# Interaction between current vitamin D supplementation and menopausal hormone therapy use on breast cancer risk: evidence from the E3N cohort[1]

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## ABSTRACT

**Background:** Experimental studies suggest protective effects of vitamin D on breast carcinogenesis, particularly on estrogen receptor–positive tumors. Epidemiologic data are less conclusive.

**Objective:** Our objective was to investigate the association between postmenopausal breast cancer risk and current or past vitamin D supplementation overall and according to the use of menopausal hormone therapy (MHT).

**Design:** Between 1995 and 2008, 2482 invasive breast cancer cases were diagnosed among 57,403 postmenopausal women from the E3N prospective cohort during 581,085 person-years. Vitamin D supplementation was assessed from biennially self-administered questionnaires sent in 1995, 2000, 2002, and 2005 and from medico-administrative data on drug reimbursements since 2004. Multivariable HRs for primary invasive breast cancer and 95% CIs were estimated by using Cox models.

**Results:** A decreased postmenopausal breast cancer risk was associated with current (HR: 0.82; 95% CI: 0.69, 0.97) but not past (HR: 1.10; 95% CI: 0.92, 1.31) vitamin D supplementation (*P*-homogeneity = 0.02). The association with current vitamin D supplementation differed according to MHT use: ever users (HR: 0.74; 95% CI: 0.60, 0.90) and never users (HR: 1.13; 95% CI: 0.89, 1.56); *P*-homogeneity = 0.02.

**Conclusions:** In this observational study, current vitamin D supplementation, mostly taken daily and combined with calcium, was associated with a decreased postmenopausal breast cancer risk in MHT users. These findings should be confirmed before considering vitamin D supplementation to partly balance the MHT-associated increased breast cancer risk. *Am J Clin Nutr* 2015;102:966–73.

**Keywords:** vitamin D, calcitriol, supplements, estrogen, menopausal hormone therapy, MHT, breast cancer, menopause, cohort, prospective study

## INTRODUCTION

In vitro and animal studies suggest that vitamin D has anticarcinogenic properties through regulation of cell proliferation, differentiation, apoptosis, and growth factor signaling in breast cells (1, 2). Vitamin D also inhibits the in vitro growth of estrogen receptor (ER)–positive (ER+) breast cancer cells through the attenuation of estrogen signaling and synthesis (2–4).

Evidence relating circulating 25-hydroxyvitamin D concentrations to breast cancer risk is not conclusive. Whereas case-control studies, with the limitation of possible reverse causation, favor an inverse association, large studies in prospective cohorts yielded mixed results: inverse association, overall (5) or only in white American (6) or premenopausal women (7), borderline inverse association (8), but mostly no association (9–18).

Nevertheless, evidence of a high proportion of subjects with concentrations below the recommended range in France (19) or in the United States (20) has raised concern. In France, few foods are fortified with vitamin D, and the mean dietary vitamin D intake is low, close to 100 IU/d in adults (21), whereas the recommended daily amount was established at 200 IU. The Research and Information Group on Osteoporosis recommends systematic vitamin D supplementation in all patients older than 65 y (22).

No clear conclusion can be drawn from available studies on the relation between breast cancer risk and vitamin D supplementation (23–26); interpretation is limited by differences in exposure assessment and population characteristics (11, 27). There is even concern about potential deleterious effects. Indeed, in a randomized trial among 36,282 women, breast cancer risk decreased in the vitamin D plus calcium supplementation group among women with low baseline intake compared with those in the placebo group but increased among women with high baseline intake (11).

Whereas experimental studies suggest that the effect of vitamin D on breast cancer risk may involve an interaction with estrogens, few epidemiologic studies have addressed this issue.

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Regarding prospective studies that considered circulating 25-hydroxyvitamin D concentrations, only 1 (15) of 3 (13, 15, 17) described an interaction with menopausal hormone therapy use (MHT); an inverse association was restricted to MHT users at the time of blood collection. Regarding vitamin D supplementation, only the Women's Health Initiative randomized trial and the Nurse's Health Study cohort considered this issue; neither observed any interaction with MHT randomization or current use (11, 26). Whereas the effect of MHT on breast cancer risk is mostly a short-term effect, temporality of use has not been considered regarding the association between vitamin D and breast cancer risk. Therefore we investigated the relation between postmenopausal breast cancer risk and current or past vitamin D supplementation, overall and according to the use of MHT.

