Dietary intake of garlic and other Allium vegetables and breast cancer risk in a prospective study of postmenopausal women

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Citation

Abstract
Because garlic and its organosulfur compounds have been shown to inhibit the occurrence of mammary tumors in animal models, we sought to examine this association in the human population. During 15 years of follow-up, 34,388 postmenopausal women completing a food frequency questionnaire (FFQ) were followed for incident breast carcinoma. Supplemental data from a nested case-control study was analyzed to obtain consumption habits of other Allium vegetables not included in the FFQ. After 15 years, higher garlic intake was not found to be associated with lower breast cancer risk. A statistically significant inverse association was noted in the first 5 years (RR=0.71), but not in the second or third 5-year periods. In the nested case-control study, some inverse relationships were noted between the consumption of Allium vegetables and breast cancer, but overall do not show that a protective effect is afforded by more frequent intake of these herbs.

GRANT SUPPORT
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INTRODUCTION
Garlic (Allium sativum) is a member of the Allium genus, which also includes species such as onions (Allium cepa), chives (Allium schoenoprasum), green onions or scallions (Allium fistulosum), leeks (Allium porrum), and shallots (Allium ascalonicum) (1). The properties of garlic and other Allium vegetables have been investigated for their possible role in the prevention of cardiovascular disease and cancer (2). Organosulfur compounds, such as S-allylcysteine sulfoxide (Aliin), allylmethlysulfide (AMS), diallylsulfide (DAS), diallyldisulfide (DADS), S-allylcysteine (SAC), and S-propenylcysteine sulfoxide, are believed to be the active constituents of garlic and other Allium vegetables (3,4), and the administration of various allyl derivatives and garlic powder to experimental animals has generally led to the inhibition of carcinogenesis in mammary glands (5). Various mechanisms have been hypothesized to explain the potential cancer-preventative effects. The addition of garlic powder to the diet of rats has been shown to increase the activity of mammary glutathione S-transferases (GST), vital enzymes in carcinogen detoxification (6). In addition, garlic powder, garlic water extract, deodorized garlic powder, garlic powder with high sulfur content, and SAC have been shown to be effective in decreasing the binding of the carcinogen 7,12-dimethylbenz[a]anthracene (DMBA) to rat mammary DNA (7). DNA adduct formation is believed to be an initial step in carcinogenesis.

Few human studies have been conducted to investigate the association of Allium vegetable consumption and breast cancer, with only five epidemiological studies published to date (8-13). Results have been far from conclusive and should be viewed with caution, as none of the case-control studies utilized population-based controls, and the cohort study ascertained garlic supplement use only (dietary garlic consumption was not assessed). With consideration to the inconsistency of findings from previous studies, we sought to examine the association of intake of garlic and other Allium vegetables on the occurrence of primary breast carcinoma. Data from the Iowa Women's Health Study, a population-based cohort of postmenopausal women in whom diet was measured at baseline, was utilized to analyze this association. Additional information was ascertained from a nested case-control study to obtain consumption habits of Allium vegetables that were not included in the original food frequency questionnaire.
MATERIALS AND METHODS

STUDY POPULATION

The methods used in the Iowa Women's Health Study have been published elsewhere (14-16). To summarize, in January 1986, a questionnaire was sent to 99,826 randomly selected women, ages 55-69 years, whose names were included on the 1985 Iowa state drivers' license list. A total of 41,836 women (42.3%) completed the questionnaire and were followed for cancer incidence and mortality. The average age of respondents was 61.7 years, and 99% of respondents were Caucasian. The rates of breast cancer among respondents and nonrespondents were virtually identical after five years of follow-up (16). The cohort members were resurveyed by mailed questionnaire in 1987, 1989, 1992, and 1997, and the response rates were high: 91%, 89%, 83%, and 79% respectively. Women with a history of cancer at baseline other than skin cancer (n=3,830), those with a prior mastectomy or lumpectomy (n=1,884), those who were peri- or premenopausal (n=569), as well as those with 30 or more blank items on the food frequency questionnaire (FFQ) or with total energy intake values of <600 or ≥ 5,000 kcal per day (n=3,102) were excluded from all analyses. After all exclusions, a total of 34,388 women were eligible for follow-up. During analysis, those with missing covariate information were also excluded.

