Tissue Antioxidants and Postmenopausal Breast Cancer: The European Community Multicentre Study on Antioxidants, Myocardial Infarction, and Cancer of the Breast (EURAMIC)\(^1\)

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Abstract

Antioxidants may protect against free radical mediated carcinogenesis. Epidemiological studies have not confirmed this hypothesis for breast cancer, possibly because of methodological limitations.

Time-integrated exposure of \(\alpha\)-tocopherol and \(\beta\)-carotene in adipose tissue, and selenium in toenails was investigated in a case-control study among postmenopausal women, ages 50–74 years, from five European countries. The study group comprised 347 incident breast cancer cases and 374 controls.

Mean antioxidant levels, adjusted for age and center, did not significantly differ for \(\alpha\)-tocopherol (cases were 4.5% higher than controls), \(\beta\)-carotene (3.0% lower), or selenium (1.8% lower). Odds ratios for highest versus lowest tertiles of exposure, adjusted for potential confounders, were 1.15 (95% confidence interval, 0.75–1.77), 0.74 (0.45–1.23), and 0.96 (0.63–1.47), respectively, without evidence for a decreasing trend. No statistically significant interactions were observed. Moreover, a provisional antioxidant score, indicating whether concentrations were above the median for zero, one, two, or all three antioxidants, yielded odds ratios of 1.00 (reference; all below median), 1.58, 1.58, and 1.21, respectively (\(\chi^2\) for association = 4.00; \(P = 0.26\)). These results do not support the hypothesis that antioxidants are important determinants of this hormone-related malignancy among postmenopausal women.

Introduction

Dietary intake of several micronutrients with antioxidant properties, such as selenium, carotenoids, and vitamins A, C, and E, has been postulated to decrease the risk of breast cancer in certain case-control and follow-up investigations (1). As a biological basis lending plausibility to this hypothesis, laboratory studies have shown that oxidative mechanisms might be implicated in age-related disorders in general and malignant tumors in particular (2).

Many epidemiological studies addressing the relationship between dietary antioxidants and cancer have been criticized because of the inherent problems of potential misclassification associated with recall of dietary habits, which in turn may not closely reflect nutritional status (3). Biomarkers offer an alternative approach that overcomes some of these problems through assessing nutrient tissue concentrations directly. Unfortunately, many biomarkers may not represent levels in the appropriate tissues. In addition, biomarkers in the most widely sampled tissue, plasma, are quite often not optimal because they may only indicate recent rather than the more robust and appropriate long-term exposures (4, 5). Further problems in observational studies arise from the relative homogeneity of the diet within study populations, leading to poor discrimination between individuals with respect to the relevant nutritional exposures (6).

In the breast cancer part of EURAMIC,\(^4\) an array of antioxidants (namely, \(\alpha\)-tocopherol and \(\beta\)-carotene levels in s.c. adipose tissue, as well as selenium in toenails) were used as long-term biomarkers of antioxidant exposure in postmenopausal women and were analyzed in a central laboratory facility (7). This study was designed to overcome many of the aforementioned potential methodological drawbacks and to explore possible interactions among these antioxidant nutrients.

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\(^3\) Member of the Project Management Group; F. J. K., project leader.
Materials and Methods

Study Population

Design. Five study centers collaborated in this case-control study (Table 1). The study design has been described in detail elsewhere (7, 8). Briefly, eligible subjects were women under 75 years of age, postmenopausal (no periods during the past 24 months), native residents, speaking the predominant or official local language, and without a medical history of malignant breast tumors. Further inclusion criteria were: no changes in the use of dietary supplements containing α-tocopherol, β-carotene, or selenium; no new or altered dietary prescription or advice for health reasons by a physician in the past year (except for prescribed changes to low-sodium or energy-restricted diets); and no weight loss over 5 kg in the previous year. Subjects with a history of treatment for alcohol or drug abuse, those diagnosed with major psychiatric disorders that would interfere with their ability to give informed consent, and institutionalized subjects were all excluded.

