Vitamin C supplement intake and postmenopausal breast cancer risk: interaction with dietary vitamin C[1,2]

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ABSTRACT

Background: Experimental and epidemiologic studies have yielded conflicting results on the relation between vitamin C intake and breast cancer risk.

Objective: We investigated the relation between vitamin C supplement intake and breast cancer risk while considering dietary vitamin C intake.

Design: Between 1995 and 2008, 2482 invasive breast cancer cases occurred in 57,403 postmenopausal women from the Etude Epidémiologique auprès de femmes de la Mutuelle Générale de l'Education Nationale (E3N) prospective cohort during 581,085 person-years. We estimated vitamin C intake from foods with the use of a validated food-frequency questionnaire that was sent to subjects in 1993–1995 and vitamin C supplement use via questionnaires sent in 1995, 2000, 2002, and 2005. Multivariable HRs (95% CIs) for primary invasive breast cancer were estimated with the use of Cox regression models. All statistical tests were 2-sided.

Results: Vitamin C supplement use (ever compared with never) was not associated with breast cancer risk overall; it was associated with higher breast cancer risk in women in the fourth quartile of vitamin C intake from foods (HR: 1.32; 95% CI: 1.04, 1.67) but not in other quartiles of dietary vitamin C intake (P-interaction = 0.03).

Conclusions: We observed that vitamin C supplement use was associated with increased postmenopausal breast cancer risk in women with high vitamin C intake from foods. Our data suggest a potential U- or J-shaped relation between total vitamin C intake and postmenopausal breast cancer risk that deserves further investigation. *Am J Clin Nutr* doi: 10.3945/ajcn.115.126326.

Keywords: breast cancer, diet, dietary supplements, interaction, prospective study, vitamin C

INTRODUCTION

Much focus has been put on antioxidants as potential preventive agents against cancer. However, experimental and epidemiologic data on vitamin C and cancer risk are still equivocal. Reviews of experimental studies have suggested both protective and detrimental effects on overall cancer risk (1, 2). Regarding the epidemiologic evidence on breast cancer risk, high vitamin C intake from foods compared with low vitamin C intake from foods was generally associated with decreased risk in case-control studies, whereas prospective studies yielded null results (3). A meta-analysis suggested that high compared with low vitamin C intake from supplements may be associated with increased breast cancer risk (3), but results were driven by a single large study (4). In a 10-y randomized trial, 500 mg vitamin C supplementation/d had no effect on breast cancer risk (5), but a potential effect modification by dietary intake was not investigated.

To our knowledge, in most previous cohort studies, vitamin C supplement use was recorded at baseline only, which may have led to misclassification and, therefore, may have limited the power to detect a potential relation between supplemental vitamin C intake and breast cancer risk. In addition, no study investigated whether this relation could differ according to vitamin C intake from foods. Vitamin C supplements are widely used in the general population and could potentially represent an easy-to-implement prevention strategy, but doses could be high and largely exceed Recommended Dietary Allowances. Therefore, we investigated the relation between supplemental vitamin C intakes and breast cancer risk with the use of regularly updated data on vitamin C supplement use and investigated a potential effect modification by dietary vitamin C.

METHODS

E3N cohort

The Etude Epidémiologique auprès de femmes de la Mutuelle Générale de l'Education Nationale (E3N) is a prospective study of 98,995 women who live in France and are covered by a national health insurance program primarily comprising teachers. Participants were 40–65 y old at recruitment in 1990. Information

[1] Supported by fellowships from the French Ministry of Research (to CC), the Mutuelle générale de l'éducation nationale, the European Community, the Ligue Nationale Contre le Cancer, the Institut Gustave Roussy, and the Institut national de la santé et de la recherche médicale.

[2] Supplemental Figure 1 is available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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about their lifestyle and health status has been collected with the use of self-administered questionnaires every 2–3 y. All study subjects signed an informed consent form in compliance with the rules of the French National Commission for Computed Data and Individual Freedom from which we obtained approval. Response rates were >75% for each follow-up questionnaire.

Identification of incident invasive breast cancers

All questionnaires inquired about cancer occurrence and type and requested the addresses of physicians and for permission to contact them. A small proportion (1%) of breast cancer cases was further identified from insurance files and death certificates. Pathology reports were obtained for 93% of incident breast cancers. In this study, we only considered confirmed invasive breast cancers as cases.

