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# DIETARY ANTIOXIDANT VITAMINS, RETINOL, AND BREAST CANCER INCIDENCE IN A COHORT OF SWEDISH WOMEN

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**Dietary antioxidant vitamins and retinol have been proposed to be protective against breast cancer on the basis of their ability to reduce oxidative DNA damage and their role in cell differentiation. Epidemiologic studies have not been convincing in supporting this hypothesis, but women with high exposure to free radicals and oxidative processes have not been specifically considered. We explored these issues in the Swedish Mammography Screening Cohort, a large population-based prospective cohort study in Sweden that comprised 59,036 women, 40–76 years of age, who were free of cancer at baseline and who had answered a validated 67-item food frequency questionnaire. During 508,267 person-years of follow-up, 1,271 cases of invasive breast cancer were diagnosed. Cox proportional hazards models were used to obtain hazard ratios (HRs) and 95% confidence intervals (CIs). There was no overall association between intake of ascorbic acid, beta-carotene, retinol or vitamin E and breast cancer incidence. High intake of ascorbic acid was inversely related to breast cancer incidence among overweight women (HR=0.61; 95% CI 0.45–0.82, for highest quintile of intake among women with body mass index>25 kg/m$^2$) and women with high consumption of linoleic acid (HR=0.72; 95% CI 0.52–1.02, for highest quintile of ascorbic acid intake and average consumption of more than 6 grams of linoleic acid per day). Among women with a body mass index of 25 or below, the hazard ratio for breast cancer incidence was 1.27 (95% CI 0.99–1.63), comparing the highest to the lowest quintile of ascorbic acid intake. Consumption of foods high in ascorbic acid may convey protection from breast cancer among women who are overweight and/or have a high intake of linoleic acid.**


**Key words:** *breast cancer; ascorbic acid; beta-carotene; retinol; vitamin E*

In the search for risk factors for breast cancer, genetic and reproductive characteristics have been identified. Many of these factors are not easily modifiable, and the quest for preventive strategies for this fairly common malignancy has targeted lifestyle factors that can be influenced. The role of diet in breast carcinogenesis and promotion of tumor formation and growth is unclear, and epidemiologic evidence is inconsistent.

Dietary carotenoids, ascorbic acid and vitamin E have been postulated to have anti-carcinogenic properties through their antioxidative action. They might neutralize free oxygen radicals, which, like other carcinogens, react with DNA.[1] Reduction of oxidative DNA damage reduces genetic mutations. Antioxidants may also influence estrogen-mediated carcinogenesis by affecting estrogen metabolism. In an animal experiment, ascorbic acid inhibited estrogen-induced carcinogenesis,[2] and it may prevent formation of reactive metabolites capable of inducing tumors by way of inhibiting oxidation of estrogens.[3] Retinol (preformed vitamin A) may affect the carcinogenic process through its role in cell differentiation.[4]

Antioxidant nutrients may interact with other nutrients, such as fatty acids, in particular. High intake of polyunsaturated fatty acids increases oxidation, since these fatty acids are highly reactive substances due to 2 or more double bonds.[5] Antioxidant vitamins may counter this oxidative process.[5] Obesity has been found to be associated with suboptimal nutrition that results in low serum concentrations of antioxidants and other essential nutrients.[6,7]

Thus, interindividual variation of exposure to free radicals and oxidative processes may leave certain individuals more vulnerable to cancer development. Dietary antioxidants may affect these processes. We used data from the Swedish Mammography Screening Cohort to prospectively study the association between dietary ascorbic acid, beta-carotene, vitamin E, retinol and diagnosis of new cases of invasive breast cancer, as well as any modification of this association by fatty acid intake and body mass index (BMI).