Cases. Patients were recruited from the surgical units of participating hospitals; they were incident cases with a diagnosis of breast cancer (ICD-O 174), histologically classified as ductal carcinoma, with primary tumors less than 5 cm, axillary lymph node stage <N3 (i.e., excluded when ipsilateral internal lymph nodes were affected), without any evidence of distant metastases at hospital discharge (M0). Histology and tumors-node-metastases were assessed by medical specialists in the pathological and clinical laboratories, with classification procedures according to International Union Against Cancer (9).

Controls. Subjects without a history of breast cancer were eligible as controls. They were recruited from the population in the catchment area and frequency matched for age according to 5-year intervals. In two centers, random samples from local population registries were used (Germany and Switzerland). In Switzerland, because of local ethical considerations, a detailed postal invitation was sent and no follow-up was allowed. In Germany, the initial contact was through a short letter, and this was followed by a telephone call. In centers where it was anticipated that low response rates would compromise internal validity, controls were selected via a random sample by the patient’s GP (the Netherlands) or from a number of GPs covering a representative area of the catchment area of the participating hospitals (Northern Ireland and Spain). These subjects were then contacted using a supportive letter from the GP; to try to improve initial response rates, this was followed by a telephone call from the study center to either the GP, as a reminder (the Netherlands), directly to the subjects (Northern Ireland), or from the GP to the subjects (Spain). Informed consent was obtained in accordance with the ethical standards of the responsible committees on human experimentation for each center. As shown in Table 1, response rates, calculated as the number of eligible subjects interviewed divided by number invited (including an unknown percentage of ineligibles), were generally higher for cases than for controls, and rates differed between centers.

Data Collection Questionnaire. Information on smoking habits; weight; height; and reproductive and medical history, including age at menarche, age at pregnancy, parity, use of oral contraceptives, age at menopause, and type of menopause, was collected for all subjects by standard questionnaires. Socioeconomic status, family history (first and second degree), and alcohol intake were assessed through locally developed questionnaires. The format of the variables used in data analysis, however, was identical in all centers. Weight and height were confirmed in most subjects, and waist and hip circumferences were measured.

Fat Aspirate. S.c. adipose tissue was taken from the buttock by needle aspiration (10). To assist in standardizing and instructing in the appropriate skills for sampling, a videotape showing the technique was distributed to all participating centers. In cases, the adipose sample was taken within 7 days of hospital admission. Samples were kept within the original plastic adaptors and immediately placed on dry ice or in liquid nitrogen, to be stored subsequently at −70°C. On average, 35 ± 15 mg of material was obtained, containing 22 ± 10 mg of fatty acids. During the study, samples were transported to the coordinating center on dry ice, which guaranteed a temperature of −56°C. Previous pilot studies had shown that this procedure did not influence vitamin levels. To monitor transport and storage conditions, pool samples were included in shipments and processed in a manner similar to the actual samples.

Vitamin levels were not associated with the time interval between hospital admission and biopsy (Pearson r = −0.002 for α-tocopherol, and r = 0.12 for β-carotene) or with storage time before analysis (r = 0.05 for α-tocopherol and −0.06 for β-carotene).

Toenails. Toenails from all toes from both feet were collected within 8 weeks after inclusion of all subjects in the study. If necessary, nails were cleaned before clipping with conventional manicure scissors; specimens were then stored in plastic bags at room temperature and were sent to the coordinating center at regular intervals.
Laboratory Analysis

Antioxidants. All biological specimens were analyzed in central laboratories. Samples from cases and controls were analyzed simultaneously by laboratory personnel blinded to disease status. Concentrations of β-carotene and α-tocopherol in adipose tissue were determined concurrently by reverse-phase high-performance liquid chromatography (11, 12) and spectrophotometric detection. Samples were protected from light during the analysis. The sample was saponified and quantitatively analyzed for vitamin and fatty acid determinations. For the duration of the study, the overall coefficients of variation for the laboratory analysis of β-carotene and α-tocopherol were 6.7 and 6.9% (at mean values of 2.13 and 84.1 μg/g in the quality control samples, respectively). Detection limits were 0.02 μg/g for β-carotene and 2 μg/g for α-tocopherol at mean sample weight. Vitamin concentrations were calculated on the basis of fatty acid concentration, which was assessed by gas-liquid chromatography (13, 14) in an aliquot of the same extract as β-carotene and α-tocopherol, and heptadecanoic acid (C17:0) was added as an internal standard to the sample before saponification. Vitamin concentrations were expressed in μg/g of total fatty acids.

