Brassica Vegetable Consumption Shifts Estrogen Metabolism in Healthy Postmenopausal Women[1]

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Abstract

Previous studies suggest that the estrogen metabolite 16α-hydroxyestrone acts as a breast tumor promoter. The alternative product of estrogen metabolism, 2-hydroxyestrone, does not exhibit estrogenic properties in breast tissue, and lower values of the ratio 2-hydroxyestrone:16α-hydroxyestrone (2:16) in urine may be an endocrine biomarker for greater breast cancer risk. Vegetables of the *Brassica* genus, such as broccoli, contain a phytochemical, which may shift estrogen metabolism and increase the 2:16 ratio. Adding 500 g/day of broccoli to a standard diet shifts 2:16 values upward in humans; however, it is unknown as to whether healthy women are able to consume a sufficient quantity of *Brassica* to affect breast cancer risk through this mechanism. In this study, 34 healthy postmenopausal women participated in an intensive intervention designed to facilitate the addition of *Brassica* to the daily diet. The diet was measured by repeated 24-h recall, and estrogen metabolites were measured by enzyme immunoassay in 24-h urine samples. In a crude analysis, there was a nonsignificant increase in the urinary 2:16 ratio associated with greater *Brassica* consumption. With adjustment for other dietary parameters, *Brassica* vegetable consumption was associated with a statistically significant increase in 2:16 values, such that for each 10-g/day increase in *Brassica* consumption, there was an increase in the 2:16 ratio of 0.08 (95% confidence interval, 0.02–0.15). To the extent that the 2:16 ratio, as measured in urine, is associated with breast cancer risk, future research should consider *Brassica* vegetable consumption as a potentially effective and acceptable dietary strategy to prevent breast cancer.

Introduction

The estrogen metabolite 16HE[3] is produced when E1 is hydroxylated on the 16th carbon. Much like E2, 16HE increases breast cell proliferation *in vitro* (1–3). 16HE binds covalently to the estrogen receptor (1), has a lower binding affinity for sex hormone-binding globulin than E2 (4), promotes mammary gland tumors in murine models of breast cancer (1), and may act directly on DNA as a mutagen (5). Alternatively, E1 may be irreversibly metabolized to 2HE by several hepatic or extrahepatic P-450 isoforms. Unlike the 16HE metabolite, 2HE has a low affinity for the estrogen receptor, and 2HE is rapidly methylated by catechol-*O*-methyl transferase in the circulation (1, 2). In addition to a lower estrogenic potential, there is evidence to suggest that the 2-hydroxylated metabolites inhibit angiogenesis (6–8). Because the metabolic pathways leading to either 2HE or 16HE are irreversible, the relative activity of these two metabolic pathways (2HE:16HE = 2:16), as measured in urine, may be an endocrine biomarker for breast cancer risk in humans (9–12). Higher urinary 2:16 scores suggest protection from breast cancer, whereas lower urinary 2:16 scores suggest greater risk (13).

IGSLs are a category of phytochemicals that are capable of shifting estrogen metabolism and increasing the urinary 2:16 ratio. IGSLs are unique to vegetables of the *Brassica* genus, the most common of which in the United States are Brussels sprouts, broccoli, cabbage, kale, turnips, collards, and cauliflower. With cutting or chewing of the vegetable, IGSLs are degraded by the plant enzyme myrosinase to a variety of indole structures, including I3C, DIM, indole-3-acetonitrile, indole-3-acetic acid, and AG (14, 15). In the body, these indole-containing compounds are either chemically or enzymatically converted to indolo[3,2-b]carbazole, a moderate aryl hydrocarbon receptor agonist (16–18). The activated aryl hydrocarbon receptor binds to specific sites on DNA and induces the expression of P-450 enzymes of the CYP1 family in hepatic and extrahepatic tissue (19–22). These P-450 enzymes hydroxylate E1 on the second carbon, leading to greater 2HE production and decreasing the pool of E1 available for conversion to 16HE, thus increasing the 2:16 ratio (21, 23–27). In three small human intervention studies, the daily administration of I3C pills (400 mg/day) or broccoli (500 g/day) significantly increased the urinary 2:16 value (21, 28, 29), consistent with reduced breast cancer risk.

