Nutrition and Cancer

Breast Cancer Risk in Premenopausal Women Is Inversely Associated with Consumption of Broccoli, a Source of Isothiocyanates, but Is Not Modified by GST Genotype

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ABSTRACT The role of vegetable consumption in relation to breast cancer risk is controversial. Anticarcinogenic compounds may be present only in specific vegetables, thereby attenuating findings for total vegetable intake. Cruciferous vegetables contain precursors of isothiocyanates (ITCs), which may be chemopreventive through potent inhibition of phase I, and induction of phase II enzymes, such as glutathione *S*-transferases (GSTs). We investigated associations between consumption of cruciferous vegetables, sources of ITCs, and breast cancer risk, and potential modification of relations by *GSTM1* and *GSTT1* genotypes. Cases ($n = 740$) were Caucasian women with incident breast cancer identified from all major hospitals in Erie and Niagara counties. Community controls ($n = 810$) were frequency matched to cases by age and county. An in-depth interview including a validated FFQ was administered in person. Odds ratios (ORs) and 95% CIs were used to estimate relative risks. Consumption of cruciferous vegetables, particularly broccoli, was marginally inversely associated with breast cancer risk in premenopausal women [4th quartile OR = 0.6, 95% CI (0.40–1.01), $P = 0.058$]. Associations were weaker or null among postmenopausal women. No significant effects of GST genotype on risk were observed in either menopausal group. These data indicate that cruciferous vegetables may play an important role in decreasing the risk of premenopausal breast cancer. J. Nutr. 134: 1134–1138, 2004.

KEY WORDS: • *breast cancer* • *cruciferous vegetables* • *isothiocyanates* • *glutathione S-transferases* • *women*

Data from epidemiologic studies indicate that diets high in vegetables reduce the risk of aerodigestive cancers (1), and may be associated with decreased risk of other cancers, including breast cancer (2). A recent meta-analysis (2) of 14 case-control studies and 3 cohort studies found that high vs. low vegetable consumption was associated with a 25% reduction in risk, but these findings were not corroborated in a pooled analysis of 8 cohort studies (Pooling Project) by Smith-Warner et al. (3).

Vegetable consumption could reduce breast cancer risk by numerous mechanisms, specific to particular vegetable families, including sources of carotenoids, vitamins A, E, and C, minerals such as selenium, and such compounds as isoflavones and lignans. Glucosinolates, found in cruciferous vegetables, may be important anticarcinogens. Interestingly, although the Pooling Project found no overall effects of total vegetable consumption on breast cancer risk, there were inverse, although nonsignificant, associations between risk and high consumption of the cruciferous vegetables, broccoli and brussels sprouts. Similarly, although total vegetable consumption did not decrease breast cancer risk in a large case-control study in Sweden (4), inverse associations were noted between risk and intake of cruciferous vegetables.

The anticarcinogenic effects of cruciferous vegetables may derive from the glucosinolates they contain; these are degraded into indoles and isothiocyanates. Indoles were studied in relation to breast cancer partly because of their effects on estrogen metabolism (5), and isothiocyanates (ITCs)[2] are chemopreventive agents in animal models, perhaps due in part to their potent effects on inhibition of phase I and induction of phase II enzymes (6).

Because ITCs, particularly sulforaphane, affect phase II enzyme activity and are also substrates for glutathione *S*-transferases (GSTs), the role of *GSTM1* and *GSTT1* polymorphisms was examined in relation to ITCs and the risk of adenomas and cancers of the colon and lung. In the majority of these studies, GST genotypes modified relations between ITCs and risk (7–10), with stronger inverse associations noted

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[2] Abbreviations used: GST, glutathione *S*-transferase; ITC, isothiocyanate; OR, odds ratio.

0022-3166/04 $8.00
Manuscript received 12 December 2003. Initial review completed 4 January 2004. Revision accepted 5 February 2004.

for those with high consumption and null genotypes, although 1 study found no association between colon cancer and *GSTM1* genotype (11).

In this study, specifically designed to evaluate associations between diet and risk of breast cancer, we investigated the effects of consumption of cruciferous vegetables on risk. We also considered the potential modulation of those associations by polymorphisms in *GSTM1* and *GSTT1*.

