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EPIDEMIOLOGY

Vegetable intake is associated with reduced breast cancer recurrence in tamoxifen users: a secondary analysis from the Women's Healthy Eating and Living Study

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Abstract The protective effect of vegetables on the risk of breast cancer recurrence is uncertain. We sought to evaluate the association between breast cancer recurrence and vegetable intake including analyses stratified on tamoxifen use. Experimental evidence of anti-carcinogenic activity of phytochemicals in cruciferous vegetables in combination with tamoxifen led to specific evaluation of this class of vegetables as well. To assess the association between vegetable intake and breast cancer recurrence, vegetable intake from repeat 24-h dietary recalls were examined as a secondary analysis of 3,080 breast cancer survivors enrolled in the Women's Healthy Eating and Living (WHEL) Study. At the time of enrollment women were, on average, 23.5 months post-diagnosis. The hazard of recurrence, controlling for relevant and significant clinical and demographic variables, with vegetable intake was assessed overall and separately for women taking tamoxifen. WHEL participants reported mean baseline intakes ($\bar{x}$, SE) of 3.1 ± 0.05 and 0.5 ± 0.02 servings/day of total and cruciferous vegetables, respectively. Baseline vegetable intake in the highest as compared to lowest tertiles was associated with an overall lower adjusted hazard ratios (HR) for recurrence of 0.69, 95% CI 0.55–0.87. Among women taking tamoxifen, the HRs were 0.56, 95% CI 0.41–0.77 for total vegetables and 0.65, 95% CI 0.47–0.89 for cruciferous vegetable intake. The hazard in women using tamoxifen who reported cruciferous vegetable intake above the median and who were within the highest tertile of total vegetable intake was HR 0.48; 95% CI 0.32–0.70. This secondary analysis in over 3,000 breast cancer survivors suggests that baseline vegetable intake may be associated with a reduction in the risk of breast cancer recurrent or new events particularly for those using tamoxifen. Such associations should be explored further as the possibility that vegetable intake is simply a surrogate for other health-promoting behaviors cannot be ruled out.

Keywords Diet · Breast cancer · Vegetables · Tamoxifen

Abbreviations

WHEL	Women's Healthy Eating and Living
BMI	Body mass index
HR	Hazards ratio
CI	Confidence interval
SERM	Select estrogen receptor modulator
US	United States
USDA	United States Department of Agriculture
NDS	Nutrient database software

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Introduction

Women treated for breast cancer comprise the largest proportion of cancer survivors in the US [1]. Substantial epidemiological evidence suggests that diets high in vegetables may reduce risk for breast cancer [2–4], although not consistently [5, 6]. Few studies have evaluated these associations in relation to breast cancer prognosis. A randomized, controlled dietary intervention, the Women's Healthy Eating and Living (WHEL) Study was undertaken in 3,088 breast cancer survivors to determine if a plant-based, low-fat, high-fiber diet could influence breast cancer recurrence rates and survival in this growing segment of the population [7]. The average duration of survivor participation was 7.3 years with the overall results showing no survival benefit [8]. Of interest, a separate sub-group analysis suggested a significantly reduced hazard for overall survival among women who consumed more than five servings of fruit and vegetables daily and were physically active at baseline [9]. These findings and the wealth of data from this large prospective cohort support a continuing effort to identify dietary variables that may be protective among breast cancer survivors.

Associations of interest include the role of vegetables as a primary source of bioactive compounds including carotenoids and isothiocyanates with demonstrated chemopreventive activity [10–16]. We therefore conducted a hypothesis-generating, secondary analysis of the WHEL Study population to test the association between baseline vegetable consumption and subsequent risk for breast cancer recurrence. Further, at the time the WHEL Study was designed, tamoxifen was not yet a routinely prescribed therapeutic agent, but by the time of enrollment a significant number of women were taking tamoxifen. We postulated that any dietary effects on recurrent disease might differ in the presence or absence of tamoxifen given the profound effects of tamoxifen in reducing the risk of recurrent disease [17]. In addition, existing mechanistic evidence is suggestive for a synergistic anti-carcinogenic action of combinational interventions with selected bioactive constituents in cruciferous and tamoxifen as well as estrogen receptor alpha [18–20]. This led us also to evaluate the independent associations between cruciferous vegetable intake and recurrent disease risk among women taking tamoxifen.