There is incomplete evidence to demonstrate that *Brassica* vegetable consumption protects against breast cancer. In animal models of breast cancer, dietary I3C (25, 30–32) or a diet with cabbage (33) reduces tumor incidence or delays tumor onset. Results from one cross-national study found that those coun-

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[3] The abbreviations used are: 16HE, 16α-hydroxyestrone; E1, estrone; E2, serum 17β-estradiol; 2HE, 2-hydroxyestrone; IGSL, indole glucosinolate; I3C, indole-3-carbinol; DIM, 3,3′-diindolylmethane; AG, ascorbigen; 24HR, 24-h recall; CI, confidence interval.

tries with higher cabbage intake had a lower breast cancer mortality rate (34). Despite the availability only of data on cabbage of the entire *Brassica* genus, intercountry differences in cabbage consumption are sufficiently large to increase the likelihood of detecting a specific protective association. Observational studies conducted within a population have not identified a consistent association between *Brassica* intake and breast cancer risk. (35–39). One of these studies did find a significant reduction in breast cancer risk with *Brassica* intake; however, there was no dose-response trend in this association [relative risk, 0.79 (0.67–0.92); Ref. 35]. These studies are limited by the very low levels of *Brassica* intake in the populations under evaluation, the limitations of individual dietary assessment techniques (*e.g.*, food frequency questionnaires), and the absence of data regarding how the vegetables are prepared before consumption.

Dietary interventions can create variability in the food consumption pattern of a targeted study group, such that it may be possible to detect a physiological response consistent with reduced cancer risk. In this study, the intervention protocol facilitated daily *Brassica* consumption among free-living postmenopausal women. The objective was to reach a level of *Brassica* intake consistent with the range and variability of amounts consumed in Japan or other Asian countries (40–42). An association between *Brassica* intake and higher urinary 2:16 values would suggest that healthy free-living postmenopausal women are able to shift their own estrogen profiles in such a way as to reduce breast cancer risk. Such a result would suggest that *Brassica* vegetables should be further evaluated as a strategy to reduce breast cancer risk.

Materials and Methods

Recruitment and Eligibility. Eligible women received a negative X-ray mammogram and a negative digital mammogram from the Department of Radiology at the University of Massachusetts Memorial Health Center within the 12 months before study entry. This institution screens and treats patients primarily residing in Worcester, Massachusetts and the surrounding communities, while a much smaller portion of patients are referred from Greater Boston or from one of the other five New England states.

The study was restricted to women >45 years of age, without a menstrual cycle in the past 12 months, without present liver or kidney disease, and without adrenalectomy. Women with a hysterectomy but without ovariectomy were at least 54 years of age. Women were excluded if they presently used any tobacco products, antibiotics, hormone replacement therapy, nonprescription hormones (*e.g.*, melatonin, dehydroepiandrosterone), black-cohosh, tamoxifen, diabetes medication, or cimetidine. Women under a physician-recommended diet or who reported a strong dislike for *Brassica* vegetables were excluded. Participants received no monetary compensation. Thirty-seven women met all eligibility criteria and started the study; however, three participants dropped-out because of family illness or scheduling conflicts. This analysis is restricted to the 34 participants who completed the intervention.

Dietary Intervention. The study population was divided into three groups of women, with between 9 and 13 women/group. The dietary intervention was administered to each of these groups, and it consisted of four classes over a 4-week period (Fig. 1). The goal of the intervention was to facilitate the incorporation of *Brassica* into the daily diet. Participants were asked to consume *Brassica* every day, at a frequency and vegetable combination that would approach a 70-mg/day intake of IGSLs. Estimated indole content for each vegetable was extracted from a review by Rosa *et al.* (43).

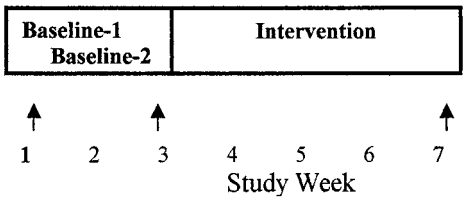

Fig. 1. Study design and data collection schedule. *Arrows*, weeks during the study in which three 24HR were administered and a 24-h urine sample and blood sample were collected.