Selenium. Selenium content of toenails was assessed by instrumental neutron activation analysis of the metastable 75Se isotope at the Interfaculty Reactor Institute, Delft University, the Netherlands (15). Sample weight was recorded and samples to be analyzed were cut into pieces less than 9 mm before analysis. Samples were irradiated for 17 s in a thermal flux of 1.2 × 10^{13} neutrons/cm^2·s. After a decay time of 20 s, γ radiation of metastable 75Se was measured for 60 s. Mean level of selenium in certified bovine liver reference material (NBS-1577A) was 0.761 ± 0.043 ppm (n = 87), against a certified value of 0.804 ± 0.036 ppm.

Data Analysis

Data Available. Data available include a total of 347 eligible cases and 374 controls (Table 1). Adipose tissue biopsies and toenails were available for 91 and 94% of the cases and for 98 and 96% of the controls, respectively. The adaptors for 26 subjects (22 cases and 4 controls) were found to contain insufficient adipose tissue in the laboratory and thus vitamin results are lacking for these subjects. Owing to small sample weight, 14 samples with unreliably high or low percentage of fat in the biopsy (weight of total fatty acids/total biopsy weight) were excluded (9 cases and 5 controls). In nine toenail clippings (four cases and five controls), selenium values were found to be below the detection level, and these were excluded from data analysis. In multivariate analyses of both aspirates and toenail concentrations, data from 266 cases (77%) and 339 controls (90%) were available. The data set used in these analyses is the EURAMIC pooled data set, BCO02.

Data Description. Crude means for major risk factors and potential confounders among cases and controls were calculated; linear regression was used to obtain age- and center-adjusted differences between cases and controls, including 95% CIs (16). α-Tocopherol, β-carotene, and selenium values were log-transformed before analysis, and results are presented in their original scale.

Multivariate Analysis. Multivariate analysis was conducted using multiple logistic regression with maximum likelihood estimation of the regression coefficients and their SEs; antioxidants were included as log-transformed variables. Relative risks were estimated as ORs for high (90th percentile) as compared to control samples, respectively. Detection limits were 0.02 pg/g (0.02; 0.15) at mean values of 2.13 and 84.1 pg/g in the quality control samples, respectively. Detection limits were 0.02 μg/g for β-carotene and 2 μg/g for α-tocopherol at mean sample weight. Vitamin concentrations were calculated on the basis of fatty acid concentration, which was assessed by gas-liquid chromatography (13, 14) in an aliquot of the same extract as β-carotene and α-tocopherol, and heptadecanoic acid (C17:0) was added as an internal standard to the sample before saponification. Vitamin concentrations were expressed in μg/g of total fatty acids.

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study center and disease status. Among controls, Switzerland had highest levels, whereas Germany and Spain tended to be at the lower side of the range. Regarding case-control differences, no consistent pattern was observed for any of the three antioxidants. The overall concentration of \( \alpha \)-tocopherol was 4% higher among cases, whereas \( \beta \)-carotene and selenium were 3 and 2% lower, respectively. After adjustment for age and center, this pattern remained similar, and none of the differences reached statistical significance.

To describe the data and identify potential confounders, the association of the antioxidants and the risk factors presented in Table 2 was studied among controls. No significant associations were observed with age at each childbirth, age at menarche, and age at menopause. Parous women, however, had lower aspirate concentrations compared with nulliparous for both \( \beta \)-carotene (21% lower; \( P < 0.01 \)) and \( \alpha \)-tocopherol (14% lower; \( P < 0.05 \)) but not for selenium (8%). \( \beta \)-Carotene was positively associated with height (using log-transformed concentrations: \( r = 0.27 \); \( P < 0.001 \)) and inversely associated with body weight (\( r = -0.11 \)), leading to an inverse association with BMI (\( r = -0.26 \); \( P < 0.001 \)). Among drinkers of alcohol, \( \beta \)-carotene and selenium levels were lower (38% lower, \( P < 0.001 \), and 6% lower, \( P < 0.05 \), respectively), whereas \( \alpha \)-tocopherol was slightly higher (12% higher; \( P = 0.05 \)), and \( \beta \)-carotene tended to be higher among smokers, but this was not significant (16% higher; \( P = 0.13 \)).