## MATERIAL AND METHODS

### *Swedish mammography screening cohort*

A population-based mammography screening program was introduced in 2 counties in central Sweden, in Västmanland county and in Uppsala county, from 1987 to 1990. In Västmanland county, all women born between 1917 and 1948 were invited by mail to participate in a mammogram screening between March 1987 and March 1989 (n=41,786). Enclosed with this invitation was a 6-page questionnaire on demographic and lifestyle factors including questions on first-degree family history of breast cancer, height (cm), weight (kg), parity, age at first birth, education and a 67-item food frequency questionnaire (FFQ); 31,735 women (76%) returned completed questionnaires. In Uppsala county all women born between 1914 and 1948 were invited to the screening and were mailed the same questionnaire between January 1988 and December 1990 (n=48,517); 34,916 women (72%) returned completed questionnaires. In total, questionnaires completed before mammography were available from 66,651 women.

Women who did not fall within the age range of 40–76 years at mammography (n=165), women with missing (n=707) or incorrect ID numbers (n=415), women with missing return date of the questionnaire (n=608), those who moved out of the study area at an unknown date (n=79) and those who had died during follow-up but for whom date of death was missing (n=16) were excluded from the study population. We also excluded women with self-reported energy intake estimates below or above 3 standard deviations of the mean $\log_e$-transformed calories (below 417 or above 3,729) kcal from this analysis (n=793). Women with a previous cancer diagnosis other than non-melanoma skin cancer at baseline were identified by linkage to the Swedish Cancer Registry and excluded from the cohort (n=2,399). A further 2,435 women with missing or unreasonable self-reported values for height (<100 cm

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or >270 cm), weight (<30 kg or > 300 kg), body mass index (<15 or > 50) and age at first birth (≤15 or ≥ 60 years of age) were excluded from this analysis, leaving a study cohort of 59,036 women.

The study was approved by the Ethical Committee at Uppsala University Hospital and by the Karolinska Institute's Regional Ethical Committee.

*Dietary assessment*

The self-administered semiquantitative FFQ[8] included 67 foods commonly consumed in Sweden. We asked how often, on average, over the past 6 months, the participants had consumed these foods. Frequency categories ranging from "never/seldom" to "4 or more times per day" were prespecified. The FFQ included questions on fruit and vegetable consumption and on the type of fat commonly used at the table (5 types) and for food preparation (5 types); we also inquired about the usual fat layer on sandwiches (thick, thin, very thin or no fat). For nutrient calculations we used age-specific portion sizes (≤52, 53–65 and ≥66 years) based on mean values of 5,922 days of weighted food records from 213 women participating in the validation of 2 food frequency questionnaires (1 of them used in the Mammography Cohort). Nutrient composition values from the Swedish National Food Administration database were used for calculation.[9]

The validity of nutrient estimates based on the self-reported food frequencies was evaluated in a subsample of 129 women from this cohort. During 4 7-day periods, 3 to 4 months apart, each of these 129 participants weighed and recorded all foods consumed. The validity of energy-adjusted micro- and macro-nutrient estimates from the FFQ (assessed by Pearson correlation coefficients between the FFQ and weighed food records-derived estimates) was as follows: ascorbic acid r=0.3, beta-carotene r=0.4, vitamin E r=0.3, retinol r=0.5, total fat r=0.5, monounsaturated fat r=0.5 and total polyunsaturated fat r=0.4. After completion of diet recording, a subcutaneous adipose tissue sample was taken from each participant by needle aspiration. The validity of polyunsaturated fat estimated from the food questionnaire (as a % of total fatty acids) in comparison to adipose tissue composition was r=0.5. Compared with the food records, the FFQ-based intakes were underestimated on average by 12% for saturated and monounsaturated fat and by 18% for polyunsaturated fat.

The mean energy intake calculated from the FFQ in the validation subsample was 1692 (±349) kcal, and mean body mass index was 24.77 (±3.72) kg/m.[2] In the whole cohort corresponding values were 1,330 (±376) kcal and 24.74 (±3.93) kg/m.[2]

*Identification of breast cancer cases and follow-up of the cohort*

Incident cases of breast cancer were identified by linkage of the study population with the national cancer registry. Deaths in the cohort and dates of death were ascertained through the Swedish Death Register, and information about the date of leaving the study area was obtained by matching of the cohort with the Swedish Population Register.

*Statistical analysis*

Incident rates of breast cancer were calculated by dividing the number of incident cases by person-years of follow-up for each study participant. Hazard rate ratios (HRs) and 95% confidence intervals (CIs) were estimated using Cox proportional hazards models.[10] Follow-up was censored at date of diagnosis of breast cancer, date of death, date of migration out of the study area or end of the follow-up period (December 31,1997).