During the intervention, participants were guided through the various theories underlying the hypothesized health benefits of *Brassica* consumption. To improve compliance, each participant completed two 3-day food diaries as a tool to self-monitor their *Brassica* vegetable intake. In addition, each participant was given written material describing the study protocol and a cookbook containing recipes that use *Brassica* vegetables. A portion of each class took place in a teaching kitchen, where participants prepared various dishes containing *Brassica* using techniques consistent with the principles of the study. During the classes, emphasis was placed on proper handling and preparation of the vegetables because vegetable preparation affects both the glucosinolate concentration and decomposition of glucosinolates within *Brassica*.

Participant Characteristics. Demographics, reproductive history, health history, tobacco use, alcohol use, and medication use were collected by questionnaire during the baseline study period. Additional questionnaires were administered at follow-up to identify any changes in tobacco or medication use. The psychological constructs "Social Approval" and "Social Desirability" were measured by questionnaire during the baseline period (44–46).

Urine and Blood Collection. The study design and sample collection schedule are illustrated in Fig. 1. Study participants provided two 24-h urine samples and two blood samples before the intervention, ~2 weeks apart. Additional urine and blood samples were collected during the last week of the intervention. Both written and oral instructions regarding the urine collection protocol were administered to all participants.

Dietary Assessment. The diet was measured by 24HR during each week that urine and blood samples were collected. Subjects were telephoned on three randomly assigned days (2 weekdays and 1 weekend day) and asked to describe the foods and portion sizes consumed during the prior day. A structured interview protocol was strictly followed, all interviews were conducted by highly trained registered dietitians, and participants were provided a two-dimensional chart of typical foods to assist with portion size estimation. Nutrient calculations were performed using the Nutrition Data System software, developed by the Nutrition Coordinating Center, University of Minnesota (Minneapolis, MN; Food Database: 13A; Nutrient Database: 28; Ref. 47). Nutrients derived from supplements were added to the dietary estimates. Data from the three 24HR administered within a given week were averaged, providing the single best estimate of each participant's dietary intake for that week.

Brassica vegetables were identified in the 24HR data. Data regarding the types of *Brassica* vegetable, the amount of vegetable, and whether the vegetable was consumed cooked or raw, were extracted. The grams of *Brassica* that were reported

as cooked were adjusted to reflect grams of raw (fresh) *Brassica*. IGSL intake (mg/day) was calculated using published IGSL concentrations in fresh/raw vegetables across the varieties of *Brassica* (43) and the amount of *Brassica* reported in the 24HR.

Body Measurements. Weight, height, and the circumferences of the waist, abdomen, and hips were measured during each week in which a urine sample was collected. Body mass index was calculated as weight (kg)/height $(m)^2$, and the waist:hip ratio was calculated by dividing the waist circumference by the hip circumference. Total body fat was calculated using the Tran and Weltman prediction equation, which combines measures of body circumference, weight, age, and height to produce an estimate of the fat (kg) in the body (48, 49). This prediction equation has been validated in women >50 years of age.

Laboratory Analyses. Urinary 2HE and 16HE were measured at the University of Massachusetts Medical School (C. Longcope) using a solid-phase enzyme immunoassay kit from Immuna Care Corporation (50). All assays were performed on samples in random order, in triplicate, within one batch, and by a single technician who was blinded as to the sequence of the sample collection. Serum E2 levels were measured by radioimmunoassay (Diagnostic Products Corp., Los Angeles, CA). There were six serum samples from four individuals that had unexpectedly high (>40 pg/ml) E2 levels. For these samples, the E2 assay was repeated, with identical results. The intraassay coefficients of variation for E2, 2HE, and 16HE were 3.4%, 4.0%, and 4.0% respectively, whereas interassay coefficients of variation were 6.8%, 10.0%, and 9.9%, respectively. Standard urine samples were from women of a similar age and estrogen level as the study participants.

Statistical Analysis. Individual changes in *Brassica* consumption or urinary 2:16 values between baseline and the intervention study phase were calculated by subtraction. Across the two baseline measurements, urinary 2:16 values and *Brassica* intake were not significantly different. Therefore, these values were pooled to provide a more stable baseline estimate. Changes in 2:16 were compared with changes in *Brassica* intake using least-squares linear regression (SAS/STAT Statistical Software, version 6.12, SAS Institute, Cary, NC). The regression coefficient (*b*) represents the change in 2:16 for each 10-g/day change in *Brassica* intake. Additionally, the pattern of the 2:16 ratio was evaluated across categories of the change in *Brassica* vegetable intake. The significance of the a linear trend was determined by inclusion into the regression model of a continuous variable with values representing each category of change in *Brassica* intake.