MATERIALS AND METHODS

Study population. Caucasian women diagnosed with incident, primary, histologically confirmed breast cancer ($n = 740$) were identified from all of the major hospitals in Erie and Niagara counties between 1986 and 1991, as previously reported (12–14). Cases were frequency matched by age and county of residence with controls ($n = 810$) randomly selected from the New York State Motor Vehicle lists (<65 y old) and the Health Care Finance administration rolls (≥65 y old). After receiving informed consent, cases and controls were interviewed in person regarding known and suspected breast cancer risk factors; all data were self-reported. The Institutional Review Board of the State University of New York at Buffalo reviewed and approved the protocol for this study.

Dietary assessment. An extensive, validated FFQ (12) was administered by the interviewer. Participants were asked about usual intake of 170 foods during the year 2 y before the diagnosis for cases and before the interview for controls. Portion size, using food models, and intake frequency were assessed to estimate grams of intake, and separate questions were asked for raw vs. cooked vegetables. Self-reported cruciferous vegetable intake was calculated as the sum of grams of intake (portion size × frequency) per month of broccoli, cabbage, sauerkraut, coleslaw, cauliflower, and brussels sprouts.

Laboratory analysis. At the conclusion of the interview, permission was requested for a subsequent blood collection; ~54% of the women provided blood samples. DNA was extracted from blood clots (14), and PCR was used to determine the polymorphic *GSTT1* and *GSTM1* genotypes, i.e., presence or absence of the allele. *GSTM1* and *GSTT1* genotypes were determined by PCR restriction fragment length polymorphism as previously described, with multiplexing of *GSTM1* and *GSTT1*, using *CYP1A1* as an internal control to confirm amplification (15–17), and with 10% duplicates included. GST genotypes were classified as either null (i.e., homozygous deletion) or present (i.e., heterozygous or homozygous for the presence of each gene).

Data analysis. Women were considered postmenopausal if they were <50 y old and had self-reported natural menopause, bilateral oophrectomy, or irradiation of the ovaries. For women ≥ 50 y old, menopausal status was assigned on the basis of self-reported cessation of menstruation, except for women taking hormone replacement therapy, who were classified as postmenopausal regardless of whether their menstrual periods had stopped. Reported BMI 2 y before the reference date was calculated as weight divided by the square of height (kg/m^2), and was included as a continuous variable. Age at menarche, age at first pregnancy, and age at menopause were all treated as continuous variables. Family history of breast cancer (yes/no) was dichotomized. Broccoli and total cruciferous vegetable intake was categorized into quartiles based on distribution in controls, and was calculated separately for pre- and postmenopausal women due to differences in consumption patterns.

Because risk factors may differ between pre- and postmenopausal women (14,18), all analyses were conducted separately by menopausal status. Student's *t* tests were utilized to determine mean case-control differences in lifestyle and reproductive factors. Odds ratios (OR) and 95% CIs were calculated from unconditional logistic regression models, using SPSSWIN version 11.0 statistical package (SPSS). ORs evaluating associations between cruciferous vegetable intake and breast cancer risk were adjusted in multivariate models for age, education, and standard breast cancer risk factors (age at menarche, age at first pregnancy, BMI, family history of breast cancer, and age at menopause for postmenopausal women). Modification of risk by *GSTT1* and *GSTM1* genotype was evaluated by calculating adjusted ORs for cruciferous vegetable intake and breast cancer risk within categories (allele present or absent) of genotypes. Associations with an α-level < 0.05 were considered significant.

TABLE 1

Monthly intake of cruciferous vegetables by pre- and postmenopausal women with and without breast cancer in the Western New York Diet Study[1]