Methods

Study design and population

The WHEL Study has been previously described [7]. Briefly, 3,088 breast cancer survivors, from four Western states, were randomized to a two-arm (dietary intervention and comparison groups) clinical trial conducted between 1995 and 2006. Eligible participants were diagnosed with and completed treatment for Stage I, II or III (AJCC VI classification) invasive breast cancer. Participants were 18–70 years of age and had a mammographic examination within the previous 12 months to confirm the absence of locoregional disease (recurrent or new primary). The study intervention diet included a daily diet of 5 vegetables, 3 fruits, 16 oz of vegetable juice, 30 g of fiber and 20% energy from fat [7]. All participants provided written informed consent as approved by the institutional Human Subjects Protection Programs in accordance with and assurances provided by the Department of Health and Human Services.

Anthropometric measurements, demographic, and lifestyle data

The WHEL Study protocol included a baseline clinic visit during which participants completed demographic and lifestyle questionnaires. Height and weight were measured at enrollment using standard procedures; and body mass index (BMI, weight [kg]/height [m^2]) was computed.

Dietary intakes for the WHEL participants were assessed using four pre-scheduled 24-h dietary recalls collected via telephone by study-trained dietary assessors over a 3-week period including weekend and weekdays [7, 21]. Standard vegetable serving variables were not available within the Minnesota Nutrient Data System (NDS) at the time the baseline estimates were derived; NDS was used to derive nutrient variables (NDS-R 4.03, 2000). By protocol a vegetable serving was defined as any half-cup serving of raw or cooked vegetables or one cup of raw leafy vegetable excluding iceberg lettuce and white potatoes [7]. To better estimate cruciferous vegetable exposure, serving weights were measured using cruciferous vegetable samples purchased from local grocery stores (raw as well as cooked samples) and USDA Food Composition Database [22] estimates per 90 g serving was applied to determine the number of servings consumed. Cruciferous vegetable intake included broccoli, broccolini, broccoflower, Bok choy, Brussels sprouts, cauliflower, cabbages, kale, radicchio, mustard/collard/turnip greens, rutabaga, sauerkraut, kohlrabi, watercress, radish, wasabi/horseradish. The mean intakes for four recalls were used to describe average intake at baseline, years 1 and 4. Physical activity was assessed using a validated 9-item measure of activity within the Women's Health Initiative Personal Habits Questionnaire [23, 24]. This questionnaire was self-administered prior to randomization to treatment group.

Breast cancer tumor characteristics and recurrence case ascertainment

All subjects were previously treated for breast cancer and diagnosis was verified by medical records review including tumor pathology; physician verification of disease-free status was required. Breast cancer recurrence events, including locoregional cancer recurrence, distant recurrence/metastasis, and development of a new contralateral primary tumor were assessed by local study coordinators during semi-annual telephone interviews and via review of the Social Security Death Index. Reports of invasive breast cancer recurrence or death triggered a confirmation interview, medical records review and central adjudication. In 7.3 years of follow-up, 518 additional recurrent breast cancer events were confirmed; 516 were included in these analyses; one case was missing diet data and another reported cruciferous vegetable intake greater than 3 standard deviations above the mean of the sample.

Statistical analysis

Descriptive statistics including mean and 95% confidence intervals or percentages were assessed for demographic, clinical and dietary variables, comparing women with/without recurrent disease using Chi-square and two-sided, independent sample Student's t tests. Dietary variables were log-transformed and Spearman correlations were used to compare relationships of vegetable intake with covariates. As both total and, to a lesser degree, cruciferous vegetable intake were exposures that were targeted and changed during the intervention trial, we present the mean and standard error of intake over time; the significance of change was assessed using diet group $\times$ time interaction terms from likelihood ratio analysis. Cox proportional hazard ratio (HR) models (analysis of time to event as outcome and including the main effects of baseline exposure) using continuous exposure and tertiles of vegetable intakes were performed to assess the relationship between intake and recurrence risk. Adjusted models were based on the most parsimonious set of variables (cut-off P value < 0.10) showing or suspected of a relationship with vegetable intakes and/or breast cancer recurrence. Although intervention assignment was not significant in models ($P = 0.44$ for total and $P = 0.57$ for cruciferous vegetables), this variable was retained to assure adjustment for time-related differences in intake by intervention status. Interactions terms between vegetable intake $\times$ intervention status were also non-significant ($P = 0.38$ and 0.34 for total and cruciferous vegetables, respectively). Interaction terms for vegetable intake $\times$ tamoxifen use were included in the models as significant tamoxifen use-specific differences ($P = 0.04$ for total and $P = 0.005$ for cruciferous vegetables) in the relationship between baseline intake and risk were identified. Cox models were therefore stratified by current tamoxifen use, excluding the tamoxifen use variable from the equations. Significance for all analysis was $\alpha = 0.05$ (two-sided). Analyses were conducted using SPSS 17.0 statistical software.