Assessment of vitamin C intake from foods

Dietary data were collected once between June 1993 and July 1995. The dietary history questionnaire, which was sent to 95,644 women, consisted of the following 2 parts: *1*) questions on consumption (quantity and frequency) of food groups and *2*) qualitative questions that allowed us to delineate the food groups into food items. The questionnaire was sent with a booklet of photographs to facilitate the estimation of portion sizes (6). The questionnaire assessed dietary consumption of 208 food items and beverages. Daily nutrient intakes were estimated with the use of a food-composition table that was derived from the updated French national database (7). The questionnaire was validated with the use of twelve 24-h recalls that were carried out monthly as the reference, and the reproducibility of the questionnaire was tested after 1 y (8). For vitamin C intake, correlation coefficients were 0.55 for validity and 0.73 for reproducibility.

Assessment of vitamin C supplement use

Vitamin C supplement use was assessed through the 1995 (baseline) and 2000, 2002, and 2005 (follow-up) questionnaires. In the first 3 questionnaires, participants were asked to check boxes for intakes of several dietary supplements, specifically of vitamin C, ≥3 times/wk. The questionnaire sent in 2005 included a more detailed questionnaire about any type of dietary supplement used ≥3 times/wk and asked subjects to check nutrient-specific boxes, especially one for vitamin C, when the supplement included the considered nutrient.

Population for analysis and follow-up

In the current analysis, follow-up started either at the date that the questionnaire sent in 1995 was returned (first questionnaire to inquire about dietary supplement intake) for women who were already postmenopausal or at the date that menopause was first reported. Participants contributed person-years of follow-up until the date of diagnosis of any cancer (other than basal cell carcinoma), the date that the last completed questionnaire was returned, or 30 June 2008 (mailing date of the last questionnaire considered for the analysis), whichever occurred first. From 98,995 E3N cohort women, we excluded women who were still premenopausal in 2005 (n = 6237), those with a prevalent cancer at the study baseline (i.e., women diagnosed with a cancer other than a basal cell carcinoma before the start of follow-up; n = 9190), women who did not answer the semiquantitative food-frequency questionnaire that was sent in 1993–1995 (n = 17,676) or the first questionnaire that inquired about dietary supplement use that was sent in 1995 (n = 6740), women who did not answer any additional questionnaire after study baseline (n = 669), and women with extreme nutritional values (i.e., those in the top and bottom 1% of the distribution of the ratio between energy intake and required energy computed with the use of age, weight, and height (n = 1080). Thus, data from 57,403 postmenopausal women were available for the study.

Statistical analysis

HRs (95% CIs) of breast cancer were estimated with the use of Cox proportional hazards models with age as the time scale. The proportional hazards hypothesis was tested by including an interaction term between each variable and age. Vitamin C intake from foods, as recorded in the 1993–1995 questionnaire, was categorized into 4 classes with the use of cutoffs that corresponded to quartiles of intake. Vitamin C supplement use was prospectively assessed and analyzed as a time-dependent variable; the information collected at the time questionnaire n and earlier from the 1995 questionnaire was used to assess vitamin C supplementation (ever compared with never) for the period between the completion of questionnaires n and n + 1. When a woman did not answer one of the follow-up questionnaires, she was considered to be exposed if she had declared exposure at any of the previous questionnaires; otherwise, she was considered to be unexposed until the date that she answered the next questionnaire. To assess the impact of potential misclassification, we verified that the exclusion of the women who did not answer one of the follow-up questionnaires had no effect on the results.

All models were stratified by 5-y–interval birth cohorts to consider a possible cohort effect. Models 2 and 3 were adjusted for the following known risk factors for breast cancer: age at menarche (continuous); age at menopause (continuous); parity and age at first full-term pregnancy (nulliparous, first full-term pregnancy at age <30 y and having 1 or 2 children, first full-term pregnancy at age <30 y and having ≥3 children, or first full-term pregnancy at age ≥30 y); use of oral contraceptives before menopause (ever or never); use of menopausal hormone therapy (ever or never; time dependent); personal history of benign breast disease (yes or no; time dependent); family history of breast cancer in first-degree relatives (yes or no); alcohol consumption (median of quartiles of intake as recorded in the 1993–1995 questionnaire); BMI (in kg/m^2; <18.5, 18.5–24.9, or ≥25; time dependent); and physical activity (<12 or ≥12 metabolic equivalents of task/wk; time dependent). In addition, we adjusted for total daily energy intake without alcohol (continuous; as recorded in the 1993–1995 questionnaire); smoking status (current smokers, never, or former smokers; time dependent); current vitamin D supplementation (yes or no; time dependent); other micronutrient supplement use (ever or never; time dependent); recent mammography (yes or no; time dependent); and educational level (undergraduate, graduate from high school, or postgraduate). In model 3, we further mutually adjusted for vitamin C intake from foods and vitamin C