	Premenopausal		Postmenopausal	
	Cases	Controls	Cases	Controls
n	301	316	396	494
Broccoli				
Intake, *g/mo*	779.6 ± 55.0	850.7 ± 46.2	639.9 ± 36.7	674.5 ± 40.2
Median, *g/mo*	512.0	624.0	356.0	370.0
Serving/mo,[2] *n*	10.0	11.0	8.0	8.5
Brussels sprouts				
Intake, *g/mo*	81.8 ± 9.3	78.0 ± 7.9	78.3 ± 7.9	86.8 ± 9.9
Median, *g/mo*	39.0	39.0	39.0	39.0
Serving/mo, *n*	1.0	1.0	1.0	1.0
Cauliflower				
Intake, *g/mo*	381.8 ± 34.1	400 ± 25	289.1 ± 16.7*	341.3 ± 20.7
Median, *g/mo*	220.0	257.0	150.0	220.0
Serving/mo, *n*	6.0	6.5	4.5	5.5
Cabbage				
Intake, *g/mo*	72.3 ± 7.1	77.0 ± 6.3	105.3 ± 9.6	108.9 ± 8.1
Median, *g/mo*	52.0	52.0	52.0	52.0
Serving/mo, *n*	1.0	1.0	1.5	1.5
Total cruciferous				
Intake, *g/mo*	1531.0 ± 85.8	1649.6 ± 69.1	1368.4 ± 52.3	1479.4 ± 59.9
Median, *g/mo*	1118.0	1287.0	1118.0	1125.5
Serving/mo, *n*	22.0	23.5	19.5	21.0

[1] Values are means ± SE unless noted otherwise. *Different from control, $P = 0.05$.
[2] Values are approximate means.

RESULTS

Consumption of cruciferous vegetables was higher overall among premenopausal women than among postmenopausal women. For both groups, controls reported higher consumption than cases, although no differences were significant (P = 0.05–0.80; **Table 1**). For the most part, this trend was evident for individual cruciferous vegetables as well as for total intake. Servings per month of broccoli and total cruciferous vegetables generally were higher than those reported in the National Health and Nutrition Examination Survey (broccoli: 4.8 and 5.3 servings/mo; total cruciferous vegetables: 9.2 and 12.3 servings/mo; women < 50 y old and ≥50 y old, respectively) (personal communication, Carlos Crespo, Ph.D., Department of Social and Preventive Medicine, University at Buffalo, Buffalo, NY).

When data were categorized and each quartile of cruciferous vegetable consumption evaluated in relation to the lowest quartile of intake (the referent group, with OR = 1.0), there was evidence of nonsignificant inverse associations (P = 0.13, 0.09, 0.07 for 2nd, 3rd, and 4th quartiles, respectively) between higher levels of consumption and premenopausal breast cancer risk (**Table 2**), with an adjusted fourth quartile OR of 0.7 (95% CI, 0.4–1.0). Similar weak relationships were observed among postmenopausal women (4th quartile OR = 0.8, 95% CI, 0.6–1.2, P = 0.41). Because broccoli is a rich source of isothiocyanates, particularly sulforophrane, we also evaluated the association between broccoli consumption and breast cancer risk. Marginally significant (P = 0.05, 0.06 for 3rd and 4th quartiles) inverse associations were noted among premenopausal women (4th quartile adjusted OR = 0.6, CI, 0.4–1.0). There was no effect of broccoli consumption among postmenopausal women (OR = 1.0, CI, 0.7–1.4).

Only a proportion of women who were interviewed agreed to provide a blood specimen, and genotype data were available for 212 premenopausal and 208 postmenopausal women. We compared putative breast cancer risk factors, cruciferous vegetable consumption, and risk associated with cruciferous vegetable consumption in this subset of women with the overall study sample (data not shown). Women with genotyping data were similar to those in the larger data set in regard to putative breast cancer risk factors, but differed in dietary practices. There were differential distributions in the tertiles of cruciferous vegetable consumption between those in the total data set and those who provided a blood specimen, and although we noted nonsignificant inverse associations between risk of breast cancer and consumption of cruciferous vegetables in the total data set, these relationships were less apparent in the subset with genotyping data. However, consumption of broccoli was similar among cases and controls who gave blood and those who refused, and similar inverse associations were noted among premenopausal women in both groups for consumption of broccoli, with no significant associations for postmenopausal women. For this reason, and because broccoli is a rich source of ITCs, only broccoli was evaluated in relation to *GSTT1* and *GSTM1* genotypes. Finally, because of the smaller numbers of women in this subset, dietary data were categorized into tertiles rather than quartiles to stabilize risk estimates.