Results

Baseline characteristics and dietary intakes

The characteristics of the study population are shown in Table 1. Participants were predominantly educated, non-smoking, sedentary white women. The majority was diagnosed with post-menopausal, early stage and estrogen receptor positive tumors and received tamoxifen. Consistent with known risk factors for disease recurrence, women who recurred were more likely to have been diagnosed at a younger age (pre-menopausal), at advanced stage, with estrogen receptor negative disease and treated with chemotherapy. Tamoxifen use was protective against recurrence.

Mean dietary intakes for the study population at baseline showed participants reported an average daily intake of 3.1 servings of vegetables (including juice but excluding white potatoes and iceberg lettuce) and 0.5 servings of cruciferous vegetable intake or 12.8% of total vegetable intake (Table 2).

Vegetable intake and recurrence

The multivariate analysis and adjusted hazards ratios (HR) for breast cancer-free survival in relation to vegetable intake are shown in Table 3. After controlling for clinical and histopathological characteristics associated with recurrence risk, women in the highest tertile of vegetable intake had a significantly lower hazard of breast cancer recurrence (HR 0.69, 95% CI 0.55–0.87), suggestive of an overall protective effect for high vegetable intake at baseline in the WHEL Study population. The HR specific to cruciferous vegetables in the full model for all women is associated with a non-significant 15% decrease in the hazard of recurrence (HR 0.85, 95% CI 0.69–1.06).

We next asked whether or not there was a differential effect of baseline vegetable intake and recurrence considering tamoxifen treatment for estrogen receptor positive disease. When stratified on tamoxifen use, we observed a significant dose-related decrease in the HR for total baseline vegetable intake for women on tamoxifen (HR 0.56, 95% CI 0.41–0.77, $P = <0.001$) that was also present for baseline cruciferous vegetable intake (HR 0.65, 95% CI 0.47–0.89, $P = 0.006$). A test for interaction between baseline dietary intakes of vegetables or crucifers and tamoxifen was also completed. To test for an independent

Table 1 Baseline demographic and clinical characteristics of WHEL Study participants ($n = 3,080$)