Several dietary macronutrients (*i.e.*, total fat, protein, carbohydrates, energy, and fiber) and body habitus measures were defined *a priori* as potentially affecting urinary 2:16 values. Although the mechanisms by which these nutrients may affect urinary estrogen metabolite levels are uncertain, these factors previously have been associated with estrogen metabolism, drug metabolism, or the excretion of estrogens and are treated as potential confounders (51–53). Adjusted regression coefficients were calculated to remove the influence of changes in these factors over time. Baseline 2:16 values were forced into the regression model to control for the possibility that large change scores result from unusual baseline values (regression to the mean).

The association between *Brassica* intake and 2:16 values were evaluated in a cross-sectional nature during the intervention study phase to explore the possibility of identifying an association between 2:16 and the diet across individuals.

Table 1 Study population characteristics[a]

Factor	*n*
Employment status[b]	
Employed	14
Unemployed	20
Living status	
Alone	9
Not alone	25
Education level	
High school	6
Any college	28
Religious affiliation	
Roman Catholic	13
Other	21
Prior breast cancer[c]	
Yes	5
No	29
Family breast cancer history	
Yes	13
No	21
Age (yr)	
≤60	14
>60	20

[a] $n = 34$.
[b] Combined part-time and full-time status.
[c] Greater than 5 years before study entry.

The impact of the dietary intervention on total estrogen production was evaluated by comparing E2 values over the study by repeated measures ANOVA. An unstructured covariance matrix was used because this approach provided the best fit of the data (54). The distribution of serum E2 was highly skewed, and E2 levels were transformed logarithmically (base e) to meet the statistical assumptions.

Results

Participants ranged in age from 49 to 77 years, and they averaged 61 years of age. Most study participants were educated beyond the high school level, were unemployed, and shared housing with another person (Table 1).

Before the intervention, *Brassica* vegetable consumption averaged ~9 g/day (Table 2). During the intervention study phase, *Brassica* consumption increased to 193 g/day. The frequency of *Brassica* vegetable consumption increased from about two servings per week at baseline to about two servings per day during the intervention phase, and estimated IGSL intake (mg/day) followed the same trend as self-reported Brassica vegetable intake.

At baseline, the average urinary 2:16 ratio was 2.27, and increased to 2.38 during the intervention (Table 2). Serum E2 levels were 20 pg/ml at baseline and 16 pg/ml during the intervention study phase. Crude urinary 2:16 levels and E2 levels did not significantly change across the two baseline measures and the intervention measurement (repeated measured ANOVA of 2:16 or E2 across time: $P = 0.31$ and $P = 0.35$, respectively). E2 levels, or changes in E2 levels over time, were not significantly associated with urinary 2:16 values. Disease history, family history of breast cancer, or other breast cancer risk factors were not consistently associated with urinary 2:16 values or adherence to the intervention guidelines (not shown).

The crude association between changes in *Brassica* intake and changes in 2:16 values indicated that urinary 2:16 values

Table 2 *Brassica* vegetable intake, IGSL intake, the 2HE:16HE ratio, and E2 levels[a]

Study phase	Mean	SD	Range
Brassica[b] (g/day)			
Baseline[c]	8.8	10.2	0–42.1
Intervention	193.5	76.7	53.6–371.5
Intervention–baseline[d]	184.6	77.3	52.1–371.5
IGSL (mg/day)[e]			
Baseline	2.2	2.6	0–10.9
Intervention	70.5	29.9	12.6–146.9
Intervention–baseline	68.3	30.3	12.2–140.7
2:16[f]			
Baseline	2.27	0.97	0.58–4.21
Intervention	2.38	1.31	0.37–5.43
Intervention–baseline	0.11	1.05	−1.78–2.53
E2 (pg/ml)[g]			
Baseline	20	22	3–114
Intervention	16	11	2–43
Intervention–baseline	−5	20	−89–25

[a] $n = 34$.
[b] *Brassica* vegetable consumption measured by 24 HRs.
[c] Baseline: data averaged across the baseline 1 and baseline 2 study phases.
[d] Intervention-baseline: change in value from the average of the baseline values to the intervention study phase.
[e] IGSL glucosinolate intake calculated from published indole concentrations and self-reported *Brassica* consumption.
[f] 2HE:16HE ratio as measured from 24-hour urine samples.
[g] Measured in a morning blood sample.