supplement use. To verify the effect of each potential confounder, we performed a stepwise procedure and verified that the inclusion or exclusion of any covariate had no major effect on the findings. Because all adjustment covariates had ≤5% of missing values, we imputed missing values to the value of the previous questionnaire if available or else to the modal category.

Absolute annual rates for each stratum of vitamin C intake from foods and vitamin C supplement use were calculated with the use of a fully adjusted model with a single reference category. We investigated a potential interaction between vitamin C supplement use and vitamin C intake from foods in a model with interaction terms (e.g., product terms between ever use of vitamin C supplements and quartiles of vitamin C intake from foods) and by stratified models. To test for a linear trend, we used the median value for each quartile of vitamin C intake from foods as a continuous variable. To investigate potential reverse causation,

TABLE 1
Characteristics of participants according to vitamin C supplement use at the end of follow-up and vitamin C intake from foods in 1993–1995 (E3N cohort: n = 57,403)[1]

	Vitamin C supplement use			Vitamin C intake from foods, mg/d				
	Never (n = 47,232)	Ever (n = 10,171)	P-difference[2]	<101.9 (n = 14,351)	101.9–135.4 (n = 14,351)	135.5–177.5 (n = 14,351)	≥177.6 (n = 14,350)	P-difference[3]
Age, y								
At end of follow-up	66.5 ± 6.7[4]	66.5 ± 6.4	0.21	65.8 ± 6.6	66.5 ± 6.6	66.7 ± 6.6	67.1 ± 6.7	<0.001
At menarche	12.8 ± 1.4	12.8 ± 1.4	0.01	12.8 ± 1.4	12.8 ± 1.4	12.8 ± 1.4	12.8 ± 1.4	0.02
At menopause	50.5 ± 3.8	50.6 ± 3.7	0.17	50.5 ± 3.7	50.6 ± 3.7	50.5 ± 3.8	50.5 ± 3.8	0.01
Educational level, %			<0.001					<0.001
Undergraduate	12.0	7.8		12.5	10.8	10.2	11.5	
Graduate from high school or postgraduate	88.0	92.2		87.6	89.2	89.8	88.5	
BMI, kg/m^2, %			<0.001					<0.001
<18.5	3.2	4.7		4.5	3.5	3.1	2.9	
18.5–24.9	64.9	71.4		67.2	68.0	65.9	63.0	
≥25	31.9	23.9		28.4	28.6	31.0	34.1	
Alcohol consumption, g ethanol/d	11.5 ± 13.8	11.1 ± 13.4	0.005	12.3 ± 15.1	11.4 ± 13.5	11.1 ± 13.0	11.2 ± 13.4	<0.001
Total energy intake without alcohol, kcal/d	2129 ± 538	2133 ± 547	0.48	1904 ± 492	2060 ± 490	2180 ± 508	2372 ± 556	<0.001
Vitamin D supplementation, %			<0.001					<0.001
Never or past	86.1	71.6		84.7	82.7	83.6	83.3	
Current	13.9	28.4		15.3	17.4	16.4	16.7	
Ever use of micronutrient supplements other than calcium, vitamin D, or vitamin C, %	36.5	92.4	<0.001	42.6	45.4	46.8	51.0	<0.001
Physical activity, MET-hours/wk	24.7 ± 18.1	26.9 ± 18.8	<0.001	22.6 ± 17.3	24.6 ± 17.8	25.8 ± 18.3	27.2 ± 19.3	<0.001
Current smokers, %	7.4	7.7	0.30	9.9	7.2	6.2	6.5	<0.001
Personal history of benign breast disease, %	34.9	42.9	<0.001	35.9	36.4	36.5	36.3	0.66
Family history of breast cancer in first-degree relatives, %	10.8	10.7	0.77	10.7	10.7	10.8	11.0	0.82
Mammography in the previous follow-up period, %	88.3	91.2	<0.001	87.8	89.4	89.5	88.6	<0.001
Parity, %			<0.001					<0.001
Nulliparous	11.4	13.1		12.3	11.8	10.9	11.8	
Parous								
First child at age <30 y, 1 or 2 children	49.9	51.2		50.0	49.5	50.8	50.4	
First child at age <30 y, ≥3 children	28.6	24.9		26.2	28.2	28.8	28.7	
First child at age ≥30 y	10.1	10.8		11.6	10.6	9.5	9.1	
Ever use of oral contraceptives before menopause, %	58.7	65.2	<0.001	61.9	59.8	59.2	58.4	<0.001
Ever use of MHT, %	69.8	73.7	<0.001	70.1	70.6	71.2	70.1	0.11
Vitamin C intake from foods, mg/d	143.3 ± 61.1	151.8 ± 66.3	<0.001	77.5 ± 18.2	118.8 ± 9.7	155.1 ± 12.0	228.0 ± 51.3	—
Ever use of vitamin C supplements, %	—	—	—	15.8	17.0	17.3	20.8	<0.001