Nutrients were adjusted for total caloric intake by using the method recommended by Willett.[11] Each nutrient was regressed on total energy intake on the logistic scale, and the antilogged residuals were used in the regression model. Total energy intake was also included in the model to account more completely for between-person variation due to total energy intake.

Intake of vitamins was divided in quintiles and the second, third, fourth and fifth quintiles of intake were each related to the first (lowest). A test for trend employed the median value for each quintile.

Hazard ratio estimates were adjusted for age (in 5-year categories) as well as family history of breast cancer (yes/no), height (continuous), body mass index (BMI) (continuous), parity (0, 1 or 2, 3 or more children), age at first birth (≤25, 26–30, ≥31), education (less than high school, high school or university), total energy intake (quintiles), alcohol (quintiles) and fiber intake (quintiles). Analyses relating antioxidant vitamin intake with breast cancer incidence were stratified by family history, age <50 or ≥50 years at mammography roughly separating pre- and post-menopausal women, BMI (<25 or ≥25 kg/m$^2$) according to the World Health Organization definition of overweight women and alcohol consumption. The association between antioxidant consumption and breast cancer incidence was also explored within high and low strata of different types of fatty acids: monounsaturated and major polyunsaturated fat, namely, linoleic acid.

## RESULTS

In our study cohort of 59,039 women, 1,271 were diagnosed with incident, invasive breast cancer during 508,267 person-years of observation between March 1987 and December 1997. The median age in this cohort was 52 at the time of routine mammography and 32,736 women were older than 50 years. The median BMI was 24.1 and the median caloric intake derived from the FFQ was 1,304 per day. A total of 4,120 women reported a family history of breast cancer.

The energy-adjusted median values for quintiles of vitamin (antioxidant and retinol) intake among participants of the Swedish Mammography Screening Cohort are given in Table I. Median intake values of fatty acids per quintile of vitamin intake are also presented. With increasing intake of vitamin E and retinol, intake of monounsaturated, polyunsaturated and linoleic acid increased. For increasing levels of ascorbic acid and beta-carotene, intake of these fatty acids decreased (Table I).

Overall, intake of ascorbic acid, beta-carotene, vitamin E and retinol was not associated with incidence of invasive breast cancer (Table II). This lack of association persisted after adjustment for age, family history of breast cancer, height, body mass index, education, parity, age at first birth, total caloric intake and consumption of alcohol, fiber and mono- and poly-unsaturated fatty acids. When analyses were stratified by BMI ≤25 and > 25 kg/m$^2$ a differential association between ascorbic acid intake and breast cancer incidence was apparent (Table III). A similar pattern was observed for vitamin E intake although it was not statistically significant (Table III). While increasing intake of ascorbic acid appeared protective for women with BMI >25 kg/m,[2] it was associated with a somewhat increased incidence of breast cancer among women with BMI ≤25 kg/m$^2$ (Table III). Stratifying the association between dietary antioxidant and breast cancer incidence by family history, age 50 at mammography or alcohol intake above 3.94 grams per day (quartile of women with highest alcohol consumption) did not reveal any difference (data not shown).

When the relation between vitamin intake and breast cancer incidence was stratified by fatty acid intake, a difference in the association was apparent only for ascorbic acid intake (Table IV). Women who consumed more than 6 grams of linoleic acid per day benefited from high ascorbic acid intake with respect to their future breast cancer risk. No association was observed for different levels of monounsaturated fat intake (data not shown). To further examine the inverse association between ascorbic acid intake and breast cancer incidence among women with high BMI and high fat intake, we considered in particular women with BMI above 25 kg/m$^2$ and high consumption of fatty acids with highly reactive double bounds (Table V). Among these women, ascorbic acid intake appeared particularly beneficial: BMI>25 kg/m$^2$ and linoleic acid>6 g/day; HR=0.43, 95% CI 0.24–0.75 (highest *vs.* lowest quintile of ascorbic acid intake); $p$ for trend = 0.01.