	All ($n = 3,080$) Mean 95% CI or n (%)	Confirmed breast cancer recurrence ($n = 516$) Mean 95% CI or n (%)	No confirmed breast cancer recurrence ($n = 2,564$) Mean 95% CI or n (%)	P
Age at randomization, Mean (95% CI)	51.2 (50.9–51.5)	49.6 (48.8–50.4)	51.5 (51.2–51.9)	<0.001
BMI (kg/m^2)	27.3 (27.0–27.5)	27.5 (27.0–28.0)	27.2 (27.0–27.4)	0.29
Physical activity (METS), Mean (SD)	868 (836–899)	813 (739–887)	879 (844–914)	0.13
Ethnicity				0.10
White	2,627 (85.3%)	438 (84.9%)	2,189 (85.4%)	
Hispanic	164 (5.3%)	28 (5.4%)	136 (5.3%)	
African American	118 (3.8%)	22 (4.3%)	9 6(3.7%)	
Asian	96 (3.1%)	9 (1.7%)	87 (3.4%)	
Pacific Islander	23 (0.7%)	6 (1.2%)	17 (0.7%)	
Other	49 (1.6%)	13 (2.5%)	36 (1.4%)	
Education				0.26
High school or less	378 (12.3%)	69 (13.4%)	309 (12.1%)	
Post-high school	87 (2.8%)	16 (3.1%)	71 (2.8%)	
Some college	944 (30.6%)	174 (33.7%)	770 (30.0%)	
College graduate	880 (28.6%)	131 (25.4%)	749 (29.2%)	
Post-college	791 (25.7%)	126 (24.4%)	665 (25.9%)	
Current smoking	137 (4.4%)	27 (5.3%)	110 (4.3%)	0.58
Years since diagnosis, Mean (SD)	2.0 (1.9–2.0)	1.9 (1.8–2.0)	2.0 (1.9–2.0)	0.23
Cancer stage				<0.001
I	1,187 (38.5%)	103 (20.0%)	1,084 (42.3%)	
IIA	1,023 (33.2%)	165 (32.0%)	858 (33.5%)	
IIB	384 (12.5%)	84 (16.3%)	300 (11.7%)	
IIIA	372 (12.1%)	107 (20.7%)	265 (10.3%)	
IIIC	114 (3.7%)	57 (11.0%)	57 (2.2%)	
Menopausal status				0.001
Premenopausal	349 (11.3%)	85 (16.5%)	264 (10.3%)	
Postmenopausal	2,442 (79.3%)	383 (74.2%)	2,059 (80.3%)	
Perimenopausal	284 (9.2%)	48 (9.3%)	236 (9.2%)	
Not sure	5 (0.2%)	0 (0.0)	5 (0.2)	
Estrogen receptor (ER)[a]				0.02
Negative	755 (24.5%)	155 (30.0%)	600 (23.4%)	
Positive	2,286 (74.2%)	355 (68.8%)	1,931 (75.3%)	
Not done/unknown	39 (1.3%)	6 (1.2%)	33 (1.3%)	
Current tamoxifen use	1,833 (59.5%)	270 (52.3%)	1,653 (61.0%)	<0.001
Chemotherapy	2,155 (70.0%)	412 (79.8%)	1,743 (68.0%)	<0.001
Radiation	1,893 (61.5%)	321 (62.2%)	1,572 (61.3%)	0.84

$n = 3,080$: with baseline dietary intake from dietary recalls

MET metabolic equivalents per week in mild, moderate, strenuous exercise or walking, mean based on 2,977 respondents

[a] Any ER+ regardless of progesterone receptor status based on 3,041 participants

contribution of cruciferous vegetable intake in relation to the reduced hazard observed with total vegetable intake in women on tamoxifen, HRs were evaluated for each tertile of total vegetable intake stratified at the median of cruciferous vegetable intake (Table 4). In this analysis total vegetable intake among women with cruciferous vegetable intake below the median was not shown to be protective. However, in women reporting baseline intake of cruciferous vegetables above the median, both the middle (HR 0.66, 95% CI 0.46–0.96) and the upper (HR 0.48, 95% CI 0.32–0.70) tertiles of total vegetable intake were protective.

Table 2 Baseline, years 1 and 4 macronutrient and vegetable intake from repeat recalls by Diet Assignment Group

	Comparison group			Intervention group		
	Baseline (n = 1,546)	Year 1 (n = 1,375)	Year 4 (n = 1,203)	Baseline (n = 1,534)	Year 1 (n = 1,289)	Year 4 (n = 1,123)
Macronutrients						
Energy (kcal)	1,724 ± 11	1,606 ± 11†	1,574 ± 11†	1,720 ± 11	1,603 ± 10†	1,553 ± 11†
Carbohydrate (g)	237.32 ± 1.67	220.75 ± 1.63†	199.15 ± 1.73†	237.57 ± 1.74	250.95 ± 1.87†	218.99 ± 1.98†
Protein (g)	68.67 ± 0.49	66.99 ± 0.49†	69.10 ± 0.56†	69.01 ± 0.50	66.98 ± 0.47†	68.92 ± 0.54†
Fat (g)	56.62 ± 0.59	52.22 ± 0.56†	56.50 ± 0.63†	55.78 ± 0.60	41.20 ± 0.47†	47.7 ± 0.58†
Fiber (g)	21.25 ± 0.22	21.06 ± 0.22	19.32 ± 0.21†	21.40 ± 0.23	28.96 ± 0.28†	25.19 ± 0.29†
Vegetables (servings/day)						
Total[a]	3.07 ± 0.05	3.12 ± 0.05	3.03 ± 0.05	3.11 ± 0.05	7.35 ± 0.09†	5.90 ± 0.09†
Cruciferous	0.45 ± 0.01	0.43 ± 0.01	0.36 ± 0.01†	0.45 ± 0.02	0.71 ± 0.02†	0.56 ± 0.02†
Non-cruciferous[a]	2.59 ± 0.04	2.69 ± 0.04††	2.66 ± 0.05	2.65 ± 0.05	6.64 ± 0.09†	5.34 ± 0.09†
Green[b]	0.36 ± 0.01	0.45 ± 0.01	0.58 ± 0.02†	0.39 ± 0.01	0.69 ± 0.02†	0.80 ± 0.02†
Orange[c]	0.40 ± 0.01	0.41 ± 0.01	0.35 ± 0.01†	0.41 ± 0.01	0.74 ± 0.02†	0.58 ± 0.02†