[1]E3N, Etude Epidémiologique auprès des femmes de la Mutuelle Générale de l'Education Nationale; MET, metabolic equivalent of task; MHT, menopausal hormone therapy.

[2]Values were estimated with the use of chi-square tests for categorical variables and Student's t tests for continuous variables.

[3]Values were estimated with the use of chi-square tests for categorical variables and ANOVA tests for continuous variables.

[4]Mean ± SD (all such values).

we performed sensitivity analyses with the exclusion of cases that were diagnosed in the first 5 y of follow-up.

All statistical tests were 2-sided, and significance was set at the 0.05 level. We performed all analyses with the use of SAS software (version 9.3; SAS Institute).

RESULTS

Characteristics of the study population

During a mean follow-up of 10.1 y, 2482 incident invasive postmenopausal breast cancer cases were diagnosed in 57,403 women. In the 2038 cases with known estrogen receptor status, 1679 cases were estrogen receptor positive, and 359 cases were estrogen receptor negative. The mean intake of vitamin C from foods was 145 mg/d. The main contributors to the total vitamin C intake from foods were vegetables (37.0%), whole fresh fruits (36.4%), and fruit juices (12.5%). In the 17.7% women who ever reported vitamin C supplement use between 1995 and end of follow-up, 59.4% of them reported use more than once (2 times: 39.4%; 3 times: 13.7%; and 4 times: 6.3%). Compared with never users of vitamin C supplements, ever users had higher vitamin C intake from foods and were more frequently ever users of other micronutrient supplements; ever users were less frequently overweight, more frequently users of menopausal hormone therapy and past users of oral contraceptives, had a higher level of physical activity, and had a higher level of education. Baseline characteristics of participants are displayed in **Table 1**.

Vitamin C intake from foods, vitamin C supplement use, and breast cancer risk

Compared with never use of vitamin C supplements, ever vitamin C supplement use was not associated with postmenopausal breast cancer risk (HR: 1.08; 95% CI: 0.94, 1.23) (**Table 2**). Overall, the highest quartile of vitamin C intake from foods compared with the lowest quartile of vitamin C intake from foods was not associated with breast cancer risk (HR: 0.91; 95% CI: 0.81, 1.03; *P*-trend = 0.20) (**Table 3**).

Vitamin C intake from foods modified the association between vitamin C supplement use and postmenopausal breast cancer risk (*P*-interaction = 0.03). Ever use compared with never use of vitamin C supplements was associated with increased breast cancer risk in women in the highest quartile of vitamin C intake from foods (HR: 1.32; 95% CI: 1.04, 1.67) but not in women in the lower quartiles (Table 2). Absolute annual rates of breast cancer observed across quartiles of vitamin C intake from foods were 450, 409, 432, and 391 cases/100,000 women with no vitamin C supplement use, and 432, 463, 364, and 531 cases/100,000 women in vitamin C supplement ever users; thus, the highest absolute rate was observed in supplement users in the highest quartile of vitamin C intake from foods. As an illustration, we

TABLE 2
HRs of invasive postmenopausal breast cancer associated with vitamin C supplement use globally and stratified by quartiles of vitamin C intake from foods (n = 57,403; E3N cohort, 1995–2008)[1]

