**Report**

**Allium vegetable consumption, garlic supplement intake, and female breast carcinoma incidence**

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**Key words:** *Allium* vegetables, breast carcinoma, cohort study, diet, garlic supplements

**Summary**

The risk of female breast carcinoma in relation to onion and leek consumption and the use of garlic supplements was evaluated in the Netherlands Cohort Study on diet and cancer. Onions, leeks, and garlic contain specific compounds which might act as antimutagens. Animal experiments also suggest a possible role for these compounds in inhibition of mammary carcinogenesis.

The Netherlands Cohort Study was started in 1986 among 120,852 Dutch men and women, aged 55–69 years, with collecting information on usual diet and important lifestyle characteristics. After 3.3 years of follow-up, 469 incident female breast carcinoma cases and 1713 female members of a randomly sampled control subcohort were available for analysis. Intake of onions or leeks was not associated with breast carcinoma risk after controlling for dietary and nondietary risk factors: the rate ratios in the highest intake categories were 0.95 (95% confidence interval 0.61–1.47) and 1.08 (95% confidence interval 0.79–1.48), respectively, compared with the lowest intake categories. The tests for trend in the rate ratios were neither significant. Garlic supplement use was also not associated with breast carcinoma incidence (rate ratio = 0.87, 95% confidence interval 0.58–1.31).

In conclusion, we found no association between the consumption of onions or leeks, or garlic supplement use, and the incidence of female breast carcinoma.

**Introduction**

Breast cancer is the most frequent cancer among women in The Netherlands. In 1989, 30.7% of all new malignant tumors in women were breast tumors [1]. In reviews of epidemiological studies, it was concluded that a high consumption of certain vegetables and fruit might reduce the risk of breast cancer [2–4]. Most evidence for an inverse association has been reported primarily in studies in which intake of a few selected micronutrients, mostly vitamin C and β-carotene, as marker for fruit or vegetable intake, was investigated [3, 4]. Nevertheless, other compounds present in vegetables and fruit, such as glutathione and flavonols, might be protective as well [3, 5–8]. Onions, leeks, garlic, and other vegetables belonging to the *Allium* genus (e.g., shallots and chives) contain not only the flavonols quercetin and kaempferol [9–11], and the cysteine-containing tripeptide glutathione [12], but also a wide variety of specific organic sulfur compounds, which are accountable for the typical odor of these
plants [13, 14]. Some of the organosulfur compounds (e.g., diallyl sulfide) act as antimutagens in in vitro laboratory experiments, and might even act as antitumor agents in vivo [15]. One of the other potential chemopreventive compounds, quercetin, has been shown to reduce the incidence and number of mammary tumors in rats induced with 7,12-dimethylbenz[a]anthracene (DMBA) and with N-nitrosomethylurea [16]. Notably, the combination of diallyl sulfide and quercetin was more effective in reducing the number of mammary tumors than single administration of these compounds in the DMBA-induced mammary carcinogenesis model [17].

Organosulfur compounds have also been detected in garlic supplements [18], the most widely used dietary supplements in elderly women in The Netherlands [19]. In the DMBA-induced mammary tumor model, increasing quantities of garlic powder from dietary supplements delayed the onset of tumors and also reduced the total number of tumors per rat. The in vivo binding of DMBA to DNA was depressed, while the activity of glutathione S-transferase, an enzyme important for detoxification of carcinogens, was enhanced [20]. In another experiment, DMBA-DNA adduct formation in mammary tissue was significantly suppressed by several types of garlic supplements, but varied depending on the processing method [21].

Recently, two case-control studies have investigated the association between Allium vegetable consumption and breast cancer risk. No significant association was found between the consumption of onions or leeks and the risk of breast cancer in Greek women [22]. In the other study, performed in Switzerland, no association was detected for fresh garlic. However, a significantly decreased risk was observed with increasing consumption of onions, which persisted after controlling for age, education, and total energy intake (odds ratio = 0.3 in the highest versus the lowest tertile) [23].

We have examined the association between onion and leek consumption, garlic supplement use, and the incidence of female breast carcinoma in the Netherlands Cohort Study (NLCS), a prospective cohort study on diet and cancer that was started in 1986 among 120,852 persons, aged 55–69 years.