**TABLE I** – ENERGY-ADJUSTED DAILY MEDIAN INTAKE OF ANTIOXIDANTS, RETINOL, AND FATTY ACIDS BY QUINTILES OF MICRONUTRIENT INTAKE AMONG 59,036 PARTICIPANTS OF THE SWEDISH MAMMOGRAPHY SCREENING COHORT

| Nutrient | Energy-adjusted intake by quintile of nutrient intake | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Ascorbic acid (mg) | 30.7 | 46.5 | 61.2 | 79.7 | 109.7 |
| Monounsaturated fat (g) | 17.9 | 17.1 | 16.5 | 15.9 | 14.7 |
| Polyunsaturated fat (g) | 7.0 | 6.5 | 6.3 | 6.1 | 5.7 |
| Linoleic acid (g) | 6.2 | 5.5 | 5.3 | 5.2 | 4.7 |
| β-carotene (mg) | 0.97 | 1.55 | 2.18 | 3.07 | 5.10 |
| Monounsaturated fat (g) | 17.3 | 16.9 | 16.5 | 16.1 | 15.2 |
| Polyunsaturated fat (g) | 6.7 | 6.4 | 6.3 | 6.2 | 6.0 |
| Linoleic acid (g) | 5.9 | 5.4 | 5.4 | 5.2 | 5.0 |
| Vitamin E (mg) | 3.8 | 5.1 | 6.3 | 7.8 | 9.3 |
| Monounsaturated fat (g) | 15.2 | 15.2 | 15.7 | 17.1 | 18.8 |
| Polyunsaturated fat (g) | 4.8 | 5.4 | 6.2 | 7.5 | 8.6 |
| Linoleic acid (g) | 3.9 | 4.5 | 5.1 | 6.5 | 7.5 |
| Retinol (mg) | 0.52 | 0.67 | 0.81 | 1.14 | 1.51 |
| Monounsaturated fat (g) | 14.7 | 16.3 | 17.1 | 16.4 | 17.3 |
| Polyunsaturated fat (g) | 5.6 | 6.2 | 6.5 | 6.3 | 6.7 |
| Linoleic acid (g) | 4.7 | 5.3 | 5.6 | 5.4 | 5.6 |

**TABLE II** – HAZARD RATIO (HR) AND 95% CONFIDENCE INTERVAL (CI) OF BREAST CANCER BY DIETARY ANTOXIDANTS AND DIETARY RETINOL AMONG 59,036 WOMEN

| | HR of breast cancer by quintile (Q) of nutrient intake | | | | | 95% CI for Q5[b] | *p* for trend* |
|---|---|---|---|---|---|---|---|
| | 1 (low) [referent] | 2 | 3 | 4 | 5 (high) | | |
| Ascorbic acid (mg/day) | | | | | | | |
| Age-adjusted | 1.00 | 0.87 | 1.10 | 0.99 | 0.95 | 0.79–1.13 | 0.96 |
| Covariate-adjusted[a] | 1.00 | 0.88 | 1.09 | 0.99 | 0.94 | 0.78–1.14 | 0.99 |
| β-carotene (mg/day) | | | | | | | |
| Age-adjusted | 1.00 | 0.95 | 1.01 | 1.06 | 1.01 | 0.85–1.21 | 0.51 |
| Covariate-adjusted[a] | 1.00 | 0.96 | 1.02 | 1.07 | 1.01 | 0.84–1.22 | 0.53 |
| Vitamin E (mg/day) | | | | | | | |
| Age-adjusted | 1.00 | 0.97 | 1.14 | 1.03 | 0.96 | 0.81–1.15 | 0.99 |
| Covariate-adjusted[a] | 1.00 | 0.96 | 1.06 | 0.88 | 0.83 | 0.60–1.14 | 0.38 |
| Retinol (mg/day) | | | | | | | |
| Age-adjusted | 1.00 | 0.95 | 0.96 | 0.96 | 1.00 | 0.84–1.19 | 0.99 |
| Covariate-adjusted[a] | 1.00 | 0.97 | 0.97 | 0.96 | 1.00 | 0.83–1.20 | 0.96 |

[a] Adjusted for age (in 5 year categories), family history of breast cancer, height, body mass index, education, parity (0, 1–2 and 3+), age at first birth (<25, 25–29 and 30+), total caloric intake and intake of alcohol, fiber and monounsaturated fatty acids.–[b] 95% confidence interval for HR comparing quintiles 1 and 5.–* *p*-value for 2-sided test for trend.