IDENTIFICATION OF CASES OF BREAST CANCER

Vital status of the cohort members was determined through computer linkage of participant identifiers with Iowa death certificates; through follow-up questionnaires mailed in 1987, 1989, 1992, and 1997; and through the National Death Index for nonrespondents. Cancer incidence was ascertained through the State Health Registry of Iowa, a part of the National Cancer Institute's Surveillance, Epidemiology, and End Results Program (17). By December 31, 2000, after 15 years of follow-up, 2031 women who met inclusion criteria were diagnosed with incident invasive or in situ breast carcinoma. Person-years were computed as the time from January 1986 to the first of a) breast cancer diagnosis, b) death (for residents of Iowa), c) midpoint of the interval between the date of last contact and the date of death (for residents outside of Iowa), d) December 31, 2000 (end of follow-up), e) emigration from Iowa (if date unknown), or f) midpoint of interval between the date of last contact and either the date of next follow-up or December 31, 2000 (if date of move was unknown). A diagnosis of breast cancer was treated as a censoring event.

EXPOSURE ASSESSMENT

Exposure was assessed at baseline by a self-administered questionnaire that solicited information on factors known or suspected to be relevant to breast cancer risk, such as family history of breast cancer, pregnancy history, menstrual history, and smoking history. Reported body weight and height were used to calculate body mass index (kg/m²). Diet was assessed using a semiquantitative food frequency questionnaire (FFQ) that was almost identical to the one used in the 1984 Nurses' Health Study (15-16). The usual intake frequency of specified portions of 127 food items, including alcoholic beverages (beer, red wine, white wine, and liquor), was ascertained for the year prior to baseline. One of the questions on the FFQ asked cohort members to provide their average use of garlic, fresh or powdered, using one clove or shake as the reference serving size. Frequency categories ranged from “never or less than once per month” to “6 or more per day.”

Food composition values used to generate nutrient intake estimates for the FFQ were obtained from the Harvard University Food Composition Database, which was derived from the US Department of Agriculture (18), and supplemented by manufacturer information and other published values. Nutrient intake was calculated by multiplying the frequency of consumption per day for each item by its nutrient content per serving and totaling the nutrient intake for all food items. In a validation study of 194 female nurses, a FFQ nearly identical to the one used in the IWHS was found to account for 93% of total caloric intake. Calorie-adjusted correlations between nutrient intake estimates from the FFQ and the criterion measure (multiple diet records) ranged from 0.28 for iron to 0.61 for total carbohydrate (19).

NESTED CASE-CONTROL STUDY

Because no information was collected at the baseline survey on the usual intake of Allium vegetables other than garlic, a supplementary survey of all common Allium vegetables was included in a case-control study conducted during the period from 1995 through 1996 in a subset of cohort members. The methodology for this study has been published previously in detail (20). Eligible case subjects included all cohort members who had breast cancer diagnosed from 1992 through 1994 (n = 453). A sample of 900 women was randomly selected from 27,186 cohort members who were alive and cancer-free...
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on January 1, 1992, and participated in the 1992 follow-up. Exclusions were made if the women were found either to have a breast cancer diagnosis during the period from 1992 through 1994 (n = 3) or to have been selected for participation in other Iowa Women's Health Study ancillary projects (n = 21), yielding 876 eligible control subjects. The subjects were asked to complete a self-administered food intake questionnaire on Allium vegetable consumption habits during the “reference” year (defined below). This questionnaire solicited information regarding the usual intake frequency and usual serving sizes of the following six Allium vegetables: chives, garlic (cloves and powder, separately), green onions or scallions, leeks, onions, and shallots. Response categories provided ranged from “never” to “1 or more times per day.” For garlic cloves, green onions, leeks, onions, and shallots, the questionnaire asked respondents to list their usual serving size rounded to the nearest tablespoon, while for garlic powder and chives, respondents were asked to provide their average serving size in teaspoons.