Materials and methods

A description of the design of the NLCS and the characteristics of the cohort has been published [24]. In brief, the NLCS was started in 1986 among 58,279 men and 62,573 women aged 55–69 years, originating from 204 municipalities in The Netherlands with computerized population registries. Accumulation of person-time in the cohort has been estimated by follow-up of a randomly selected subcohort of 1688 men and 1812 women. Information on cancer incidence has been collected for the entire cohort by record linkage with all nine cancer registries in The Netherlands and with PALGA, the Dutch network and National Database for Pathology. The method of record linkage has been published [25]. The present analysis is restricted to female breast carcinoma incidence in the first 3.3 years of follow-up (from baseline in September 1986 to December 1989). Completeness of follow-up in this period is estimated to be 95% [26]. After excluding those subjects from the entire group of breast cancer cases who reported any prevalent cancer other than skin cancer, and those with incident in situ breast cancer, with breast cancer other than carcinoma (sarcoma, lymphoma, unspecified morphology), or without at least a microscopically confirmed diagnosis, 469 incident primary breast carcinoma cases were available for analysis. From the 1812 women in the subcohort, 1716 did not report prevalent cancer (excl. skin) at the start of the study.

A 150-item semi-quantitative food frequency questionnaire was used to collect information on the usual intake of foods and beverages in the year preceding the start of the study [27]. Questionnaire data of all cases and subcohort members have been key-entered twice and processed blinded with respect to case/subcohort status to prevent random and systematic coding errors.

Subjects were categorized into four categories of onion consumption (0, ≤ 0.25, 0.25–0.5, and ≥ 0.5 onions per day) and three categories of leek consumption (0, ≤ 2, and > 2 times per month) based on information collected by asking how many onions they usually consumed per week and how often they consumed leeks in summer and winter. A question on the consumption of fresh garlic was not in-
included in the questionnaire. The semiquantitative food frequency questionnaire has been validated against three 3-day diet records [27]. Garlic supplement users were defined as those women who reported daily use of any garlic supplement for at least one year in the five-year period before baseline. Recall of garlic supplement use was evaluated by comparing questionnaire data with information from three personal interviews as reference [28].

Analyses on onion and leek consumption are based on cases and subcohort members with complete dietary data [26], while analyses on garlic supplement use are based on subjects with complete information on dietary supplement use. As potential confounders we considered: age, parity, age at menarche, age at first birth, age at menopause, artificially-induced menopause, oral contraceptive use, history of benign breast disease, breast cancer in mother or sister(s), alcohol consumption, Quetelet index, highest level of education, smoking status, and dietary intake of vitamin C and β-carotene (computed by using the Dutch food composition table [29]). Case-cohort analyses were performed based on the assumption that survival times were exponentially distributed in this follow-up period [26]. In age-adjusted stratified analyses, we computed Mantel-Haenszel rate ratios and 95% confidence intervals for each category of onion and leek consumption and for garlic supplement use. In the multivariable analyses, we further adjusted for other covariables. The rate ratios for garlic supplement use were not only computed for all consumers of garlic supplements, but also for those who exclusively consumed garlic supplements (i.e. without other supplements) and for those who consumed garlic supplements together with any other supplement. The 95% confidence intervals of the estimated rate ratios were corrected for the additional variance introduced by using a subcohort instead of the complete cohort. Tests for trend in the rate ratios were based on likelihood ratio tests. Since prediagnostic disease symptoms might have influenced dietary habits, analyses were not only conducted for all cases diagnosed in 3.3 years of follow-up, but also for those diagnosed after the first year.

Results

As is presented in Table 1, the proportion of subjects not consuming onions was slightly higher among cases than in the subcohort, while the proportions of subjects in the two highest intake categories were somewhat lower in the case group. The differences between the distributions of onion consumption in cases diagnosed after the first year of follow-up and in the subcohort were smaller. The frequency of leek consumption was equally distributed in cases and subcohort. No differences were seen between cases and subcohort regarding overall use of dietary supplements. Nearly 35% of the cases used dietary supplements, 10.2% used garlic supplements, and 24.6% any other supplement. Twenty-four of the 48 garlic supplement users took also other supplements: vitamin B (9.4%), vitamin C (7.8%), brewers' yeast (10.9%), calcium (9.4%), and multivitamins/minerals (7.9%). Cases using any other supplement took vitamin B (21.1%), vitamin C (15.8%), brewers' yeast (14.0%), calcium (12.3%), and multivitamins/minerals (12.2%). The 5.1% of the subcohort who used garlic together with any other supplement took vitamin B (7.8%), vitamin C (8.8%), brewers' yeast (7.5%), calcium, and multivitamin/minerals (both 7.0%). The distribution of supplements among subcohort members who used any other supplement was: vitamin B (24.4%), vitamin C (15.9%), calcium (12.0%), and multivitamins/minerals (13.4%).