**TABLE III** – ANTIOXIDANT AND RETINOL INTAKE AND COVARIATE-ADJUSTED HAZARD RATIO (HR) AND 95% CONFIDENCE INTERVAL (CI) OF BREAST CANCER BY BODY MASS INDEX (BMI) AT MAMMOGRAPHY AMONG 59,036 WOMEN

| | HR[a] of breast cancer by quintile (Q) of nutrient intake | | | | | 95% CI[a] for Q5[b] | *p* for trend* |
|---|---|---|---|---|---|---|---|
| | 1 (low) [referent] | 2 | 3 | 4 | 5 (high) | | |
| Ascorbic acid | | | | | | | |
| BMI > 25 (554 cases) | 1.00 | 0.78 | 0.92 | 0.79 | 0.61 | 0.45–0.82 | 0.004 |
| BMI ≤ 25 (717 cases) | 1.00 | 0.96 | 1.23 | 1.17 | 1.27 | 0.99–1.63 | 0.02 |
| β-carotene | | | | | | | |
| BMI > 25 | 1.00 | 0.87 | 0.86 | 0.98 | 0.86 | 0.65–1.14 | 0.60 |
| BMI ≤ 25 | 1.00 | 1.03 | 1.14 | 1.13 | 1.14 | 0.89–1.47 | 0.21 |
| Vitamin E | | | | | | | |
| BMI > 25 | 1.00 | 0.84 | 0.84 | 0.83 | 0.74 | 0.45–1.19 | 0.27 |
| BMI ≤ 25 | 1.00 | 1.03 | 1.22 | 0.87 | 0.86 | 0.56–1.32 | 0.73 |
| Retinol | | | | | | | |
| BMI > 25 | 1.00 | 0.96 | 1.09 | 0.91 | 0.93 | 0.69–1.24 | 0.49 |
| BMI ≤ 25 | 1.00 | 0.96 | 0.87 | 1.00 | 1.05 | 0.83–1.33 | 0.59 |

[a] Adjusted for age (in 5 year categories), family history of breast cancer, height, body mass index, education, parity (0, 1–2 and 3+), age at first birth (<25, 25–29 and 30+), total caloric intake and intake of alcohol, fiber and mono- and poly-unsaturated fatty acids.–[b] 95% confidence interval for HR comparing quintiles 1 and 5.–* *p*-value for 2-sided test for trend.

## DISCUSSION

Among this cohort of women assembled in two counties of central Sweden who completed a food frequency questionnaire at the occasion of a routine mammography, we found no overall association between dietary ascorbic acid, beta-carotene, vitamin E or retinol and subsequent breast cancer incidence. We found an inverse association between self-reported ascorbic acid intake and breast cancer incidence among women with high BMI and/or high intake of linoleic acid.

A number of epidemiologic studies have considered the association between dietary antioxidant vitamins and breast cancer risk. In several individual case-control studies[12–14] as well as in a

**TABLE IV** – ANTIOXIDANT AND RETINOL INTAKE AND COVARIATE-ADJUSTED HAZARD RATIO (HR) AND 95% CONFIDENCE INTERVAL (CI) OF BREAST CANCER BY EXTREMES OF INTAKE OF LINOLEIC ACID