## METHODS

### The E3N cohort

E3N is a prospective study of 98,995 women living in France and covered by a national health insurance program primarily comprising teachers. Participants were 40–65 y of age at recruitment in 1990. Information about their lifestyle and state of health has been collected by self-administered questionnaires every 2–3 y. The study was approved by the French National Commission for Data Protection and Privacy. On 30 June 2008, the vital status was unknown for 3% of participants. Average follow-up for each questionnaire has been 83%.

### Identification of incident invasive breast cancers

All questionnaires inquired about cancer occurrence and type, requesting addresses of physicians and permission to contact them. A small proportion (1%) of breast cancer cases were 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. Information on ER status was extracted from pathology reports, and cases were classified into 2 categories: ER+ and ER negative (ER−).

### Assessment of vitamin D and calcium supplementation

Vitamin D supplementation was assessed with an algorithm by using several complementary sources of information. First, participants were asked whether they were currently taking vitamin D supplements in the follow-up questionnaires sent in 1995, 2000, 2002, and 2005. We additionally identified vitamin D supplements among medications against osteoporosis reported in the 1993 and 1995 questionnaires and currently used medications reported in the 1997 and 2005 questionnaires. Finally, reimbursed vitamin D supplements were extracted from the database of all reimbursements for health expenditures, which has been made available for all study participants by the health insurance fund since 1 January 2004; we considered participants as users at the questionnaire sent in 2005 if they had a reimbursement for vitamin D supplements in the 3 mo preceding the date the questionnaire was completed. Identification of vitamin D supplements used the online Thériaque database (theriaque.org) (28) and websites of dietary supplement producers or retailers. Similar information was used to assess exposure to calcium supplementation.

Information on vitamin D supplement doses was available only in the 2005 questionnaire, in which women were asked to report names of vitamin D supplements. Of those who declared to be vitamin D supplement users, 71% reported names for medications available on medical prescription and with doses ≥400 IU/d or had a reimbursement in the past 6 mo for medications with doses ≥400 IU/d.

### Assessment of dietary intakes

A validated 208-item food-frequency questionnaire administered between 1993 and 1995 assessed usual diet over the previous year (29). Daily nutrient intakes were estimated by using a food-composition table derived from the updated French national database (30).

### Assessment of menopause and use of MHT

Menopausal status and date of menopause were determined from updated data on self-reported menopausal status and menopausal symptoms, menstrual periods, hysterectomy, oophorectomy, and use of MHT, as detailed elsewhere (31). The 1992 questionnaire requested information on lifetime use of MHT. Information was updated in all subsequent questionnaires. MHT included any nonvaginal use of estrogens (except estriol) or tibolone.

### Population study

Follow-up started either at the date the 1995 questionnaire was completed for women already postmenopausal or at the date when the questionnaire in which menopause was first reported was completed. Participants contributed person-years of follow-up until the date of diagnosis of any cancer (other than basal cell carcinoma), return of the last completed questionnaire, or 30 June 2008, whichever occurred first. We excluded women who were still premenopausal at the 2005 questionnaire ($n$ = 6237), received a diagnosed of cancer (other than basal cell carcinoma) before baseline ($n$ = 9190), did not answer the 1993 dietary questionnaire ($n$ = 17,676) or the 1995 questionnaire ($n$ = 6740), did not answer any further questionnaire ($n$ = 669), or had extreme values (top and bottom 1%) for the ratio of energy intake to required energy (computed on the basis of age, weight, and height) ($n$ = 1080). Thus 57,403 postmenopausal women were available for the study.

### Statistical analyses

HRs of breast cancer and 95% CIs were estimated by using Cox proportional hazards models, with age as the time scale. We used information at the time questionnaire $n$ was completed to prospectively categorize exposure to vitamin D supplementation as current, past, never, or missing for the period between completions of questionnaires $n$ and $n+1$.