Age-adjusted Mantel-Haenszel rate ratios according to various known risk factors have already been published [26]. In short, breast cancer risk was positively associated with early menarche and late menopause, a history of benign breast disease, and breast cancer in mother or sisters(s). No association was observed for artificially induced menopause, oral contraceptive use, education, current cigarette smoking, or Quetelet Index. A decreasing risk was observed with parity and early age at first birth [26]. Associations among subcohort members between these and other potential risk factors on the one hand and onion or leek consumption and the use of garlic supplements on the other hand, are presented in Table 2. We observed no association between onion or leek intake and age, age at menarche or
menopause, Quetelet index, breast cancer in mother, or artificially induced menopause. Compared to the lower consumption categories, more women in the highest categories of onion and leek consumption had a higher mean intake of vitamin C and \( \beta \)-carotene, and a history of benign breast disease, and did not have a sister with a history of breast cancer. A larger proportion of women in the lowest consumption categories were nulliparous, did not consume alcohol, and had never smoked. Garlic supplement use was not associated with age or age at menarche or menopause. The mean vitamin C or \( \beta \)-carotene intake and Quetelet index were higher among garlic supplement users than among those not consuming dietary supplements. A higher percentage of garlic supplement users was nulliparous, had ever been diagnosed with benign breast disease, or had a mother with a history of breast cancer, while a lower percentage had never smoked, did not use alcohol, had an artificially induced menopause, a sister with breast cancer, or reported the use of oral contraceptives.

In age-stratified analysis, lower risks were seen for those women who consumed onions, compared to those not consuming onions (Table 3). However, none of the Mantel-Haenszel rate ratios were significantly different from one. After further adjustment for other risk factors in multivariable analysis, the rate ratios stayed below the null-value, but were also not statistically significant. Onion consumption was not associated with breast carcinoma risk after exclusion of cases diagnosed during the first year of follow-up (results not shown). None of the tests for trend in the rate ratios were statistically significant.

Leek consumption was also not associated with breast carcinoma risk: none of the RRs either in the age-adjusted stratified analysis or in the multivariate analysis, were different from unity. The tests for trend were not statistically significant. Exclusion of cases diagnosed in the first year of follow-up did not alter the results (data not shown).

The use of garlic supplements was not associated with breast carcinoma risk (RR = 0.87, 95% CI 0.58–1.31). When the association with garlic supplement use was evaluated for each of the two subcate-
categories (exclusively garlic supplements with no supplements as reference category, or garlic supplements plus any other supplement with any other supplement as reference), none of the RRs (in stratified and multivariable analysis) were statistically significant. After entering parity, age at menarche, Quetelet index, and education in the model, the RR associated with exclusively garlic supplement use decreased to 0.77 (95% CI 0.49–1.23). The RR was estimated at 0.75 (95% CI 0.41–1.38) after further adjustment for other risk factors.

**Discussion**

The NLCS is the first prospective cohort study investigating the association between *Allium* vegetable consumption, garlic supplement use, and breast cancer risk in postmenopausal women. We found no evidence that consumption of onions or leeks, or the use of garlic supplements, is associated with a lower risk of female breast carcinoma.

Our results correspond with outcomes from a hospital-based case-control study in Greece, in which no association was observed between consumption of onions or leeks and breast cancer risk [22]. In contrast, in a case-control study in Switzerland, a significant protective effect was reported for increasing onion intake. The investigators noted, however, that their results should be viewed with caution, since they did not collect complete dietary information and used hospital controls. Comparison of our results with those found in Switzerland is further complicated by the fact that in the Swiss study the association between onion consumption and breast cancer risk was not evaluated for pre- and postmenopausal women separately [23].

Unfortunately, we were not able to evaluate in the NLCS if fresh garlic consumption is associated with breast carcinoma risk, since a question on fresh garlic consumption was not included in the baseline questionnaire. However, consumption of fresh garlic by female subcohort members was probably not very high: in a follow-up questionnaire mailed in 1990 to subcohort members only, 69.7% of the subjects reported that they never used fresh garlic, 12.9% consumed up to 1 clove of garlic per week. Only 1.4% of the subcohort members consumed at least 1 garlic clove per day. In the Swiss case-control study, fresh garlic consumption was not associated with breast cancer risk (age-adjusted odds ratios for garlic consumption in increasing frequency consumption tertiles (low, intermediate, high) were 1.0, 0.7, and 0.6 (trend test $\chi^2 = 1.7$)) [23].
Table 3: Rate ratios of breast carcinoma according to *Allium* vegetable consumption and garlic supplement use