Table 3 Change in the 2HE:16HE ratio by level of change in *Brassica* from baseline to intervention consumption levels (g/day)[a]

Brassica[b]		Crude[c]		Adjusted[d]	
n	Range (g/d)	2:16[e]	(95% CI)	2:16	(95% CI)
8	52–108	−0.19	(−0.96 to 0.57)	−0.51	(−1.33 to 0.31)
9	109–173	−0.16	(−0.87 to 0.56)	−0.16	(−0.78 to 0.46)
9	174–246	0.48	(−0.24 to 1.21)	0.25	(−0.40 to 0.90)
8	247–372	0.33	(−0.43 to 1.10)	0.91	(0.13 to 1.70)
	P trend[f]		0.17		0.02

[a] $n = 34$.
[b] *Brassica* vegetable intake measured by 24 HR.
[c] Adjusted for baseline 2:16 only.
[d] Adjusted for baseline 2:16, fiber (g of water-soluble), protein (g), energy (kcal), social approval score.
[e] 2:16, change in 2HE:16HE (intervention-baseline), as measured in 24-h urine samples.
[f] Two-sided trend test using a continuous variable with values representing each category of *Brassica* intake.

increased 0.03 for every 10 g of *Brassica* consumed (regression coefficient = 0.03; 95% CI, −0.02 to 0.06).

Several dietary variables were considered as potential sources of bias, including changes in dietary protein (g), fat (g), carbohydrates (g), energy (kcal), and fiber (g), as well as previously defined body habitus measures and psychosocial scales that have been linked with dietary misreport. Dietary fat intake, carbohydrate intake, and measures of body habitus did not alter the *Brassica*-2:16 association and were not significant predictors of 2:16, and these parameters were excluded from the final model to improve the precision of the analysis. With adjustment for dietary fiber, protein, energy, and scores of social approval, the increase in *Brassica* consumption from baseline to intervention was significantly associated with an increase in the 2:16 ratio, such that each 10-g/day increase in *Brassica* consumption increased the 2:16 ratio by 0.08 (regression coefficient = 0.08; 95% CI, 0.02–0.15). Similarly, estimated indole intake shifted the urinary 2:16 ratio upward (regression coefficient = 0.21; 95% CI, 0.03–0.39 for 10 mg/day of IGSL).

Participant compliance from baseline to the intervention study phase was categorized into four groups to explore any dose-response relationship. Changes in *Brassica* intake were categorized across quartiles of the distribution, and crude and adjusted changes in urinary 2:16 values were calculated. Those participants who consumed less *Brassica* did not show any increase in urinary 2:16 values (Table 3). Those participants who consumed more *Brassica* had a greater increase in urinary 2:16 values, with a significant linear dose-response trend in the adjusted analysis.

In a cross-sectional analysis during the intervention phases of the study, *Brassica* consumption was generally associated with higher urinary 2:16 values, but no association was statistically significant [regression coefficient = 0.03; 95% CI, −0.05 to 0.12; adjusted for energy (kcal), fiber (g), protein (g), and social approval score]. In contrast to *Brassica* intake, the IGSL index was not associated with greater 2:16 values, [regression coefficient = −0.02, 95% CI, −0.21 to 0.16 for each 10 mg/day in IGSL intake; adjusted for energy (kcal), fiber (g), protein (g), and social approval score]. When *Brassica* intake or IGSL values were categorized at the median level within the intervention study phase, urinary 2:16 levels were about 0.3–0.5 greater among participants reporting higher *Brassica* intake or higher IGSL intake (Table 4), consistent with a shift in estrogen metabolism toward 2HE in response to IGSL consumption.