Values are mean ± SE

$P < 0.001$ for interaction between intake change over time and intervention assignment for carbohydrate, fat, fiber, and total, cruciferous, non-cruciferous, green, and orange vegetables

† $P \leq 0.001$ for change from baseline

†† $P < 0.05$ for change from baseline

[a] Excluding iceberg lettuce and white potatoes but including juice

[b] E.g., leaf lettuce (excluding iceberg lettuce), spinach, green beans

[c] E.g., carrots, yams, winter squash

Discussion

In a secondary analysis of the WHEL Study population, we found that women with the highest reported intakes of total vegetable intake at their baseline measure had an overall lower hazard for breast cancer recurrence or new primary breast cancer [9]. Here, we report the first evidence that this effect of baseline high dietary vegetable consumption was greater in women receiving tamoxifen and was greatest in women who consumed high levels of cruciferous vegetables in their diets. A test for interaction between tamoxifen use and vegetable consumption for lowering hazard of recurrence was significant ($P = 0.04$) as well as cruciferous vegetables ($P = 0.005$) and suggests a previously unrecognized interaction between diet and tamoxifen on breast cancer outcomes.

The overall protective effect of high baseline vegetable intake is consistent with our earlier work demonstrating higher vegetable/fruit intake and physical activity promotes improved cancer-free survival [9]. The results also replicate earlier evidence from the WHEL Study suggesting that plasma carotenoid concentrations at baseline (independent of intervention assignment or change in vegetable intake over the trial) are associated with reduced risk for recurrent or new breast cancer [25, 26]. Our observation of an overall effect of total vegetable intake at baseline on recurrence risk is expected given the high correlation between total vegetable intake and plasma carotenoid concentrations.

The finding that a protective association was found within the highest tertile of vegetable intake at baseline, but not in relation to a substantial increase in intake with assignment to the intervention group, suggests that at baseline women have already achieved a sufficient threshold of exposure to achieve dietary benefits against recurrent disease. Women were enrolled in the WHEL Study on average 23.5 months post-breast cancer diagnosis. Early post-diagnosis change in diet, which included higher vegetable intake, has been described for this population [27] and may have influenced breast cancer outcomes. Our findings are not supported by a recent study where higher overall vegetable intake reported 1–2 years prior to breast cancer diagnosis was not associated with increased survival [28]. No stratification to assess specific associations with tamoxifen was included in the analysis.

The reduction in hazard risk for recurrence attributed to higher vegetable intake was most significant in women taking the selective estrogen receptor modulating drug, tamoxifen. The differential associations for tamoxifen use in WHEL have not been evaluated previously [8] and the secular trend in chemotherapy and tamoxifen use during

Table 3 Hazard ratios (HR) for breast cancer recurrence by tertiles of baseline vegetable intake (full models and models stratified by tamoxifen use) for WHEL women reporting dietary intake with repeat dietary recalls[a]