We first used models stratified by year of birth to consider a possible cohort effect (model 1). Multivariate models (model 2) were further adjusted for known breast cancer risk factors: age at menarche, age at menopause, parity and age at first full-term pregnancy, use of oral contraceptives, use of menopausal

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**TABLE 1**
Characteristics of participants according to vitamin D supplementation status at the end of follow-up in the E3N Cohort ($n$ = 57,403)[1]

| | Never vitamin D supplementation ($n$ = 41,775) | Current vitamin D supplementation ($n$ = 9267) | Past vitamin D supplementation ($n$ = 3143) |
|---|---|---|---|
| Age at the end of follow-up, y | 65.9 ± 6.5[2] | 68.4 ± 6.5 | 68.8 ± 6.8 |
| Age at menarche, y | 12.8 ± 1.4 | 12.9 ± 1.4 | 12.8 ± 1.4 |
| Age at menopause, y | 50.6 ± 3.7 | 50.3 ± 3.8 | 50.0 ± 4.1 |
| Educational level, % | | | |
| Undergraduate | 11.4 | 8.6 | 12.7 |
| Graduate from high school or postgraduate | 88.6 | 91.4 | 87.3 |
| BMI, kg/m$^2$ | 24.0 ± 3.8 | 22.8 ± 3.3 | 23.5 ± 3.8 |
| Alcohol consumption, g ethanol/d | 11.6 ± 13.8 | 10.6 ± 12.9 | 11.0 ± 13.6 |
| Total energy intake without alcohol, kcal/d | 2135.2 ± 540.0 | 2101.7 ± 524.4 | 2125.2 ± 543.9 |
| Dietary calcium intake, mg/d | 1066.4 ± 363.2 | 1052.7 ± 364.3 | 1101.5 ± 381.7 |
| Calcium supplementation, % | | | |
| Never | 86.8 | 8.3 | 32.5 |
| Current | 5.1 | 89.0 | 17.9 |
| Past | 8.1 | 2.7 | 49.6 |
| Supplementation with micronutrients other than calcium and vitamin D, % | | | |
| Never | 59.6 | 24.1 | 27.2 |
| Ever | 40.4 | 75.9 | 72.8 |
| Physical activity, MET-h/wk | 25.0 ± 18.2 | 25.6 ± 17.9 | 25.8 ± 19.2 |
| Smoking status, % | | | |
| Former or never smokers | 92.4 | 94.6 | 93.5 |
| Current | 7.6 | 5.4 | 6.6 |
| Skin complexion, % | | | |
| Very fair to medium | 58.8 | 60.2 | 59.9 |
| Dark to very dark | 41.2 | 39.8 | 40.1 |
| Ultraviolet radiation dose exposure at place of residence, kJ · m$^{-2}$ · d$^{-1}$ | 1.54 ± 0.19 | 1.55 ± 0.19 | 1.55 ± 0.19 |
| History of breast cancer in first-degree relatives, % | | | |
| No | 89.2 | 88.6 | 89.3 |
| Yes | 10.8 | 11.5 | 10.7 |
| Personal history of benign breast disease, % | | | |
| No | 64.8 | 59.5 | 60.0 |
| Yes | 35.2 | 40.5 | 40.0 |
| Mammography in the previous follow-up period, % | | | |
| No | 11.1 | 7.8 | 12.8 |
| Yes | 88.9 | 92.2 | 87.2 |
| Parity, % | | | |
| Nulliparous | 11.2 | 12.8 | 13.3 |
| Parous, first child before age 30 y, 1 or 2 children | 50.1 | 50.5 | 49.6 |
| Parous, first child before age 30 y, ≥3 children | 28.4 | 26.4 | 27.1 |
| Parous, first child after age 30 y | 10.2 | 10.3 | 10.0 |
| Use of oral contraceptives before menopause, % | | | |
| Never | 38.8 | 43.4 | 47.0 |
| Ever | 61.2 | 56.6 | 53.0 |
| Use of MHT, % | | | |
| Never | 29.9 | 26.2 | 31.3 |
| Current | 30.6 | 20.8 | 24.5 |
| Past | 39.5 | 53.0 | 44.2 |
| Dietary vitamin D intake, μg/d | 2.59 ± 1.22 | 2.53 ± 1.22 | 2.65 ± 1.27 |

[1]At the end of follow-up, current exposure to vitamin D supplementation was unknown for 3218 women. MET, metabolic equivalent of task; MHT, menopausal hormone therapy.
[2]Mean ± SD (all such values).

hormone therapy, personal history of benign breast disease, family history of breast cancer, alcohol consumption, BMI, and physical activity (see legend of Table 2 for further details); they were also adjusted for factors that may affect vitamin D status, i.e., dietary vitamin D intake, skin complexion, and ultraviolet radiation dose exposure at the place of residence. We also

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adjusted models for total daily energy intake, dietary calcium intake or supplements, other supplements, smoking status, recent mammography, and educational level. Because all covariates had ≤5% missing values, we imputed missing values to the value of the previous questionnaire if answered or else to the modal category. Model 2 was used to calculate absolute risks of breast cancer associated with vitamin D supplementation and MHT use.