	Cases, *n*	HR (95% CI)[2]	HR (95% CI)[3]	HR (95% CI)[4]	HR (95% CI)[5]
All women					
Never use of vitamin C supplements	2175	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Ever use of vitamin C supplements	307	1.09 (0.97, 1.23)	1.08 (0.96, 1.23)	1.07 (0.94, 1.23)	1.08 (0.94, 1.23)
Women with vitamin C intake from foods <101.9 mg/d					
Never use of vitamin C supplements	556	1 (reference)	1 (reference)	1 (reference)	—
Ever use of vitamin C supplements	66	1.05 (0.81, 1.36)	1.03 (0.79, 1.34)	0.98 (0.74, 1.30)	—
Women with vitamin C intake from foods within 101.9–135.4 mg/d					
Never use of vitamin C supplements	529	1 (reference)	1 (reference)	1 (reference)	—
Ever use of vitamin C supplements	72	1.11 (0.87, 1.42)	1.10 (0.86, 1.43)	1.12 (0.85, 1.47)	—
Women with vitamin C intake from foods within 135.5–177.5 mg/d					
Never use of vitamin C supplements	574	1 (reference)	1 (reference)	1 (reference)	—
Ever use of vitamin C supplements	57	0.81 (0.62, 1.07)	0.83 (0.63, 1.10)	0.83 (0.62, 1.11)	—
Women with vitamin C intake from foods ≥177.6 mg/d					
Never use of vitamin C supplements	516	1 (reference)	1 (reference)	1 (reference)	—
Ever use of vitamin C supplements	112	1.36 (1.11, 1.67)	1.31 (1.06, 1.62)	1.32 (1.04, 1.67)	—

[1]Values were estimated with the use of Cox proportional hazard models. *P*-interaction = 0.03. E3N, Etude Epidémiologique auprès des femmes de la Mutuelle Générale de l'Education Nationale.

[2]Adjusted for age (time scale) and stratified by year of birth (1925–1930, 1930–1935, 1935–1940, 1940–1945, or 1945–1950).

[3]Further adjusted for BMI (in kg/m^2; <18.5, 18.5–24.9, or ≥25; time dependent); use of oral contraceptives before menopause (ever or never); use of menopausal hormone therapy (ever or never; time dependent); parity and age at first full-term pregnancy (nulliparous, first full-term pregnancy at age <30 y and 1 or 2 children, first full-term pregnancy at age <30 y and ≥3 children, or first full-term pregnancy at age ≥30 y); age at menarche (y; continuous); age at menopause (y; continuous); total energy intake without alcohol (kcal/d; continuous; as recorded in the 1993–1995 questionnaire); current vitamin D supplementation (yes or no; time dependent); alcohol consumption (median of quartiles of intake as recorded in the 1993–1995 questionnaire); physical activity (<12 or ≥12 metabolic equivalent of tasks/wk; time dependent); personal history of benign breast disease (yes or no; time dependent); smoking status (current smokers, never or former smokers; time dependent); mammography in the previous follow-up period (yes or no; time dependent); family history of breast cancer in first-degree relatives (yes or no); and educational level (undergraduate, graduate from high school, or postgraduate).

[4]Further adjusted for use of micronutrient supplements other than vitamin C, calcium, or vitamin D (ever or never; time dependent).

[5]Further adjusted for vitamin C intake from foods (quartiles; as recorded in the 1993–1995 questionnaire).

TABLE 3
HRs of invasive postmenopausal breast cancer associated with quartiles of vitamin C intake from foods globally and stratified by vitamin C supplement use (n = 57,403; E3N cohort, 1995–2008)[1]