Total vegetable intake	All women [2,940(487)[b]]	Women not using tamoxifen [1,175(230)[b]]	Women using tamoxifen [1,765(257)[b]]
P value for tamoxifen interaction in full model = 0.04			
Lowest tertile	1.00	1.00	1.00
	981 (190)[b]	392 (82)[b]	589 (105)[b]
Middle tertile	0.87 (0.70–1.07)	0.90 (0.66–1.25)	0.78 (0.58–1.04)
	980 (163)[b]	391 (74)[b]	589 (89)[b]
Highest tertile	**0.69 (0.55–0.87)**	0.77 (0.56–1.08)	**0.56 (0.41–0.77)**
	979(134)[b]	392(74)[b]	587(63)[b]
Cruciferous vegetable intake	All women [2,940(487)[b]]	Women not using tamoxifen [1,175(230)[b]]	Women using tamoxifen [1,765(257)[b]]
P value for tamoxifen interaction in full model = 0.005			
Lowest tertile	1.00	1.00	1.00
	981 (181)[b]	392 (79)[b]	589 (102)[b]
Middle tertile	0.84 (0.68–1.04)	0.81 (0.58–1.13)	0.86 (0.64–1.14)
	980 (154)[b]	391 (65)[b]	589 (89)[b]
Highest tertile	0.85 (0.69–1.06)	1.08 (0.79–1.47)	**0.65 (0.47–0.89)**
	979 (152)[b]	392 (86)[b]	587 (66)[b]

Values are HR (95% CI)

Significant ($P \leq 0.05$) HRs are in bold

[a] Models adjusted for time from diagnosis to study entry, menopausal status, intervention status, cancer stage, estrogen receptor status, chemotherapy, BMI, physical activity, clinical site, and tamoxifen use in the full model

[b] *n* (events)

Table 4 Hazard ratios (HR)[a] for cruciferous vegetable intake groups stratified by tertiles of total daily vegetable intake among women taking tamoxifen ($n = 1{,}765$)

	Low cruciferous vegetable intake Mean = 0.08 ± 0.09[b]	High cruciferous vegetable intake Mean = 0.81 ± 0.52[b]
Tertiles of total vegetable intake		
Low		
1.47 (1.43–1.51)[b]	1.00 (reference)	0.80 (0.50–1.27)
Range: 0.00–2.16	432 (82)[c]	156 (23)[c]
Middle		
2.77 (2.74–2.80)[b]	0.82 (0.57–1.19)	**0.66 (0.46–0.96)**
Range: 2.16–3.45	263 (44)[c]	325 (45)[c]
High		
5.12 (4.99–5.26)[b]	0.66 (0.41–1.07)	**0.48 (0.32–0.70)**
Range 3.46–16.11	181 (22)[c]	408 (41)[c]

Values are HR (95% CI)

Significant ($P \leq 0.05$) HRs are in bold

[a] Models adjusted for menopausal status, cancer stage, estrogen receptor status, chemotherapy, BMI, physical activity, intervention assignment, time from diagnosis to study entry, and clinical site

[b] In servings per day with SD or 95% CI; cruciferous vegetable intake is split at median intake (0.285 servings)

[c] *n* (events)

the study may have influenced the findings. Importantly, tests for interactions with estrogen receptor positive status were not significant (data not shown) and suggest that 'tamoxifen use' is driving the observed protective associations. The reason for this observed effect in tamoxifen users is not known. We speculate that these results may reflect a potential enhancement of tamoxifen efficacy or complimentary cancer-preventive bioactivity of vegetable-derived chemopreventive compounds. There also may be confounding between baseline tumor characteristics in women with healthy diet behaviors (i.e., mammography use, physical activity and lower BMI) or some unmeasured effect of tamoxifen compliance among this same subgroup of women.

Interpretation of the findings related to the risk modification demonstrated for vegetable consumption supports the in vitro and in vivo for anti-tumor properties of bioactives in vegetables and, in particular, cruciferous vegetables. For example, experimental studies testing cruciferous vegetable bioactive constituents in combination with tamoxifen provide a biologic mechanism for the potential protective effects of cruciferous vegetable. First, indole-3-carbinol, the primary indole found in broccoli, in combination with tamoxifen induces a potent pro-apoptotic response in the estrogen responsive MCF-7 breast cancer cell line resulting in a 30% increase in overall inhibition of cell growth when the agents are combined compared to tamoxifen or indole-3-carbinol alone [19]. Second, indole-3-carbinol (I3C) + tamoxifen in combination have been associated with a fourfold reduction in DMBA-induced mammary tumors in rats, a response that was accompanied by prolonged tumor latency [18]. Finally, a study in Fisher rats showed diindolylmethane (DIM), the major metabolic end-product of indole-3-carbinol, down-regulated flavin-containing monooxygenase activity (FMO) in liver tissue [29] and when co-administered with tamoxifen resulted in an inhibition in tamoxifen N-oxide. Reduced production of tamoxifen N-oxide has been suggested to allow the parent tamoxifen compound to undergo preferential metabolism to 4-hydroxy tamoxifen, the more biologically active metabolite of tamoxifen.