Use of vitamin C supplements was related (all \( P < 0.001 \)) to higher levels of \( \beta \)-carotene (43% higher) and \( \alpha \)-tocopherol (35% higher), but not to selenium (4% higher; \( P = 0.15 \)).

Table 4 presents the basic analysis of the associations of interest. Clearly, no significantly decreased ORs are observed in the higher tertiles of the three antioxidants. Point estimates of the OR in the highest tertile differed less than 25% from 1.0, with highest estimates for \( \alpha \)-tocopherol and lowest for \( \beta \)-carotene. Table 5 presents these ORs for exposure contrasts representing the 90th versus 10th percentile for each antioxidant. Age- and center-adjusted ORs showed no significant associations for any of the antioxidants. For \( \alpha \)-tocopherol, the OR = 1.25 varied from 0.56 (0.19–1.67) in Switzerland to 2.96 (1.08–8.15) in Spain; for \( \beta \)-carotene, estimates varied around the overall OR of 0.86 from 0.62 (0.25–1.51) in Switzerland to 1.19 (0.41–3.46) in Spain; for selenium, the adjusted OR = 0.77 ranged from 0.40 (0.13–1.19) in Spain to 1.60 (0.63–4.03) in the Netherlands. Owing to study size per center, however, the variability of these estimates can easily be attributed to sampling variability [\( x^2 \)-test for interaction with 4 degrees of freedom was 6.10 (\( P > 0.10 \)) for \( \alpha \)-tocopherol, 0.51 (\( P > 0.90 \)) for \( \beta \)-carotene, and 5.35 (\( P > 0.25 \)) for selenium].

Adjustment for vitamin C supplement use did not essentially alter the overall estimates. Adjustment for reproductive factors and alcohol (\( \alpha \)-tocopherol and selenium) and BMI and smoking (\( \beta \)-carotene) yielded similar results (Table 5). Logistic models including all three antioxidants and the aforementioned potential confounders failed to show any consistent pattern; owing to the positive association of aspirate \( \alpha \)-tocopherol and \( \beta \)-carotene (\( r = 0.39 \)), the estimate for the former increased at the expense of the estimate for \( \beta \)-carotene, as compared with separate modeling of the antioxidants.

Interactions were studied using two complementary approaches, both using dummy variables for high (above median) versus low (below median) antioxidant concentrations. First, two- and three-way interaction terms between the antioxidant

### Table 3

<table>
<thead>
<tr>
<th>Country (center)</th>
<th>( \alpha )-tocopherol (( \mu )g/g) Cases</th>
<th>Controls</th>
<th>( \beta )-carotene (( \mu )g/g) Cases</th>
<th>Controls</th>
<th>Selenium (( \mu )g/g) Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germany (Berlin)</td>
<td>254</td>
<td>251</td>
<td>0.98</td>
<td>1.00</td>
<td>0.506</td>
<td>0.500</td>
</tr>
<tr>
<td>The Netherlands (Zeist)</td>
<td>279</td>
<td>286</td>
<td>1.17</td>
<td>1.22</td>
<td>0.537</td>
<td>0.520</td>
</tr>
<tr>
<td>Northern Ireland (Coleraine)</td>
<td>243</td>
<td>225</td>
<td>1.01</td>
<td>1.01</td>
<td>0.572</td>
<td>0.591</td>
</tr>
<tr>
<td>Switzerland (Zurich)</td>
<td>367</td>
<td>398</td>
<td>1.11</td>
<td>1.26</td>
<td>0.594</td>
<td>0.618</td>
</tr>
<tr>
<td>Spain (Malaga)</td>
<td>328</td>
<td>266</td>
<td>0.46</td>
<td>0.44</td>
<td>0.558</td>
<td>0.588</td>
</tr>
<tr>
<td>All centers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude mean</td>
<td>288</td>
<td>277</td>
<td>0.91</td>
<td>0.94</td>
<td>0.555</td>
<td>0.567</td>
</tr>
<tr>
<td>95% CI</td>
<td>270–308</td>
<td>263–292</td>
<td>0.84–0.99</td>
<td>0.87–1.01</td>
<td>0.544–0.567</td>
<td>0.556–0.579</td>
</tr>
<tr>
<td>Age- and center-adjusted %</td>
<td>4.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% CI of difference, %</td>
<td>-3.5 to 13.2</td>
<td></td>
<td></td>
<td></td>
<td>-1.8</td>
<td></td>
</tr>
</tbody>
</table>