| | HR[a] of breast cancer by quintile (Q) of nutrient intake | | | | | 95% CI[a] for Q5[b] | *p* for trend* |
|---|---|---|---|---|---|---|---|
| | 1 (low) [referent] | 2 | 3 | 4 | 5 (high) | | |
| Ascorbic acid | | | | | | | |
| Linoleic acid > 6 g/d (468 cases) | 1.00 | 0.84 | 1.16 | 0.84 | 0.72 | 0.52–1.02 | 0.17 |
| Linoleic acid < 4 g/d (199 cases) | 1.00 | 0.66 | 0.83 | 0.67 | 0.97 | 0.63–1.49 | 0.61 |
| β-carotene | | | | | | | |
| Linoleic acid > 6 g/d | 1.00 | 1.08 | 1.26 | 1.22 | 0.93 | 0.67–1.29 | 0.77 |
| Linoleic acid < 4 g/d | 1.00 | 0.92 | 1.14 | 1.14 | 1.06 | 0.66–1.70 | 0.59 |
| Vitamin E | | | | | | | |
| Linoleic acid > 6 g/d | 1.00 | 0.77 | 0.91 | 0.74 | 0.69 | 0.40–1.19 | 0.09 |
| Linoleic acid < 4 g/d | 1.00 | 1.01 | 1.16 | 1.02 | 1.62 | 0.95–2.76 | 0.15 |
| Retinol | | | | | | | |
| Linoleic acid > 6 g/d | 1.00 | 0.95 | 0.92 | 0.90 | 0.93 | 0.68–1.28 | 0.62 |
| Linoleic acid < 4 g/d | 1.00 | 1.00 | 0.91 | 1.32 | 1.17 | 0.75–1.82 | 0.21 |

[a] Adjusted for age (in 5 year categories), family history of breast cancer, height, body mass index, education, parity (0, 1–2 and 3+), age at first birth (<25, 25–29 and 30+), total caloric intake and intake of alcohol, fiber and monounsaturated fatty acids.–[b] 95% confidence interval for HR comparing quintiles 1 and 5.–* *p*-value for 2-sided test for trend.

**TABLE V** – RETINOL AND ANTIOXIDANT INTAKE AND COVARIATE-ADJUSTED HAZARD RATIO (HR) AND 95% CONFIDENCE INTERVAL (CI) OF BREAST CANCER (210 CASES) AMONG 59,036 WOMEN WITH BODY MASS INDEX GREATER THAN 25 KG/M$^2$ AND HIGHEST INTAKE OF LINOLEIC ACID

| | HR[a] of breast cancer by quintile (Q) of nutrient intake | | | | | 95% CI[a] for Q5[b] | *p* for trend* |
|---|---|---|---|---|---|---|---|
| | 1 (low) [referent] | 2 | 3 | 4 | 5 (high) | | |
| Ascorbic acid | | | | | | | |
| Linoleic acid > 6 g/d | 1.00 | 0.71 | 0.97 | 0.69 | 0.43 | 0.24–0.75 | 0.01 |
| β-carotene | | | | | | | |
| Linoleic acid > 6 g/d | 1.00 | 0.96 | 1.15 | 1.00 | 0.96 | 0.61–1.52 | 0.95 |
| Vitamin E | | | | | | | |
| Linoleic acid > 6 g/d | 1.00 | 0.35 | 0.69 | 0.72 | 0.61 | 0.22–1.67 | 0.46 |
| Retinol | | | | | | | |
| Linoleic acid > 6 g/d | 1.00 | 1.12 | 1.22 | 1.00 | 0.93 | 0.56–1.55 | 0.50 |

[a] Adjusted for age (in 5 year categories), family history of breast cancer, height, body mass index, education, parity (0, 1–2 and 3+), age at first birth (<25, 25–29 and 30+), total caloric intake and intake of alcohol, fiber and monounsaturated fatty acids.–[b] 95% confidence interval for HR comparing quintiles 1 and 5.–* *p*-value for 2-sided test for trend.

compilation of case-control studies,[15] a significant inverse association between ascorbic acid intake and breast cancer risk was observed. Among prospective cohort studies,[16–21] a significant inverse association between some micronutrients and breast cancer incidence was found only in the Nurses' Health Study,[21] namely, ascorbic acid, carotenoids and vitamin A were associated with lower risk among women with a family history of breast cancer, and beta-carotene and vitamin A among premenopausal women. Four of the cohort studies were suggestive of an inverse association between ascorbic acid consumption and breast cancer incidence; this was strongest in the Netherlands Cohort Study.[19] Besides the Nurses' Health Study, stratified analyses were presented only for the Netherlands Cohort Study; stratification by intake of polyunsaturated fatty acids exhibited non-significant inverse trends for ascorbic acid and beta-carotene among women with high consumption of polyunsaturated fatty acids defined as at least 15.89 grams per day.[20] Analyses stratified by body mass index were not presented in any of the studies.