<table>
<thead>
<tr>
<th>Allium consumption</th>
<th>Stratified analysis(^1)</th>
<th>Multi-variable analysis(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RR (95% CI)</td>
<td>RR (95% CI)</td>
</tr>
<tr>
<td>Onion (number/day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.00</td>
<td>1.00(^1)</td>
</tr>
<tr>
<td>≤ 0.25</td>
<td>0.91 (0.67-1.22)</td>
<td>1.02 (0.72-1.44)</td>
</tr>
<tr>
<td>0.25-0.5</td>
<td>0.82 (0.62-1.09)</td>
<td>0.87 (0.62-1.27)</td>
</tr>
<tr>
<td>≥ 0.6</td>
<td>0.86 (0.60-1.24)</td>
<td>0.95 (0.61-1.47)</td>
</tr>
<tr>
<td>Trend test (z) (P-value)</td>
<td>1.40 (0.24)</td>
<td>0.65 (0.42)</td>
</tr>
<tr>
<td>Leek (freq/month)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.00</td>
<td>1.00(^1)</td>
</tr>
<tr>
<td>≤ 2</td>
<td>0.98 (0.75-1.27)</td>
<td>0.96 (0.71-1.30)</td>
</tr>
<tr>
<td>&gt; 2</td>
<td>1.01 (0.77-1.31)</td>
<td>1.08 (0.79-1.48)</td>
</tr>
<tr>
<td>Trend test (z) (P-value)</td>
<td>0.004 (0.95)</td>
<td>0.32 (0.57)</td>
</tr>
<tr>
<td>Garlic supplement use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No supplements</td>
<td>1.00</td>
<td>1.00(^1)</td>
</tr>
<tr>
<td>Garlic supplements</td>
<td>1.00 [1.71-1.41]</td>
<td>0.87 [0.58-1.31]</td>
</tr>
<tr>
<td>No supplements</td>
<td>1.00</td>
<td>1.00(^1)</td>
</tr>
<tr>
<td>Exclusively garlic</td>
<td>1.01 [1.63-1.61]</td>
<td>0.75 [0.41-1.38]</td>
</tr>
<tr>
<td>Any other supplement</td>
<td>1.00</td>
<td>1.00(^1)</td>
</tr>
<tr>
<td>Garlic + any other</td>
<td>0.98 [0.59-1.63]</td>
<td>1.12 [0.63-1.99]</td>
</tr>
</tbody>
</table>

Reference:
\(^1\) Adjusted for age in three categories (55-59, 60-64, 65-69).
\(^2\) Adjusted for age continuous, parity, age at menarche, age at first birth, age at menopause, artificially induced menopause, oral contraceptive use, history of benign breast disease, breast cancer in mother, breast cancer in sister(s), alcohol consumption, Quetelet index, highest level of personal education, smoking status, dietary intake of vitamin C and β-carotene (both continuous).

There is no reason to assume that selection bias is responsible for not finding an association: completeness of follow-up of cancer incidence in the entire cohort was estimated at 95%, and certification of person time in the subcohort was 100% [26]. We also adjusted for confounding by including known risk factors (dietary and nondietary) in the analysis. If preclinical symptoms of breast carcinoma had an effect on the habitual intake of *Allium* vegetables or garlic supplements, we might have observed other RR after excluding cases diagnosed during the first year of follow-up. However, the results were similar to those observed for the entire case group. A potential limitation of our study is that we are not entirely sure that the absence of a relation between onion and leek consumption and breast carcinoma risk has not been caused by nondifferential misclassification of exposure. Although the validity of the semi-quantitative food frequency questionnaire has been assessed for vegetables, information on its validity regarding onion and leek consumption is not available [27]. Estimates on dietary supplement use and intake of garlic supplements, however, were considered to be accurate enough to classify individuals as user [28].

The strongest indication up to now that *Allium* vegetable consumption might be inversely associated with cancer development is derived from case-control studies. Most of these studies reported a decreasing risk with increasing intake of *Allium* vegetables, mainly for cancer in the digestive tract [2, 3]. Experimental research indicates that certain specific *Allium* compounds (e.g. diallyl sulfide) act antimutagenic, and may even act anticarcinogenic in vivo against several carcinogens [15]. Antimutagenic and anticarcinogenic action have also been suggested for other compounds detected in *Allium* vegetables, such as the flavonols quercetin and kaempferol, and the tripeptide glutathione [7, 8, 12, 16]. However, there is only little information on the ab-
sorption, metabolism, and bioavailability of the potentially chemopreventive compounds in humans. A few studies have been published on the bioavailability of flavonoids [30–31], but the uptake of glutathione has to our knowledge only been evaluated in animals [32]. Specific data on the bioavailability of organosulfur compounds from onions, leeks, and garlic supplements are also not available for humans. If these compounds are not absorbed in the intestinal tract, as is the case with quercetin [30,31], they can only act anticarcinogenically by detoxifying or scavenging ingested carcinogens or preventing formation of carcinogens from precursor compounds within the digestive tract [5]. The strongest inhibitory action might then be expected for intestinal cancers, not for hormone related sites such as breast cancer.

In conclusion, this study did not provide evidence that neither onion and leek consumption, or the use of garlic supplements, protects against breast carcinoma in postmenopausal women.

References