	Cases, *n*	HR (95% CI)[2]	HR (95% CI)[3]	HR (95% CI)[4]	HR (95% CI)[5]
All women, mg/d					
<101.9	622	1 (reference)	1 (reference)	1 (reference)	1 (reference)
101.9–135.4	601	0.94 (0.84, 1.05)	0.92 (0.82, 1.03)	0.92 (0.82, 1.03)	0.92 (0.82, 1.03)
135.5–177.5	631	0.97 (0.87, 1.09)	0.95 (0.84, 1.06)	0.94 (0.84, 1.06)	0.94 (0.84, 1.06)
≥177.6	628	0.95 (0.85, 1.07)	0.92 (0.81, 1.03)	0.91 (0.81, 1.03)	0.91 (0.81, 1.03)
P-trend	—	0.58	0.23	0.21	0.20
Never users of vitamin C supplements, mg/d					
<101.9	556	1 (reference)	1 (reference)	1 (reference)	—
101.9–135.4	529	0.93 (0.82, 1.04)	0.91 (0.81, 1.03)	0.91 (0.81, 1.03)	—
135.5–177.5	574	1.00 (0.89, 1.12)	0.96 (0.86, 1.09)	0.96 (0.85, 1.09)	—
≥177.6	516	0.91 (0.81, 1.03)	0.87 (0.77, 0.99)	0.87 (0.77, 0.99)	—
P-trend	—	0.24	0.07	0.07	—
Ever users of vitamin C supplements, mg/d					
<101.9	66	1 (reference)	1 (reference)	1 (reference)	—
101.9–135.4	72	1.00 (0.71, 1.39)	0.99 (0.70, 1.38)	0.99 (0.70, 1.38)	—
135.5–177.5	57	0.77 (0.54, 1.10)	0.78 (0.54, 1.11)	0.78 (0.54, 1.11)	—
≥177.6	112	1.21 (0.89, 1.64)	1.18 (0.85, 1.63)	1.17 (0.85, 1.62)	—
P-trend	—	0.19	0.27	0.29	—

[1]Values were estimated with the use of Cox proportional hazard models. *P*-interaction = 0.03. E3N, Etude Epidémiologique auprès des femmes de la Mutuelle Générale de l'Education Nationale.

[2]Adjusted for age (time scale) and stratified by year of birth (1925–1930, 1930–1935, 1935–1940, 1940–1945, or 1945–1950).

[3]Further adjusted for BMI (in kg/m^2; <18.5, 18.5–24.9, or ≥25; time dependent); use of oral contraceptives before menopause (ever or never); use of menopausal hormone therapy (ever or never; time dependent); parity and age at first full-term pregnancy (nulliparous, first full-term pregnancy at age <30 y and 1 or 2 children, first full-term pregnancy at age <30 y and ≥3 children, or first full-term pregnancy at age ≥30 y); age at menarche (y; continuous); age at menopause (y; continuous); total energy intake without alcohol (kcal/d; continuous; as recorded in the 1993–1995 questionnaire); current vitamin D supplementation (yes or no; time dependent); alcohol consumption (median of quartiles of intake as recorded in the 1993–1995 questionnaire); physical activity (<12 or ≥12 metabolic equivalent of tasks/wk; time dependent); personal history of benign breast disease (yes or no; time dependent); smoking status (current smokers, never, or former smokers; time dependent); mammography in the previous follow-up period (yes or no; time dependent); family history of breast cancer in first-degree relatives (yes or no); and educational level (undergraduate, graduate from high school, or postgraduate).

[4]Further adjusted for use of micronutrient supplements other than vitamin C, calcium, or vitamin D (ever or never; time dependent).

[5]Further adjusted for vitamin C supplement use (ever or never; time dependent).

plotted absolute breast cancer rates in deciles of vitamin C intake from foods according to never or ever vitamin C supplement use (**Supplemental Figure 1**). In never users of vitamin C supplements, the highest quartile of vitamin C intake from foods compared with the lowest quartile of vitamin C intake from foods was associated with reduced breast cancer risk (HR: 0.87; 95% CI: 0.77, 0.99; *P*-trend = 0.07) (Table 3). In ever users of vitamin C supplements, quartiles of vitamin C intake from foods were not associated with postmenopausal breast cancer risk (*P*-trend = 0.29).

Sensitivity analysis

When cases that were diagnosed in the first 5 y of follow-up were excluded (n = 1060 cases), the positive association that was associated with ever vitamin C supplement use in the highest quartile of vitamin C intake from foods was not attenuated (HR: 1.38; 95% CI: 1.04, 1.85). For the first, second, and third quartiles of vitamin C intake from foods, HRs (95% CIs) for ever vitamin C supplement use compared with never vitamin C supplement use were 0.96 (0.68, 1.34), 0.93 (0.65, 1.32), and 0.88 (0.61, 1.26), respectively (*P*-interaction = 0.17).

DISCUSSION

In this large prospective study of postmenopausal French women, ever use of vitamin C supplements was not associated with invasive breast cancer risk overall. However, ever use of vitamin C supplements was associated with increased risk in women with high vitamin C intake from foods. In never users of vitamin C supplements, high vitamin C intake from foods compared with low vitamin C intake from foods was associated with reduced breast cancer risk.