Dietary habits may differ between the countries for which data are available. The intake of ascorbic acid from food sources was highest among study cohorts in the United States, ranging from <112 mg per day in the lowest quintile and ≥392 mg per day in the highest quintile in the Iowa Women's Health Study,[19] to a median intake of 70 mg per day in the lowest quintile, and 205 mg per day in the highest quintile in the Nurses' Health Study.[21] In the Netherlands Cohort Study, the median intake in the lowest quintile was 58.6 mg per day and in the highest quintile 165.3 per day.[20] In our study, the median ascorbic acid intake was 30.7 mg per day in the lowest quintile and 109.7 mg per day in the highest quintile (Table I). It is possible that the women in our cohort with the lowest ascorbic acid intake were deficient in this vitamin and thus, if they were also overweight and/or consumed high amounts of linoleic acid, were at increased risk of breast cancer due to their high oxidative stress.[22,23] Most of the protection, however, was concentrated among women with ascorbic acid intake of more than 90 mg per day (highest quintile). We have previously found in this cohort that high intake of monounsaturated fat was associated with lower incidence of breast cancer and high consumption of polyunsaturated fat was associated with higher incidence of breast cancer, while saturated fat intake was nor associated with breast cancer incidence.[23] Linoleic acid is a major polyunsaturated fatty acid in the diet.

In the present data, ascorbic acid intake exhibited an inverse association with breast cancer incidence and a similar pattern, although not statistically significant, was observed for vitamin E. Orange juice has been reported to inhibit mammary tumorigenesis induced in female rats[24] and to effectively inhibit the *in vitro* proliferation of human breast cancer cells.[25] Citrus flavonoids were found to mediate these reactions. Flavonoids have been reported to inhibit cancer development.[26,27] We did not have measures of flavonoids available from the present data, but, since a high correlation between ascorbic acid and citrus flavonoids is likely, flavonoids may have a part in the protective association with ascorbic acid.

This population-based study comprised more than half a million years of follow-up and with more than 200 breast cancer cases per quintile of nutrient intake had sufficient power to detect any association between micronutrient vitamin intake and breast cancer incidence. Unfortunately, we could not account for vitamin intake from supplements, however, since this information was not available to us.

Measurement error is inherent in questionnaire-based dietary assessment. Owing to its structured form, the FFQ generally leads to an underestimation of intake as reflected in the relatively low total caloric intake calculated (Table I). When characterizing individuals according to quintiles of intake, absolute intake is of less relevance and systematic underestimation of intake across the entire study population will only bias the measure of association if the degree of underestimation differs considerably between individuals. Random within-person variation, however, might misclassify true average intake and could affect ranking of individuals; such misclassification would lead to an underestimation of the association with disease outcome (regression dilution bias). This random measurement error is also reflected in the relatively low correlations between nutrients assessed with the FFQ and the diet record in validation studies. Thus, it is possible that an association between antioxidant or retinol intake and breast cancer incidence was not detected in our study due to random measurement error. Furthermore, systematic bias due to correlated errors in reporting of foods is likely to impact on the measure of observation in an unpredictable direction. Nevertheless, the FFQ should be able to roughly separate individuals with extreme intake, *e.g.*, women with very high intake of retinol from women with low intake of retinol. Therefore, comparison of extreme quintiles of intake should be informative. Given the complex structure of correlated errors in questionnaire-based diet assessment, however, results from epidemiologic studies have to be interpreted cautiously. Finally, with any subgroup analysis such as the stratification by BMI, the possibility of a chance finding has to be considered.

In conclusion, we found no overall association between dietary ascorbic acid, beta-carotene, vitamin E or retinol and subsequent breast cancer incidence. Women who were overweight and consumed foods rich in ascorbic acid had a lower incidence of breast cancer, in particular if they also had high intake of linoleic acid.

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