As previously reported for *GSTM1* (15,17), the *GSTM1* and *GSTT1* null genotypes were not associated with breast

TABLE 2

Odds ratios and 95% CI for risk of breast cancer associated with cruciferous vegetable intake among pre- and postmenopausal women in the Western New York Diet Study[1,2]

	Case	Control	OR (95% CI)[3]
n	301	316	
	n (%)		
Premenopausal			
Total cruciferous vegetables, *g/mo*			
≤809	98 (32)	79 (25)	1.0
810–1282	72 (24)	79 (25)	0.7 (0.5–1.2)
1283–2041	68 (23)	79 (25)	0.7 (0.5–1.2)
>2041	63 (21)	79 (25)	0.7 (0.5–1.2)
Broccoli, *g/mo*			
≤305	94 (31)	77 (25)	1.0
305–624	82 (27)	82 (25)	0.8 (0.5–1.3)
625–1024	64 (21)	78 (25)	0.6 (0.4–1.0)
>1024	61 (20)	79 (25)	0.6 (0.4–1.0)
	n (%)		
Postmenopausal			
n	396	494	
Total cruciferous vegetables, *g/mo*			
≤659	121 (28)	123 (25)	1.0
659–1125	115 (26)	124 (25)	1.0 (0.7–1.4)
1126–1878	97 (22)	123 (25)	0.8 (0.5–1.1)
>1879	63 (24)	124 (25)	0.8 (0.6–1.2)
Broccoli, *g/mo*			
≤140	106 (24)	121 (25)	1.0
141–367	117 (27)	126 (25)	1.0 (0.7–1.5)
368–799	97 (22)	117 (25)	0.9 (0.6–1.3)
>800	114 (26)	123 (25)	1.0 (0.7–1.4)

[1] Values are OR and 95% CI.

[2] Not all numbers in cells sum to same total due to missing data.

[3] Adjusted for age, education, age at menarche, age at first pregnancy, family history of breast cancer, BMI, and age at menopause for postmenopausal women.

cancer risk in either pre- or postmenopausal women (data not shown). When associations were evaluated by *GSTT1* genotype, inverse associations with broccoli consumption tended to be greater among premenopausal women with null genotypes (3rd tertile OR = 0.3, 0.1–1.6), than among those with present alleles [OR = 0.7 (0.3–1.8); **Table 3**]. There were no inverse associations among postmenopausal women. In fact, there tended to be an increase in risk among those with null *GSTT1* with the highest reported intake of broccoli, although these risk relationships were not significant ($P = 0.55$) and the CIs were wide. For *GSTM1*, contrary to our hypothesis and findings for *GSTT1* among premenopausal women, inverse associations were evident only among women with present alleles although, again, associations were not significant ($P = 0.63$). Among postmenopausal women, inverse associations with high consumption were noted among those with null *GSTM1*, but not *GSTM1* present genotypes, as hypothesized.

DISCUSSION

In this case-control study specifically designed to examine associations between diet and breast cancer, we found that consumption of cruciferous vegetables, particularly broccoli intake, was associated with a reduced risk of premenopausal breast cancer. Significant risk reduction was not noted for postmenopausal women. The findings of inverse associations between cruciferous vegetable consumption and premenopausal breast cancer risk support those of a Swedish case-control study (4) and the Pooling Project (3).

Much of the focus in the past on compounds in cruciferous vegetables that could reduce risk of breast cancer has been on indole-3-carbinol. Michnovicz and Bradlow (19) and others hypothesized that indole-3-carbinol has antiestrogenic effects, primarily through induction of 2-hydroxylation of estradiol, resulting in nonestrogenic metabolites. Indole-3-carbinol can bind to the estrogen receptor with low affinity (20), which exerts antiestrogenic activities, represses 17β-estradiol activated ERα signaling, and downregulates the expression of estrogen-responsive genes (21).