We investigated whether the association differed by tumor hormone receptor status with competitive risk models to deal with multiple censoring types and performing Wald chi-square tests for homogeneity. Cases with missing information on hormone receptor status were excluded from the corresponding analyses. We tested a potential interaction between vitamin D supplementation and MHT use (ever, never and current, past, never) by stratifying analyses accordingly and performing Wald chi-square tests for homogeneity. All statistical tests were 2 sided, and significance was set at the 0.05 level. We performed all analyses using SAS software version 9.3 (SAS Institute).

## RESULTS

### Characteristics of the study population

A total of 2482 invasive postmenopausal breast cancers were diagnosed during 581,085 person-years of follow-up. Of the 2038 cases with known hormone receptor status, 1679 were ER+ (82%) and 359 were ER− (18%). Mean dietary vitamin D intake of cohort women was 104 IU/d; it was <200 IU/d for 96% of them. At the end of follow-up, current and past users of vitamin D supplements were older than never users. They were more frequently ever users of other supplements and more frequently had a personal history of benign breast disease. Current users of vitamin D supplements were thinner. They were more frequently ever users of MHT, but were less frequently current users. Characteristics of the participants are shown in **Table 1**.

The proportion of women who reported current vitamin D supplementation steadily increased from 1995 to 2005; overall, 22% of the study population was estimated to be ever exposed to vitamin D supplementation during this period. During follow-up, 61–90% of women with current vitamin D supplementation also reported current calcium supplementation; 72% of users reported vitamin D supplementation only once during follow-up.

### Breast cancer risk by hormone receptor status

A decreased postmenopausal breast cancer risk was associated with current (HR: 0.82; 95% CI: 0.69, 0.97) but not past (HR: 1.10; 95% CI: 0.92, 1.31) vitamin D supplementation (*P*-homogeneity = 0.02; **Table 2**). The decrease in risk associated with current vitamin D supplementation was stronger for ER+ tumors (HR: 0.73; 95% CI: 0.58, 0.91) than for ER− tumors (HR: 0.89; 95% CI: 0.57, 1.38), but there was no statistical heterogeneity (*P*-homogeneity = 0.44; Table 2).

### Interaction between vitamin D supplementation and MHT use

Current vitamin D supplementation was associated with a decreased postmenopausal breast cancer risk only among MHT

**TABLE 2**
HRs of invasive postmenopausal breast cancer associated with vitamin D supplementation overall (E3N cohort, 1995–2008; $n$ = 57,403) and according to the ER+/ER− status of the tumor (E3N cohort, 1995–2008; $n$ = 56,959)[1]

| | All cases | | | | ER+ cases | | ER− cases | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. of person-years | No. of cases | HR (95% CI)[2] | HR (95% CI)[3] | No. of cases | HR (95% CI)[2] | No. of cases | HR (95% CI)[3] | *P*-homogeneity between ER+ and ER− tumors |
| Vitamin D supplementation | | | | | | | | | |
| Never | 407,662 | 2119 | 1 (ref) | 1 (ref) | 1441 | 1 (ref) | 316 | 1 (ref) | |
| Current | 104,815 | 162 | 0.84 (0.71, 0.99) | 0.82 (0.69, 0.97) | 93 | 0.73 (0.59, 0.91) | 24 | 0.89 (0.57, 1.38) | 0.44 |
| Past | 34,275 | 137 | 1.12 (0.94, 1.33) | 1.10 (0.92, 1.31) | 102 | 1.23 (1.00, 1.51) | 16 | 0.93 (0.55, 1.55) | 0.32 |
| *P*-homogeneity between current and past use | | | | 0.02 | | <0.001 | | 0.89 | |

[1]Sixty-four cases, including 43 ER+ and 3 ER− cases, were diagnosed among women for whom current use of vitamin D supplementation was unknown. Women with unknown exposure to current vitamin D supplementation at the end of follow-up contributed 34,333 person-years. ER, estrogen receptor; MET, metabolic equivalent of task; MHT, menopausal hormone therapy; ref, reference.