In order to reduce potential effects of breast cancer diagnosis and pre-diagnostic disease symptoms on dietary intake, information was obtained for case subjects on usual dietary habits one year before cancer diagnosis. Because breast carcinoma cases were diagnosed during the period from 1992 through 1994, and dietary assessment was conducted during the period from 1995 through 1996, three reference years (1991, 1992, and 1993) were identified, corresponding to the years immediately before breast cancer diagnosis. Control subjects were randomly divided into three corresponding groups of approximately equal sample sizes to obtain their dietary habits during these three reference years. To help the women recall their eating habits that year, a list of national and international events that occurred during the reference year was provided with the questionnaire. Of those eligible for the study, 273 (60.3%) case subjects and 657 (75.0%) control subjects participated. The major reasons for nonparticipation were refusal (29.1% of cases, 18.7% of controls), inability to locate (4.9% of cases, 3.8% of controls), and death before contacting (5.7% of cases, 2.5% of controls).

STATISTICAL ANALYSIS: COHORT STUDY

The frequency of garlic consumption did not lend itself to the formation of quartiles, due to a large number of non-consumers/low consumers. Instead, consumption was categorized into four groups (<1 clove or shake/month, 1-3 cloves or shakes/month, 1 clove or shake/week, ≥2 cloves or shakes/week). Proportional hazards regression was used to derive relative risks (RRs) and 95% confidence intervals (CIs) adjusted for age and other potentially confounding variables (Ω), derived from a list that included well-confirmed risk factors for breast cancer (Ω). A linear trend test using the χ² statistic was performed to examine trends in risk ratios across levels of garlic consumption (coded ordinarily).

STATISTICAL ANALYSIS: NESTED CASE-CONTROL STUDY

Although the questionnaire administered to case-control study participants requested the “usual serving size” for each Allium vegetable, many respondents left this portion blank. As a result, rather than attempting to quantify intake, frequency of consumption was calculated. The intake frequency of each Allium vegetable was originally categorized into three groups (<1 time/month, 1-3 times/month, ≥1 time/month). The consumption frequency of chives was alternately categorized (0 times/month, 0.5-1 time/month, ≥2 times/month) to account for the large number of non-consumers. Leek and shallot consumption was dichotomized (never, ever) due to low intake among study participants. Due to the high frequency of intake of onions among respondents, relative to the other Allium vegetables, the highest consumption frequency category was split to yield four groups (<1 time/month, 1-3 times/month, 1-2 times/week, ≥3 times/week). Consumption frequencies for individual Allium vegetables were summed for each participant, yielding total scores, which were organized into quartiles (<4.5 times/month, 4.5-10.49 times/month, 10.5-21 times/month, >21 times/month). Similar to the cohort analysis, variables considered for inclusion as covariates included education, family history of breast cancer, history of benign breast disease, prior or current use of hormone replacement therapy, body mass index, age at menarche, age at first live birth, age at menopause, parity, smoking history, and dietary intake of alcohol, total energy, total fat, and total fruits & vegetables. Odds ratios (ORs) were used to measure the strength of association between exposures to Allium vegetables and breast cancer risk (Ω). Unconditional logistic regression was used to control for potential confounders, and to derive adjusted ORs and 95% confidence intervals (CIs). A linear trend test using the χ² statistic was performed to examine trends in risk ratios across levels of Allium vegetable consumption (coded ordinarily). Reported P values (two sided) were from χ² tests (for categorical variables) and
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Wilcoxon rank-sum tests (for continuous variables).