Isothiocyanates, formed from glucosinolates in cruciferous vegetables, could also be responsible for inverse associations between cruciferous vegetable consumption and breast cancer risk. The chemopreventive effect of ITCs observed in animal models is likely due in part to their indirect effects on metabolism of xenobiotics, through inhibition of phase I activating enzymes (e.g., cytochrome P_{450}), and induction of phase II detoxifying enzymes (e.g., GSTs) (6). Through this mechanism, increased induction of phase II enzymes could lead to quicker and more extensive excretion of reactive intermediates, whether their source is chemical carcinogens, reactive oxygen species, or hormone metabolites that are substrates for GSTs. ITCs are also substrates for metabolism by GSTs, and it was reported (22) that *GSTM1* converts sulforaphane to the GSH conjugate and subsequent excretion. Based on the hypothesis that *GSTM1* can metabolize ITCs, particularly sulforaphane, and abrogate their chemopreventive effects, studies were conducted to investigate interactions between GST genotypes, cruciferous vegetable intake or urinary ITCs, and risk of cancer and adenomatous polyps. Lin et al. (7) first evaluated this hypothesis, finding inverse associations between broccoli consumption and risk of colorectal adenomas, which were attributed to those with *GSTM1* null genotypes. This was not found in relation to colon cancer, however (11).

Associations were also investigated in relation to lung cancer risk for which carcinogen exposure is a known risk factor, and the detoxifying effects of the GSTs may play a more important role. London and colleagues (8) found that individuals with detectable urinary isothiocyanates were at decreased risk of lung cancer, and risk was lowest among those who carried deletions in *GSTM1* and *GSTT1*. Spitz et al. (9) found that risk was greatest among those with GST null genotypes who were low consumers of ITCs. Among higher consumers of ITCs, risk was greater for those with null alleles than those with present genotypes. These data argue that the

TABLE 3

Odds ratios and 95% CI for risk of breast cancer associated with broccoli intake by GST genotypes among pre- and postmenopausal women in the Western New York Diet Study[1]

	Cases	Controls	OR (95% CI)[2]	OR (95% CI)
	n			
			Premenopausal	
Broccoli intake, *g/mo*			GSTT1−	GSTT1+
≤380	34	35	1.0	1.0
380–966	36	28	1.1 (0.3–4.2)	0.7 (0.5–1.2)
>966	35	44	0.3 (0.1–1.6)	0.7 (0.3–1.8)
			GSTM1−	GSTM1+
≤380	40	38	1.0	1.0
380–966	43	31	1.3 (0.5–3.5)	0.9 (0.3–2.3)
>966	22	38	1.0 (0.4–3.1)	0.7 (0.1–1.2)
			Postmenopausal	
			GSTT1−	GSTT1+
≤234	32	24	1.0	1.0
234–635	36	45	0.7 (0.2–2.9)	0.8 (0.3–1.9)
>635	35	36	2.1 (0.5–9.7)	0.7 (0.3–1.7)
			GSTM1−	GSTM1+
≤234	32	24	1.0	1.0
234–635	36	45	0.7 (0.2–2.1)	0.6 (0.2–1.6)
>635	35	36	0.5 (0.2–1.5)	1.0 (0.4–3.0)

[1] Values are OR and 95% CI.

[2] Adjusted for age, education, age at menarche, age at first pregnancy, family history of breast cancer, BMI, and age at menopause for postmenopausal women.

inducing effects of ITCs on GSTs may be more important than their role in the metabolism and excretion of the chemopreventive agents. Among nonsmoking women in China, however, the strongest inverse associations with ITC intake on lung cancer risk were among those with *GSTM1* and *GSTT1* null genotypes (10). Inconsistencies between studies, the majority of which had adequate sample size and were well designed, could be attributed to study design issues, or to chance. However, GST genotypes encode for enzymes that are highly inducible by numerous exposures and processes, and that participate in extremely complex pathways. Thus, associations for 1 cancer site may vary from those for another; other exposures that are also substrates for GSTs and the weight of those exposures in cancer risk may ultimately determine the effects of GST genotypes on relationships between cruciferous vegetable intake and cancer risk.

Associations between breast cancer, cruciferous vegetable intake, and GST genotypes may be even more complex because of the strong role of estrogens in breast cancer etiology. A recent case-control study (23) evaluated associations between urinary ITCs and breast cancer risk, finding inverse associations among pre- and postmenopausal women, with the greatest inverse associations among those with deletions in *GSTM1* and *GSTT1*, although relations were not significant. However, this study relied on a one-time measure of excreted ITCs, collected from women after a diagnosis of breast cancer. The validity and reliability of a single measure of prediagnostic levels are not clear. Habitual consumption of cruciferous vegetables before diagnosis was not associated with decreased breast cancer risk, although urinary ITC levels were validated against a FFQ (24). In contrast, when we assessed usual diet 2 y before the interview, we found that cruciferous vegetable intake, particularly broccoli, was associated with a decreased risk of premenopausal breast cancer. For diseases with long latencies, such as cancer, estimation of usual diet by FFQ more accurately reflects long-term exposure to dietary components. Although there is likely some degree of misclassification of consumption using FFQs, and the most important time period within which diet would affect risk is not known, questionnaire assessment may be more appropriate than use of a biomarker that reflects exposure in only the last 2–3 d.