Note: Retransformed from means on the log scale.

Note: As a percentage of the mean value of controls.

### Table 4

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
<th>df = 2 (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha )-Tocopherol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median exposure, ( \mu )g</td>
<td>173</td>
<td>287</td>
<td>448</td>
<td></td>
</tr>
<tr>
<td>No. of cases/controls</td>
<td>94/121</td>
<td>89/121</td>
<td>113/123</td>
<td></td>
</tr>
<tr>
<td>Age- and center-adjusted OR</td>
<td>1.00</td>
<td>0.98</td>
<td>1.25</td>
<td>1.75 (0.42)</td>
</tr>
<tr>
<td>Multivariate OR</td>
<td>1.00</td>
<td>0.87</td>
<td>1.15</td>
<td>1.73 (0.42)</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.56–1.34</td>
<td>0.75–1.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \beta )-Carotene</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median exposure, ( \mu )g</td>
<td>0.47</td>
<td>0.96</td>
<td>1.80</td>
<td></td>
</tr>
<tr>
<td>No. of cases/controls</td>
<td>99/119</td>
<td>107/122</td>
<td>90/124</td>
<td></td>
</tr>
<tr>
<td>Age- and center-adjusted OR</td>
<td>1.00</td>
<td>1.06</td>
<td>0.84</td>
<td>1.46 (0.48)</td>
</tr>
<tr>
<td>Multivariate OR</td>
<td>1.00</td>
<td>1.03</td>
<td>0.74</td>
<td>2.32 (0.31)</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.65–1.62</td>
<td>0.45–1.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selenium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median exposure, ( \mu )g</td>
<td>0.469</td>
<td>0.561</td>
<td>0.698</td>
<td></td>
</tr>
<tr>
<td>No. of cases/controls</td>
<td>111/119</td>
<td>117/120</td>
<td>98/120</td>
<td></td>
</tr>
<tr>
<td>Age- and center-adjusted OR</td>
<td>1.00</td>
<td>1.05</td>
<td>0.92</td>
<td>0.46 (0.80)</td>
</tr>
<tr>
<td>Multivariate OR</td>
<td>1.00</td>
<td>1.02</td>
<td>0.98</td>
<td>0.08 (0.96)</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.67–1.53</td>
<td>0.63–1.47</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Tertile cutpoints: 231, 350 (\( \alpha \)-tocopherol); 0.69, 1.33 (\( \beta \)-carotene); 0.521, 0.613 (selenium).
* Adjusted for age, center, reproductive factors, and alcohol.
* Adjusted for age, center, reproductive factors, alcohol, BMI, and smoking.
compounds were included in the age- and center-adjusted model. Neither three-way ($P = 0.21$) nor two-way interactions reached statistical significance ($\chi^2$, 3 degrees of freedom = 5.04; $P > 0.10$); the two-way interaction terms in this model were all below 1.0, but the three-way interaction term exceeded 1.0, thereby diminishing the overall OR for combined exposure. Without these interaction terms, the age- and center-adjusted ORs were 1.24 (95% CI, 0.87–1.75) for $\alpha$-tocopherol, 1.02 (0.64–1.27) for $\beta$-carotene, and 0.90 (0.64–1.27) for selenium, essentially similar to Table 5. Second, because the former approach to interaction does not readily give interpretable estimates of association, we also calculated an antioxidant score, indicating whether one had high versus low exposure to zero, one, two, or all three antioxidants. Age- and center-adjusted ORs were in the opposite direction from that expected under the study hypothesis [i.e., 1.00 (reference), 1.58 (0.92–2.71), 1.58 (0.92–2.72), and 1.21 (0.62–2.36), respectively]. Further adjustment for reproductive factors, alcohol, BMI, and smoking did not essentially alter these estimates.