The shift in 2:16 values appeared sensitive to the types of vegetables consumed. When the amount of broccoli, cabbage, Brussels sprouts, or other *Brassica* are considered simultaneously in a multivariable model, an increase of 10 g of cabbage was associated with an increase of 0.07 (95% CI, −0.04 to 0.19) in the urinary 2:16 value, whereas an increase of 10 g of broccoli was associated with an increase of 0.01 (95% CI, −0.09 to 0.11) in 2:16 values. However, lightly cooked and raw *Brassica* appear equally able to shift the 2:16 ratio [cooked: regression coefficient = 0.03, 95% CI, −0.03 to 0.10); raw: regression coefficient = 0.03, 95% CI, −0.08 to 0.13; for each 10-g/day vegetable].

Discussion

When studies are conducted in animals or when widely divergent populations are compared, the dietary differences are very large, and an association between diet and breast cancer is more easily detected (55). On the other hand, a causal relationship between a dietary component and breast cancer may be rendered undetectable when using an imprecise measure in a population having little variability in the dietary component of interest (56, 57). An intensive intervention was used to greatly increase the consumption of *Brassica* vegetables, a dietary factor potentially important in modifying breast cancer risk (29, 34, 35, 58), within a highly selected and targeted study group. Additionally, this protocol provided the opportunity to guide participants through the dietary change and to informally monitor dietary compliance and health-related consequences of the intervention. *Brassica* vegetable consumption increased about 20-fold among free-living women in response to the intervention protocol. There were no serious side-affects associated with adherence. The minority of participants who reported minor gastrointestinal discomfort ($n = 15$) generally found that this side-effect diminished with time and with experimentation

Table 4 Crude and adjusted mean 2HE:16HE ratio values within levels of *Brassica* intake or IGSL intake during the intervention study phase; with 95% CIs and *P*s for difference in 2:16 across categories[a]

Intake[b]	Range	*n*	Mean 2:16	Difference in 2:16	95% CI	*P*[g]
Brassica[c] (g/day)						
132.4	53–181	17	2.16[e]	0.45[e]	−0.45 to 1.37	0.32
254.5	182–371	17	2.61[e]			
IGSL[d] (mg/day)						
52.6	2–67	17	2.12[e]	0.53[e]	−0.38 to 1.44	0.25
85.1	68–146	17	2.65[e]			
Brassica[c] (g/day)						
132.4	53–181	17	2.25[f]	0.27[f]	−0.94 to 1.48	0.65
254.5	182–371	17	2.52[f]			
IGSL[d] (mg/day)						
52.6	2–67	17	2.15[f]	0.47[f]	−0.54 to 1.49	0.35
85.1	68–146	17	2.62[f]			

[a] $n = 34$.
[b] Median *Brassica* or IGS intake within each category.
[c] Derived from 24 HRs.
[d] Estimated IGSL intake calculated from published estimates and self-reported *Brassica* consumption.
[e] Crude: Adjusted for baseline 2:16 only.
[f] Adjusted: mean 2:16 values and differences in 2:16 adjusted for energy (kcal), protein (g), fiber (g), and social approval scores.
[g] Two-sided *P* under null hypothesis that the difference equals 0.

of different vegetables. The results of this study indicate that the consumption of *Brassica* vegetables, as prepared and consumed by healthy postmenopausal women in the United States, was significantly associated with higher urinary 2:16 values, suggesting that *Brassica* vegetables should be further explored as an additional dietary strategy to reduce breast cancer risk in the population.

Kall *et al.* (21) found the urinary 2:16 ratio increased 0.40 with administration of 500 g of broccoli/day to a small group of young men and women, and Bradlow *et al.* (29) observed a 0.40 shift in 2:16 with the daily administration of pills containing I3C. Unlike pills, *Brassica* vegetables expose the body to a complex mixture of indole structures, including I3C, AG, and DIM; and AG or DIM may be more potent than I3C alone (18, 59, 60). In this study, different vegetable types appeared to be effective in shifting the urinary 2:16 ratio, with cabbage intake having the strongest effect. Finally, the larger response observed in this study might be specific to postmenopausal women, or advances in the laboratory procedures used to detect estrogen metabolites in urine.