Biological mechanisms

As an antioxidant, vitamin C is expected to prevent cells from oxidative DNA damage and thereby protect against carcinogenesis (1). Some trials using vitamin C supplementation reported a reduction of oxidative DNA damage but only in specific groups with a high exposure to oxidative stress or low baseline plasma vitamin C concentrations (1, 9). However, most trials have reported no effect on various biomarkers of oxidative DNA damage (1, 10). Vitamin C has also been shown to regulate cell differentiation and inhibit the growth of several tumor cell lines (1).

Some in vitro studies have suggested other protective mechanisms such as an influence on redox-sensitive signaling pathways (1, 11) and on gene expression (1, 12). Paradoxically, other in vitro studies have reported that vitamin C acts as a pro-oxidant in the presence of iron or copper (1) and may increase DNA damage mediated by lipid hydroperoxide (13–15). Two in vitro studies reported on the effect of vitamin C on lipid peroxidation

with opposite findings (16, 17). However, the interpretation of in vitro studies that used vitamin C is limited by technical considerations (e.g., the stability of vitamin C under typical incubation conditions and differences in the cell models) (1, 18).

Evidence from previous epidemiologic studies

Previous cohort studies on vitamin C intake and postmenopausal breast cancer risk have generally reported on vitamin C supplement use at baseline only. Few prospective studies have considered the exposure to vitamin C intake both from foods and from supplements in relation to risk of postmenopausal breast cancer risk, and no studies, to our knowledge, have previously investigated a potential interaction between those 2 sources of vitamin C. In a previous prospective study, the highest quintile of total vitamin C intake from foods and supplements compared with lowest quintile of total vitamin C intake from foods and supplements ($\geq$686 compared with $<$97 mg/d, respectively) was associated with higher postmenopausal breast cancer risk (4). In other studies that compared similar intakes (19) or doses $>$203 compared with $\leq$98 mg/d (20), no association was reported.

When considering studies on vitamin C intake from supplements only, one study that compared vitamin C supplement use at doses $\geq$250 mg/d compared with none reported higher risk of postmenopausal breast cancer risk (21). Other studies that compared lower doses ($>$50 mg/d compared with none) (22) or did not take into account doses (23) did not report any association. A randomized clinical trial reported no effect of a 10-y 500-mg vitamin C supplementation/d (5). Similarly, previous prospective studies on the association between vitamin C intake from foods and breast cancer risk reported null results (4, 19, 20, 22–24).

Strengths and limitations

Strengths of our study include its prospective design, large size, long follow-up with minimal loss, and case ascertainment through pathology reports. Vitamin C intake from foods was assessed from a validated dietary questionnaire that was designed to assess the usual dietary intake. In the validation study, the correlation coefficients for vitamin C intake were 0.73 for reproducibility and 0.55 for validity. For the latter, although not very high, figures were of the same order of magnitude as in other similarly sized cohort studies (25) and could reflect that monthly 24-h recalls may not appropriately capture true vitamin C intake especially of seasonal specificities.

Vitamin C supplement use was assessed several times during follow-up. However, information on the duration and doses of vitamin C supplements was not available. In the 1995, 2000, and 2002 questionnaires, women were asked to report specific dietary supplements, in particular of vitamin C. Thus, the exposure may reflect the use of single vitamin C supplements (i.e., usually doses of 500 or 1000 mg/d at the time that the study was conducted); the use of multivitamins that contained lower doses of vitamin C may have been underreported, but the impact of such underreporting would have been limited because multivitamin use became very common in France only after the early 2000s. Vitamin C intake from foods was self-reported and assessed at baseline only, whereas some participants may have changed their diets during follow-up. Thus, misclassification for ever vitamin C supplement use and vitamin C intake from foods might have existed and, therefore, resulted in the underestimation of risks. Last, we could not exclude residual confounding although we adjusted for a large set of potential confounders with minimal changes from the nonadjusted model.

In conclusion, we observed an interaction between vitamin C supplement use and vitamin C intake from foods on postmenopausal breast cancer risk. Vitamin C supplement use was associated with increased risk in women with already high vitamin C intake from foods. Our findings suggest that vitamin C supplement users should be excluded from analyses on vitamin C intake from foods and cancer risk. Additional studies are needed to investigate a potential U- or J-shaped relation between total vitamin C intake and postmenopausal breast cancer risk and to clarify involved mechanisms.

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