**Discussion**

In this multicenter European case-control study, no evidence was found for an inverse association of breast cancer with adipose tissue levels of $\alpha$-tocopherol and $\beta$-carotene and toenail selenium levels, either alone or in combination. Results regarding family history of cancer, anamnesis of benign breast disease, and reproductive variables were in line with the literature, whereas age at menarche and age at menopause, which are generally less consistent in epidemiological studies and more difficult to measure, were not associated with breast cancer.

Previous epidemiological findings between either estimates of dietary antioxidant intakes or blood measurements of antioxidant levels and breast cancer have been inconsistent (17). For example, for high versus low vitamin E intakes, ORs ranged from 0.6 to 1.3 in case-control studies, whereas a prospective study found no association with breast cancer (18). Moreover, the limited data from case-control and prospective studies of blood $\alpha$-tocopherol levels do not favor a relationship with breast cancer (reviewed in Ref. 17). Similarly, comparison of blood levels of selenium, which has close biological relationships with vitamin E, and breast cancer in case-control and prospective studies has indicated both elevated risk and protective effects (reviewed in Ref. 18). Null results have been observed in case-control (19) and prospective (20, 21) studies that assessed selenium in toenails as a time-integrating measure of selenium status (22). Cancer risk and dietary intake of $\beta$-carotene has often been studied in combination with vitamin A, because of both their function in cell integrity and antioxidant capacity. Although most studies showed an inverse association, it is not clear whether this is attributed to preformed vitamin A or carotenoid-derived vitamin A (reviewed in Ref. 23). From observational epidemiology, ORs for blood levels of $\beta$-carotene, which generally reflect dietary intake, are inconsistent, ranging from 0.3 to 3.3 (17, 23).

Many associations reported in the epidemiological literature may partially be a result of methodological pitfalls, quite frequently related to exposure assessment in the case-control design and often made worse in multicenter studies. In EURAMIC, comparability of exposure, both between cases and controls and between centers, was maintained by the use of biomarkers. Aspiration equipment and procedures were standardized, samples were stored and transported at $-56^\circ$C (on dry ice). Laboratory analysis was blinded with respect to disease status, analytical runs contained similar numbers of cases and controls, and vitamin concentrations are expressed relative to the amount of fat tissue obtained.

Disease-induced adaptations in dietary habits or alterations in metabolism may in theory have affected exposure assessment in cases. This potential problem is minimized by the study choice of breast cancer, rather than gastro-intestinal cancer, and the exclusion of cases with advanced disease. The choice of time-integrating biomarker media such as adipose and toenails, rather than blood, plasma (24), or antioxidant intake data (3), will have reduced such biases further. Adipose tissue concentrations of $\beta$-carotene are affected by intake (25), and almost 90% of the body pool of $\alpha$-tocopherol is concentrated in adipose tissue (26). In a separate study (25), we observed that $\beta$-carotene does indeed enter the body fat stores, and that $\beta$-carotene and $\alpha$-tocopherol are associated with plasma levels. In addition, measurements in gluteal aspirates taken from women 4 months apart are clearly correlated ($r = 0.62$ for $\beta$-carotene and $r = 0.86$ for $\alpha$-tocopherol). Toenail selenium concentration, similarly, appears to indicate long-term intake (22). Finally, exclusion of cases and controls who lost over 5 kg weight over the past year and those who started the use of supplements with $\alpha$-tocopherol, $\beta$-carotene, or selenium restricted the population to those with relatively stable food habits, without recent health-related reasons to alter these habits.

Subclinical disease or adapted health habits are therefore unlikely to have distorted our findings substantially. As long-standing supplement use increases the exposure contrast (and biomarker levels) in the population and simultaneously affects disease risk, these subjects were included.