[2]Model 1: adjusted for age (time scale) and stratified by year of birth (1925–1930, 1930–1935, 1935–1940, 1940–1945, 1945–1950).

[3]Model 2: adjusted for age (time scale), BMI (in kg/m$^2$; <18.5, 18.5–24.9, ≥25; time-dependent), use of oral contraceptives before menopause (ever, never), use of MHT (ever, 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, 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 questionnaire), dietary vitamin D intake (tertiles: 1.95, 1.95–2.85, ≥2.85 $\mu$g/d; as recorded in the 1993 questionnaire), calcium intakes (2 categories: dietary intake as recorded in the 1993 questionnaire <1014 mg/d, and dietary intake as recorded in the 1993 questionnaire ≥1014 mg/d or calcium supplementation; time dependent), supplementation with micronutrients other than calcium and vitamin D (ever, never; time-dependent), skin complexion (very fair to medium, dark to very dark), ultraviolet radiation dose exposure at place of residence (quartiles: <1.40, 1.40–1.49, 1.50–1.68, ≥1.69 kJ · m$^{-2}$ · d$^{-1}$; as recorded in the 1995 questionnaire), alcohol consumption (median of quartiles of intake; as recorded in the 1993 questionnaire), smoking status (current smoker, former smoker, never smoker; time-dependent), physical activity (<12, ≥12 MET/d; time-dependent), personal history of benign breast disease (yes, no; time-dependent), mammography in the previous follow-up period (yes, no; time-dependent), family history of breast cancer in first-degree relatives (yes, no), and educational level (undergraduate, graduate from high school, postgraduate); further stratified by year of birth (1925–1930, 1930–1935, 1935–1940, 1940–1945, 1945–1950).

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**TABLE 3**
HRs of invasive postmenopausal breast cancer associated with vitamin D supplementation, stratified by ever use of MHT, in the E3N cohort, 1995–2008 ($n$ = 57,403)[1]

| | Never MHT use | | | | Ever MHT use | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. of person-years | No. of cases | HR (95% CI)[2] | HR (95% CI)[3] | No. of person-years | No. of cases | HR (95% CI)[2] | HR (95% CI)[3] | *P*-homogeneity between never and ever use of MHT |
| Vitamin D supplementation | | | | | | | | | |
| Never | 110, 410 | 451 | 1 (ref) | 1 (ref) | 297,252 | 1668 | 1 (ref) | 1 (ref) | — |
| Current | 25,866 | 49 | 1.19 (0.88, 1.61) | 1.13 (0.89, 1.56) | 78,948 | 113 | 0.73 (0.60, 0.89) | 0.74 (0.60, 0.90) | 0.02 |
| Past | 10,193 | 40 | 1.46 (1.05, 2.02) | 1.42 (1.02, 1.98) | 24,083 | 97 | 1.00 (0.82, 1.23) | 1.00 (0.81, 1.24) | 0.08 |
| *P*-homogeneity between current and past use of vitamin D supplementation | | | | 0.34 | | | | 0.04 | |

[1]64 cases were diagnosed among women for whom current use of vitamin D supplementation was not known. Women with unknown exposure to current vitamin D supplementation at the end of follow-up contributed 34,333 person-years. MET, metabolic equivalent of task; MHT, menopausal hormone therapy; ref, reference.

[2]Model 1: adjusted for age (time scale) and stratified by year of birth (1925–1930, 1930–1935, 1935–1940, 1940–1945, 1945–1950).

[3]Model 2: adjusted for age (time scale), BMI (in kg/m$^2$; <18.5, 18.5–24.9, ≥25; time-dependent), use of oral contraceptives before menopause (ever, never), use of MHT (ever, 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, 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 questionnaire), dietary vitamin D intake (tertiles: 1.95, 1.95–2.85, ≥2.85 $\mu$g/d; as recorded in the 1993 questionnaire), calcium intakes (2 categories: dietary intake as recorded in the 1993 questionnaire <1014 mg/d, and dietary intake as recorded in the 1993 questionnaire ≥1014 mg/d or calcium supplementation; time dependent), supplementation with micronutrients other than calcium and vitamin D (ever, never; time-dependent), skin complexion (very fair to medium, dark to very dark), ultraviolet radiation dose exposure at place of residence (quartiles: <1.40, 1.40–1.49, 1.50–1.68, ≥1.69 kJ · m$^{-2}$ · d$^{-1}$; as recorded in the 1995 questionnaire), alcohol consumption (median of quartiles of intake; as recorded in the 1993 questionnaire), smoking status (current smoker, former smoker, never smoker; time-dependent), physical activity (<12, ≥12 MET/d; time-dependent), personal history of benign breast disease (yes, no; time-dependent), mammography in the previous follow-up period (yes, no; time-dependent), family history of breast cancer in first-degree relatives (yes, no), and education level (undergraduate, graduate from high school, postgraduate); further stratified by year of birth (1925–1930, 1930–1935, 1935–1940, 1940–1945, 1945–1950).