RESULTS

The distribution of demographic and risk factors from the baseline survey conducted in 1986 are shown for the eligible cohort population (left three columns) and the case-control population (right three columns) (Table 1). Among the cohort population, age, education, family history of breast cancer, history of benign breast disease, body mass index, age at first live birth, age at menopause, parity, and alcohol consumption showed at least a borderline significant association with risk for breast cancer (P <0.10), and were included in the adjusted analyses. In the case-control population, family history of breast cancer, history of benign breast disease, use of hormone replacement therapy, body mass index, and parity were positively associated with breast cancer risk (P <0.05), and were included in the multivariate analyses. Though not significantly associated with breast cancer in both study groups, total energy intake was also included as a covariate in the analyses to reduce potential variation that may have arisen from overreporting or underreporting of food intake. Total fruit and vegetable intake was positively associated with garlic consumption in both populations, but the variable was not included as a covariate in the cohort and case-control analyses as no independent statistical association was found between it and breast cancer in our analyses as well as in past studies of this cohort (26,27).

Figure 1

Table 1: Comparison of cohort sample, stratified by breast cancer diagnosis, and nested case-control sample on selected demographic factors and risk factors for breast cancer.

Table 2: Relative risks (RRs) and 95% confidence intervals (CIs) of incident breast cancer according to intake of dietary garlic among postmenopausal women enrolled in the Iowa Women’s Health Study, 1986-2000.

Intake frequencies of the six Allium vegetables with their associations with incident breast cancer are shown for the nested case-control sample (Table 3). Though statistical analysis was performed on the consumption frequencies of leeks and shallots, low consumption prevented us from making meaningful assessments between high and low intake frequency groups. The only statistically significant associations observed were the odds ratio of the middle garlic powder intake frequency category relative to the reference group (RRmultivariate = 0.63, 95% CI = 0.45-0.90) and the odds ratio of the middle green onion intake frequency...
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category relative to the reference group (RR\textsubscript{multivariate} = 0.70, 95% CI = 0.49-0.99). In comparisons of the highest intake frequency groups with the lowest intake frequency groups, multivariate adjusted ORs were 0.77, 0.77, 1.07, 0.84, and 0.73 for total garlic, garlic powder only, chives, green onions, and onions respectively. None of these OR estimates or any of the trend tests were statistically significant. In order to evaluate the potential relationship between breast carcinoma and total intake of Allium vegetables, as well to take into account the low consumption of Allium vegetables among study participants, consumption frequency was summed for all Allium vegetables and categorized into quartiles. ORs for the upper three categories were estimated relative to the lowest category. Reduced risks ratios were observed with increased intake frequency of these vegetables, with adjusted odds ratios of approximately 0.72 to 0.78 in the upper three intake groups (P\textsubscript{trend} = 0.29); however, none of these results reached statistical significance.

Figure 3

Table 3: Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for the association of breast cancer with the consumption of vegetables.

<table>
<thead>
<tr>
<th>Total Garlic (Dosages and Period)</th>
<th>No. of case subjects(^a)</th>
<th>No. of control subjects(^a)</th>
<th>Age adjusted OR (95% CI)</th>
<th>Multivariate adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1 time/month</td>
<td>25</td>
<td>35</td>
<td>0.75 (0.53-1.08)</td>
<td>0.75 (0.49-1.16)</td>
</tr>
<tr>
<td>1 time/month or more/mo</td>
<td>75</td>
<td>24</td>
<td>0.79 (0.61-1.01)</td>
<td>0.79 (0.56-1.10)</td>
</tr>
<tr>
<td>P\textsubscript{value}</td>
<td>0.16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Garlic Powder</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1 time/month</td>
<td>60</td>
<td>58</td>
<td>0.70 (0.49-1.00)</td>
<td>0.70 (0.46-1.07)</td>
</tr>
<tr>
<td>1 time/month or more/mo</td>
<td>10</td>
<td>12</td>
<td>0.75 (0.54-1.04)</td>
<td>0.75 (0.51-1.10)</td>
</tr>
<tr>
<td>P\textsubscript{value}</td>
<td>0.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chives</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1 time/month</td>
<td>149</td>
<td>162</td>
<td>1.01 (0.67-1.52)</td>
<td>1.01 (0.65-1.57)</td>
</tr>
<tr>
<td>1 time/month or more/mo</td>
<td>75</td>
<td>194</td>
<td>0.87 (0.80-1.99)</td>
<td>0.87 (0.80-1.99)</td>
</tr>
<tr>
<td>P\textsubscript{value}</td>
<td>0.72</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green Onions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1 time/month</td>
<td>147</td>
<td>182</td>
<td>1.01 (0.67-1.52)</td>
<td>1.01 (0.65-1.57)</td>
</tr>
<tr>
<td>1 time/month or more/mo</td>
<td>57</td>
<td>137</td>
<td>1.03 (0.90-1.19)</td>
<td>1.03 (0.90-1.19)</td>
</tr>
<tr>
<td>P\textsubscript{value}</td>
<td>0.91</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1 time/month</td>
<td>42</td>
<td>50</td>
<td>1.00 (0.69-1.49)</td>
<td>1.00 (0.69-1.49)</td>
</tr>
<tr>
<td>1 time/month or more/mo</td>
<td>22</td>
<td>38</td>
<td>0.71 (0.47-1.10)</td>
<td>0.71 (0.47-1.10)</td>
</tr>
<tr>
<td>P\textsubscript{value}</td>
<td>0.31</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1 time/month</td>
<td>13</td>
<td>19</td>
<td>0.67 (0.40-1.15)</td>
<td>0.67 (0.40-1.15)</td>
</tr>
<tr>
<td>1 time/month or more/mo</td>
<td>57</td>
<td>137</td>
<td>0.98 (0.83-1.15)</td>
<td>0.98 (0.83-1.15)</td>
</tr>
<tr>
<td>P\textsubscript{value}</td>
<td>0.71</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