While the data support a protective role for vegetable intake in tamoxifen users, our data cannot clearly ascertain if cruciferous vegetable intake, independent of total vegetable intake, can reduce the risk for future breast cancer events. In Table 4, we explored the relationship between cruciferous vegetable intake (cut at median) and recurrent or new breast events across tertiles of total vegetable intake. These results are suggestive of a non-significant dose response for greater vegetable intake regardless of whether women report cruciferous vegetable intake below or above the median; however, the greatest protection and the only evidence for a statistically significant protective association is among women reporting vegetable intake within the highest tertile and also who report cruciferous vegetable above the median. Thus, there appears to be a synergistic or additive efficacy associated with a diet higher in all vegetables that includes a significant contribution from cruciferous vegetables at least in women taking tamoxifen. This warrants further study.

Numerous anti-breast cancer properties have been attributed to bioactive food components of vegetables rich in carotenoids and/or cruciferous vegetables. Our previous work argues that the most important mechanisms for the reduction of breast cancer recurrence are those that influence circulating and bioavailable estrogens [30, 31]. Carotenoid-rich and cruciferous vegetables, commonly consumed by the WHEL Study women, have been associated with reductions in estrogen [10, 32] and to induce apoptosis of estrogen receptor expressing mammary tumor cells [11]. Additional or complementary mechanisms of cancer-preventive bioactivity must also be considered [14–16] since SERMS such as tamoxifen substantially suppress tissue-specific estrogen exposure and thus any additional suppression either in these target tissues or perhaps systemically would not likely account for the totality of risk reduction demonstrated. Further, the 33% reduction in the risk for recurrence seen in women not taking tamoxifen who demonstrate high baseline vegetable intake, although borderline in significance ($P = 0.13$), certainly suggests that alternative mechanisms may also be important.

Repeat 24-h dietary recalls, although limited by the reliance on self-report, pre-scheduled rather than unannounced, and collected over a relatively short interval of 3 weeks time, were selected for use here. Though a potential limitation, repeat recalls were available for a larger number of WHEL women and were more highly correlated with plasma carotenoid concentrations than the food frequency questionnaire [34]. The observed associations were constant when data were analyzed as continuous variables and as tertiles of exposure. The significant *P*-for-trend across tertiles in women taking tamoxifen suggests a possible dose–response, which favors a biological relationship. However, the potential for confounding with correlated lifestyle factors (higher physical activity and lower BMI) as well as unmeasured factors, risk of false positive discovery associated with multiple comparisons and the secondary nature of these analyses are significant limitations to the study design. This is the first study to suggest a possible beneficial effect of vegetable intakes above the average intakes in the US population [33] for breast cancer patients taking tamoxifen. The relatively high and wider range of total and cruciferous vegetable intake in our study population and the sample size are strengths of our study and afforded a unique opportunity to evaluate these associations.

We recognize that a "healthy cohort" effect cannot be ruled out, especially given the strong correlations between vegetable intake and other healthy lifestyle choices in our population. It is important to emphasize the possibility here that high vegetable intake is a surrogate for other potentially risk lowering behaviors including compliance to tamoxifen use. This is especially relevant given the commonality of lifestyle change among women diagnosed with breast cancer [35]. In our study total and cruciferous vegetable intakes were associated with lower BMI and greater physical activity. For this reason lifestyle factors were included in the adjusted models as was intervention group assignment. Interestingly, these did not modify the associations and like the dose effect suggests potential biological effects of bioactives in the diet.

Our findings from these secondary analyses cannot be generalized to the patient care setting and need to be interpreted with caution and investigated in other cohorts. In spite of the identifiable limitations, our results are the first to explore and offer evidence that a diet rich in vegetables may alter breast cancer outcomes in the context of tamoxifen treatment. The results suggest a protective association for high vegetable intake among tamoxifen users and warrant replication studies. These findings suggest the need for additional research to better understand the mechanisms of treatment responsiveness when bioactives or bioactive-rich whole foods are delivered concurrent with pharmaceuticals in an effort to reduce breast cancer recurrence risk.

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