In summary, our findings and those from other recent studies, suggest that specific vegetables, such as those from the Brassica family, may have important effects on breast cancer risk. These data support a rationale for public health efforts to encourage individuals to maintain diets high in vegetables, which are sources of numerous anticarcinogenic compounds.

ACKNOWLEDGMENT

We thank Carlos Crespo, University at Buffalo, Buffalo, NY) for providing data on broccoli and cruciferous vegetable intakes from NHANES.

LITERATURE CITED

1. (1997) Food, Nutrition and the Prevention of Cancer: A Global Perspective. American Institute for Cancer Research, Washington, DC.

2. Gandini, S., Merzenich, H., Robertson, C. & Boyle, P. (2000) Meta-analysis of studies on breast cancer risk and diet: the role of fruit and vegetable consumption and the intake of associated micronutrients. Eur. J. Cancer 36: 636–646.

3. Smith-Warner, S. A., Spiegelman, D., Yaun, S. S., Adami, H. O., Beeson, W. L., van den Brandt, P. A., Folsom, A. R., Fraser, G. E., Freudenheim, J. L., Goldbohm, R. A., Graham, S., Miller, A. B., Potter, J. D., Rohan, T. E., Speizer, F. E., Toniolo, P., Willett, W. C., Wolk, A., Zeleniuch-Jacquotte, A. & Hunter, D. J. (2001) Intake of fruits and vegetables and risk of breast cancer: a pooled analysis of cohort studies. J. Am. Med. Assoc. 285: 769–776.

4. Terry, P., Wolk, A., Persson, I. & Magnusson, C. (2001) Brassica vegetables and breast cancer risk. J. Am. Med. Assoc. 285: 2975–2976.

5. Fowke, J. H., Longcope, C. & Hebert, J. R. (2000) Brassica vegetable consumption shifts estrogen metabolism in healthy postmenopausal women. Cancer Epidemiol. Biomark. Prev. 9: 773–779.

6. Talalay, P. & Fahey, J. D. (2001) Phytochemicals from cruciferous plants protect against cancer by modulating carcinogen metabolism. J. Nutr. 131: 3027S–3033S.

7. Lin, H. J., Probst-Hensch, N. M., Louie, A. D., Kau, I. H., Witte, J. S., Ingles, S. A., Frankl, H. D., Lee, E. R. & Haile, R. W. (1998) Glutathione transferase null genotype, broccoli, and lower prevalence of colorectal adenomas. Cancer Epidemiol. Biomark. Prev. 7: 647–652.

8. London, S. J., Yuan, J. M., Chung, F. L., Gao, Y. T., Coetzee, G. A., Ross, R. K. & Yu, M. C. (2000) Isothiocyanates, glutathione S-transferase M1 and T1 polymorphisms and lung cancer risk: a prospective study of men in Shanghai, China. Lancet 356: 724–729.

9. Spitz, M. R., Duphorne, C. M., Detry, M. A., Pillow, P. C., Amos, C. I., Lei, L., deAndrade, M., Gu, X., Hong, W. K. & Wu. X. (2000) Dietary intake of isothiocyanates: evidence of a joint effect with glutathione *S*-transferase polymorphisms in lung cancer risk. Cancer Epidemiol. Biomark. Prev. 9: 1017–1020.

10. Zhao, B., Seow, A., Lee, E. J. D., Poh, W.-T., Teh, M., Eng, P., Wang, Y.-T., Tan, W.-C., Yu, M. C. & Lee, H.-P. (2001) Dietary isothiocyanates, glutathione *S*-transferase-M1, -T1 polymorphisms and lung cancer risk among Chinese women in Singapore. Cancer Epidemiol. Biomark. Prev. 10: 1063–1067.