After 15 years of follow-up, high frequency of garlic consumption (≥ 2 cloves or shakes/week) was not found to be significantly associated with lower breast cancer incidence. A statistically significant association was observed during the first 5 years of follow-up, though not in the second or third 5-year periods. A possible reason for this discrepancy might be that the first five years most accurately reflected the dietary patterns as indicated on the food frequency questionnaire because habits changed over time. These observations may suggest a need to reassess diet periodically to accurately measure consumption. Another possibility for the discrepancy in the results between periods might be that those not yet diagnosed with breast cancer at baseline, but who in actuality had advanced disease, might have had symptoms that influenced dietary habits and responses on the food frequency questionnaire; results from this effect would be most notable when analyzing the first 5-years, given the mortality rates associated with these individuals. To minimize this effect, our FFQ asked for usual intake in the year prior to baseline.

Although a few isolated statistically significant inverse associations between Allium vegetable consumption and breast cancer occurrence were observed in the nested case-control study, there were no significant associations found comparing the high consumption frequency groups with reference groups. A variety of study design and methodological issues could explain why the inverse associations observed did not achieve statistical significance. The most apparent factor was that consumption levels of Allium vegetables were low within the study participants, with frequency categories of the individual herbs between <1 time/month and ≥1 time/week. In comparison, a French case-control study reporting significant inverse associations between garlic and onion intake frequency and breast cancer categorized their high intake frequency group at >16 times/week (12).

A second potential explanation for the predominately null findings is low statistical power due to the limited number of case subjects (273) available for the analysis. A third possible explanation is that measurement errors in the assessment of dietary intake may have attenuated the risk estimates. The Allium vegetable consumption questions in the case-control study were not evaluated for reliability or validity. However, there is no reason to speculate that those with breast cancer would differentially recall intake compared to those without breast cancer. Thus, one can reasonably conclude that any measurement errors in diet would most likely lead to non-differential misclassification.
As with all case-control studies, selection bias may be a concern, especially if participation was somehow related to both exposure and outcome. Our participation rates (60.3% for cases, 75.0% for controls) are comparable to most contemporaneous case-control studies. We compared study subjects with all eligible subject participants, and found the two groups generally similar in virtually all baseline risk factors and dietary habits (\(T_2\)). This suggests little selection bias in our sample. In consideration of the multiple statistical tests and comparisons conducted in this study, it is possible that the isolated statistically significant results observed are chance findings. This possible explanation is important to consider given the fact that breast cancer is believed to be mainly associated with hormonal and reproductive factors as opposed to dietary risk factors (\(T_3\)).