Confounding was controlled by multivariate analysis. As hormone-related factors were not associated with antioxidant levels, only life-style factors were taken into account. In this respect, it is interesting to note that adipose antioxidant levels were lower in parous women. Similar observations have been reported for fat-soluble xenobiotics, known to be excreted by breast-feeding. Furthermore, vitamin C supplement use, associated with both disease and antioxidant levels, did not affect our estimates. In view of

**Table 5** OR of breast cancer for high versus low biomarker levels of micronutrients

<table>
<thead>
<tr>
<th>Exposure to</th>
<th>Exposure range*</th>
<th>OR and 95% CI of breast cancer in various models</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P10–P90</td>
<td>Ratio</td>
</tr>
<tr>
<td>$\alpha$-Tocopherol</td>
<td>139–500</td>
<td>3.6</td>
</tr>
<tr>
<td>$\beta$-Carotene</td>
<td>0.39–2.10</td>
<td>5.4</td>
</tr>
<tr>
<td>Selenium</td>
<td>0.44–0.73</td>
<td>1.7</td>
</tr>
</tbody>
</table>

* 90th ($P90$) and 10th ($P10$) percentiles among controls and their ratio ($P90/P10$).
* Adjusted for age, center, reproductive factors, alcohol; for $\beta$-carotene also adjusted for BMI and smoking.
* Adjusted for age, center, reproductive factors, alcohol, BMI and smoking.

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For a detailed understanding of the data presented in Table 5, please refer to the original publication. The table details the odds ratios (OR) and 95% confidence intervals (CI) for breast cancer in various models, comparing high versus low biomarker levels of micronutrients such as $\alpha$-tocopherol, $\beta$-carotene, and selenium. The analysis adjusts for age, center, reproductive factors, alcohol, BMI, and smoking. The table illustrates the relative risk associated with different exposure ranges, highlighting the impact on breast cancer risk.
Antioxidants and Breast Cancer: EURAMIC

Antioxidant status.

In contrast with other malignant tumors, is not related to controls (Table 3). To the extent that response rates might have no indication that case-control differences were smaller in between antioxidants and breast cancer. There was, however, oxidants, would tend to strengthen any inverse association bias resulting from preferential participation of health-conscientious control women, who may have greater intake of antioxidants, would tend to strengthen any inverse association between antioxidants and breast cancer. There was, however, no indication that case-control differences were smaller in centers that used population controls rather than OP-biased controls (Table 3). To the extent that response rates might have been related to any other risk factors or general health-related factors (such as smoking, drinking, and BMI), these were taken into account in the analysis.

Thus, given the lack of clear and consistent results in the literature, at least partially attributable to methodological pitfalls, and the results presented, it might be that breast cancer, in contrast with other malignant tumors, is not related to antioxidant status.

First, there are underlying differences between the hormone-related malignancies and the other malignancies, in particular for breast cancer. Apart from a positive family history of the disease (27, 28), there are few established strong and consistent risk factors (29), either with respect to hormonal and reproductive factors (30–32) or with respect to lifestyle and dietary factors (29).

Second, an important lifestyle factor in chronic disease etiology (e.g., cancer and cardiovascular disease) is cigarette smoking. There is an apparent lack of association between smoking and breast cancer risk, however (33). This may give further support for the contention that antioxidant protection against oxidant damage is not central to the etiology of breast cancer. In addition, because smoking is not a risk factor of breast cancer, we do not have to consider the potential for residual confounding in our effect estimates by such a strong background risk factor, as would be the case for lung cancer or myocardial infarction, for example (34).

Third, the higher adipose antioxidant levels in these female EURAMIC controls compared with the male controls from the myocardial infarction study (8) may exceed critically low levels and provide sufficient protection against oxidative mechanisms. Thus, the results of this study, although covering a reasonably wide range of tissue levels, do not exclude increased risks at lower levels, especially when combined with factors related to higher oxidant stress or periods of hormone-mediated increased susceptibility of mammary tissue during early life.

In conclusion, although certain aspects of diet are believed to be determinants of breast cancer risk, results from this and other studies would suggest that these diet-related antioxidants do not greatly influence risk, either alone or in combination.

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References


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