsen S, Møller P, Sørensen H. Factors influencing the separation and quantitation of intact glucosinolates and desulphoglucosinolates by micellar electrokinetic capillary chromatography. *J Chromatogr* 1992;608:363-74.
- Wheeler CR, Salzman JA, Elsayed NM, Omaye ST, Korte DW Jr. Automated assays for superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase activity. *Anal Biochem* 1990;184:193-9.
- Vang O, Jensen H, Autrup H. Induction of cytochrome P-450IA1, IA2, IIB1, IIB2 and IIE1 by broccoli in rat liver and colon. *Chem Biol Interact* 1991;78:85-96.
- Kall M, Vang O, Andersen O, Clausen J. In vivo induction of human phase I and phase II enzymes by diet [Abstract]. *J Cell Biochem Suppl* 1995;19A:197.
- Wortelboer HM, De Kruif CA, Van Iersel AAJ, Noordhoek J, Blaauboer BJ, Van Bladeren PJ, et al. Effects of cooked brussels sprouts on cytochrome P-450 profile and phase II enzymes in liver and small intestinal mucosa of the rat. *Food Chem Toxicol* 1992;30:17-27.
- Bogaards JJ, Verhagen H, Willems MI, van Poppel G, Van Bladeren PJ. Consumption of brussels sprouts results in elevated alpha-class glutathione *S*-transferase levels in human blood plasma. *Carcinogenesis* 1994;15:1073-5.
- Chen MF, Chen LT, Boyce HW. Cruciferous vegetables and glutathione: their effects on colon mucosal glutathione level and colon tumor development in rats induced by DMH. *Nutr Cancer* 1995;23:77-83.
- Shertzer HG, Sainsbury M. Chemoprotective and hepatic enzyme induction properties of indole and indenoindole antioxidants in rats. *Food Chem Toxicol* 1991;29:391-400.

Comet Assay: New Biomarker for Detecting Genotoxicity Induced by Chemicals and Radiation, Ulla Plappert,¹ Klaus Raddatz, Wolfgang Rieth, and Theodor