Glucosinolates and glucosinolate break-down products are hydrophilic, and as much as 63% of the glucosinolate content of a vegetable may leach into the cooking water during boiling (20, 26). However, vegetable preparation did not diminish the ability of the vegetables to shift estrogen metabolism in this study. Participants were instructed to cook the *Brassica* only lightly, by either light steaming or as stir-fry, and they were provided guided practice in vegetable preparation techniques. Steaming provides less opportunity for leaching, and stir-fried vegetables retain glucosinolate levels (61). Light cooking may disrupt plant cell membranes without leaching of the indoles into the cooking medium, providing the opportunity for myrosinase to release these indoles for eventual conversion to indolo[3,2-b]carbazole (15, 18, 20, 26, 62, 63).

Another potential source of error in a study such as this is improper collection of biological samples. There was no indication that the 24-h urine samples were collected improperly. All participants understood the urine collection procedure, and participants recorded the time and dates of urine collection. The relationship between *Brassica* consumption and the 2:16 ratio was not significantly modified by the time period between urine collection and storage (*i.e.*, urine age), and there was no evidence of differences in sample handling over time. However, there was no way to be sure of whether samples were contaminated or whether urine samples represented a complete 24-h collection.

The single-armed design could not control for any unmeasured factors. Duplicate baseline measures were collected to produce a stable estimate of the usual dietary intake and hormone levels. There were no significant differences in nutrient or hormone values between these baseline time points, suggesting that these factors, at the very least, were consistent across the short term within study population. Prescription medication use was monitored throughout the study, and participants followed a stable drug regime. Soy food and bean food consumption was very low in this Central Massachusetts study population consisting primarily of European-Americans, with consumption consistently estimated at about 0.25 g/day, on average (only eight participants ate any bean or soy products). Not surprisingly, this very low soy-food intake was not associated with urinary 2:16 values. The short duration of the intervention (4 weeks) minimized the opportunity for the influences of seasonal variation in the diet or a social trend that might affect 2:16 values.

The laboratory data suggest the hypothesis that 2:16 is important in breast cancer etiologically, but epidemiological studies evaluating the role of 2:16 and breast cancer risk are inconsistent. Four case-control studies found significantly lower 2:16 levels or higher 16HE levels among breast cancer cases (13, 64–66), whereas a recent analysis of a prospective study reported a nonsignificant 30% reduction in breast cancer risk with higher urinary 2:16 values (67). Recently, Ursin *et al.* (68) reported an inconsistent finding, where only women who were in the middle tertile of the 2:16 distribution were less likely to be diagnosed with breast cancer [$OR_{T2\ versus\ T1} = 0.34$ (0.12–0.98); $OR_{T3\ versus\ T1} = 1.13$ (0.46–2.78)]. At present, it is not possible to conclude that 2:16 is a valid endocrine biomarker for breast cancer risk. Further studies should be conducted to evaluate this relationship.

To the extent that the urinary 2:16 ratio is etiologically relevant to breast cancer, frequent *Brassica* intake may be able to reduce breast cancer risk. Studies evaluating urinary 2:16

values and breast cancer risk identify differences in 2:16 values between the case and control series ranging from 0.1 to 0.7. A shift of 0.08 in the 2:16 ratio for every 10 g/day of *Brassica* suggests that the population would need to increase *Brassica* consumption between 12.5 g/day to 75 g/day to move 2:16 ratios to a favorable level to affect the causal mechanism leading to breast cancer. Of course, caution must be used when comparing results across laboratories, and the above interpretation should be considered only as a rough guideline. *Brassica* vegetable consumption in the United States is estimated between 5 and 11 g/day (fresh; Refs. 41 and 69). This suggests that even a small increase in *Brassica* vegetable consumption across the population could have an impact on the incidence of breast cancer.

Presently, there are three approaches to risk reduction: prophylactic surgery, pharmaceuticals, and behavioral change. None of these options are universally acceptable or appropriate. It would be ideal to have a variety of strategies that could be tailored to an individual's characteristics and risk profile. *Brassica* vegetable consumption appears to shift estrogen metabolism in a way consistent with reduced breast cancer risk. Future work should clarify the relationship between the 2:16 endocrine biomarker and breast cancer and the relationship between *Brassica* intake and breast cancer risk, and it should identify those women most susceptible to the beneficial action of increased *Brassica* consumption.

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