11. Slattery, M. L., Kampman, E., Samowitz, W., Caan, B. J. & Potter, J. D. (2000) Interplay between dietary inducers of GST and the GSTM-1 genotype in colon cancer. Int. J. Cancer. 87: 728–733.

12. Graham, S., Hellmann, R., Marshall, J., Freudenheim, J., Vena, J., Swanson, M., Zielezny, M., Nemoto, T., Stubbe, N. & Raimondo, T. (1991) Nutritional epidemiology of postmenopausal breast cancer in western New York. Am. J. Epidemiol. 134: 552–566.

13. Freudenheim, J. L., Marshall, J. R., Vena, J. E., Laughlin, R., Brasure, J. R., Swanson, M., Nemoto, T. & Graham, S. (1996) Premenopausal breast cancer risk and intake of vegetables, fruits, and related nutrients. J. Natl. Cancer Inst. 88: 340–348.

14. Ambrosone, C. B., Freudenheim, J. L., Graham, S., Marshall, J. R., Vena, J. E., Brasure, J. R., Michalek, A. M., Laughlin, R., Nemoto, T., Gillenwater, K., Harrington, A. M. & Shields, P. G. (1996) Cigarette smoking, *N*-acetyltransferase 2 genetic polymorphisms, and breast cancer risk. J. Am. Med. Assoc. 276: 1494–1501.

15. Ambrosone, C. B., Freudenheim, J. L., Graham, S., Marshall, J. R., Vena, J. E., Brasure, J. R., Laughlin, R., Nemoto, T., Michalek, A. M. & Harrington, A. (1995) Cytochrome P4501A1 and glutathione *S*-transferase (M1) genetic polymorphisms and postmenopausal breast cancer risk. Cancer Res 55: 3483–3485.

16. Pemble, S., Schroeder, K. R., Spencer, S. R., Meyer, D. J., Hallier, E., Bolt, H. M., Ketterer, B. & Taylor, J. B. (1994) Human glutathione *S*-transferase theta (GSTT1) cDNA cloning and the characterization of a genetic polymorphism. Biochemistry 300: 271–276.

17. Ambrosone, C. B., Coles, B. F., Freudenheim, J. L. & Shields, P. G. (1999) Glutathione *S*-transferase (GSTM1) genetic polymorphisms do not affect human breast cancer risk, regardless of dietary antioxidants. J. Nutr. 129: 565S–568S.

18. Janerich, D. T. & Hoff, M. B. (1982) Evidence for a crossover in breast cancer risk factors. Am. J. Epidemiol. 116: 737–742.

19. Michnovicz, J. J. & Bradlow, H. L. (1990) Dietary and pharmacological control of estradiol metabolism in humans. Ann. N.Y. Acad. Sci. 595: 291–299.

20. Yuan, F., Chen, D. Z., Liu, K., Sepkovic, D. W., Bradlow, H. L. & Auborn, K. (1999) Anti-estrogenic activities of indole-3-carbinol in cervical cells: implication for prevention of cervical cancer. Anticancer Res. 19: 1673–1680.

21. Meng, Q., Yuan, F., Goldberg, I. D., Rosen, E. M., Auborn, K. & Fan, S. (2000) Indole-3-carbinol is a negative regulator of estrogen receptor α signaling in human tumor cells. J. Nutr. 130: 2927–2931.

22. Ketterer, B. (1998) Dietary isothiocyanates as confounding factors in the molecular epidemiology of colon cancer. Cancer Epidemiol. Biomark. Prev. 7: 645–646.

23. Fowke, J. H., Chung, F.-L., Jin, F., Qi, D., Cai, Q., Conaway, C., Cheng, J.-R. Shu, X.-O., Gao, Y.-T. & Zheng, W. (2003) Urinary isothiocyanate levels, brassica, and human breast cancer. Cancer Res. 63: 3980–3986.

24. Seow, A., Shi, C. Y., Chung, F. L., Jiao, D., Hankin, J. H., Lee, H. P., Coetzee, G. A. & Yu, M. C. (1998) Urinary total isothiocyanate (ITC) in a population-based sample of middle-aged and older Chinese in Singapore: relationship with dietary total ITC and glutathione *S*-transferase M1/T1/P1 genotypes. Cancer Epidemiol Biomark. Prev. 7: 775–781.