Our findings contribute to a limited body of literature, consisting of only five epidemiological studies demonstrating conflicting results. Dorant et al. (11) recorded 469 incident breast cancer cases among their Dutch cohort of postmenopausal women during the first 3.3 years of follow-up; these cases were compared to 1713 cancer-free subcohort members who had previously been randomly chosen for follow-up. High intake of onions, leeks, and garlic supplements was not found to be significantly associated with breast cancer occurrence after controlling for dietary and nondietary risk factors (\(R_{\text{onions}} = 0.95, 95\% \text{ CI} = 0.61-1.47, R_{\text{leeks}} = 1.08, 95\% \text{ CI} = 0.79-1.48, \) and \(R_{\text{garlic supplement}} = 0.87, 95\% \text{ CI} = 0.58-1.31\)). Dietary garlic (clove or powder) consumption was not evaluated. Leek consumption was categorized as 0 times/month, \(\leqslant 2\) times/month, and \(\geqslant 2\) times/month. Onion consumption was categorized as 0 onions/day, \(\leqslant 0.25\) onions/day, 0.25-0.5 onions/day, and \(>0.5\) onions/day. Garlic supplement use was dichotomized (yes/no). In Switzerland, Levi et al. (12) reported in a study of 107 cases and 318 hospital-based control cases an inverse association with frequency of onion intake, with an age-adjusted OR of 0.5 for high consumption (\(P_{\text{trend}} < 0.01\)); no significant association was found for garlic. Intake frequency categories for the herbs were not provided.

More recently, Challier et al. (13) investigated the frequency of garlic and onion consumption in 345 breast cancer patients and 345 age and socio-economic status matched controls in France, reporting a highly significant inverse association between the combined consumption of garlic and onions and breast cancer (\(P_{\text{trend}} = <10^{-6}\)). The high consumption frequency group (\(>16\) times/week) had an odds ratio of 0.30 (95\% CI = 0.17-0.52). Frequency categories were reported between \(\leqslant 6\) times/week and \(>16\) times/week. Katsouyanni et al.’s (11) small Greek case-control study of 120 confirmed breast cancer patients and 120 hospital-matched controls reported no significant association between onion and leek consumption and breast cancer occurrence. The authors did not report intake categories. Galeone et al. (13) studied onion and garlic consumption in a case-control study of 2936 histologically confirmed breast cancer cases and 3122 hospital-matched controls in southern Europe, finding no inverse associations after multivariate analyses comparing the highest consumers to the reference group (\(OR_{\text{onion}} = 0.75, 95\% \text{ CI} = 0.50-1.12, \) and \(OR_{\text{garlic}} = 0.90, 95\% \text{ CI} = 0.77-1.05\)). Frequency categories were reported between 0 and \(\geqslant 7\) portions/week for onions, and from “nonuse or low use” to “high use” for garlic.

Varying intake frequencies amongst the different study populations may account for the contradictory results. Unfortunately, none of the studies, including our own, quantitatively assessed the amount of Allium vegetables consumed. Thus, there is limited ability to ascertain whether or not dose differences explain the conflicting findings. In our prospective analysis, the baseline FFQ provided a reference serving size for garlic intake (one clove or shake). In our nested case-control study, the supplemental questionnaire requested the “usual serving size” for each Allium vegetable, but many respondents left this portion blank, preventing meaningful attempts to quantify intake.

To our knowledge, our study is only the second prospective study to investigate the association between Allium vegetables and breast carcinoma, and the first cohort analysis to investigate dietary garlic and breast cancer risk. Overall, our study did not find an association between the consumption of garlic and other Allium vegetables and breast cancer risk. Strengths of our study include its population-based design, the large cohort size, the use of a nested case-control study to supplement information not found in the original FFQ, the ability to adjust for multiple potential confounders, and the use of a validated FFQ. This study is also of interest as it is the first one conducted in the United States to report on this issue. The results may have been affected by the low frequency of consumption of these herbs among the study population. More research, including studies conducted in groups with higher Allium vegetable consumption and with better measures of intake, is needed to...
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clarify the association between Allium vegetables and breast cancer risk.

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