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ever users (HR: 0.74; 95% CI: 0.60, 0.90) and not among never users (HR: 1.13; 95% CI: 0.89, 1.56); *P*-homogeneity = 0.02 (**Table 3**). There was no heterogeneity of the association according to current or past use of MHT (data not shown). Past vitamin D supplementation was associated with an increased postmenopausal breast cancer risk among never users of MHT (HR: 1.42; 95% CI: 1.02, 1.98) but not among ever users of MHT (HR: 1.00; 95% CI: 0.81, 1.24); *P*-homogeneity = 0.08 (Table 3).

The absolute annual rates for breast cancer for never, current, and past vitamin D supplementation were 408/100,000 person-years, 498/100,000 person-years, and 596/100,000 person-years, respectively, in MHT never users and 682/100,000 person-years, 490/100,000 person-years, and 682/100,000 person-years, respectively, in MHT ever users (**Table 4**).

## DISCUSSION

In France, very few foods are fortified with vitamin D, and the mean dietary vitamin D intake is low, close to 100 IU/d (21), whereas the recommended daily amount was established at 200 IU for adults. In this large prospective study of postmenopausal French women, current but not past vitamin D supplementation—mostly taken daily and combined with calcium—was associated with a decreased breast cancer risk in MHT ever users.

### Evidence from other epidemiologic studies

Most large prospective studies reported null results regarding an association between postmenopausal breast cancer risk and circulating 25-hydroxyvitamin D concentrations (7–17), dietary vitamin D intake (23–26, 32, 33), or vitamin D intake from supplements at doses ≥400 IU/d (23, 26). Two others have suggested an inverse association between 25-hydroxyvitamin D concentrations and postmenopausal breast cancer risk (5, 6).

However, circulating 25-hydroxyvitamin D concentrations or vitamin D intake were generally assessed at baseline only, so that a potential short-term effect may have been missed. Consistent with our results, 2 observational studies suggested a short-term effect of vitamin D supplementation on postmenopausal breast cancer risk. In the Iowa Women's Study, total intake >800 compared with <400 IU/d was associated with a 34% reduced risk of postmenopausal breast cancer, but in the first 5 y after exposure assessment only (25). In a case-control study, supplementation ≥400 IU/d in the previous 2 y compared with nonuse was associated with a 24% decreased risk in pre- and postmenopausal breast cancer (34).

In the Women's Health Initiative randomized trial, daily supplementation with 1000 mg calcium and 400 IU vitamin D was not associated with postmenopausal breast cancer risk (11). However, risk estimates were modified by total vitamin D intakes at baseline: the intervention was associated with a 21% decreased risk among women with baseline intakes <200 IU/d and a 34% increased risk among women with baseline intakes ≥600 IU/d. The interpretation of these results is still being debated (35).

In a previous nested case-control study in our cohort, we observed an increased breast cancer risk in the 33% of women with serum vitamin D concentrations <49.5 nmol/L compared with the 33% with concentrations >67.5 nmol/L (5). It must be emphasized that the dietary vitamin D intake in France is quite low because of little food fortification; indeed, it was <200 IU/d in our study population. Thus, our findings of an inverse association between breast cancer risk and vitamin D supplements may only apply to women with insufficient baseline vitamin D status and in some high-risk groups, such as MHT users, whereas potentially deleterious effects cannot be ruled out in other settings.

**TABLE 4**
HRs of invasive postmenopausal breast cancer associated with vitamin D supplementation and MHT use in the E3N cohort, 1995–2008 ($n$ = 57,403)[1]

| | No. of person-years | No. of cases | HR (95% CI)[2] | HR (95% CI)[3] | Absolute risks per 100,000 person-years |
|---|---|---|---|---|---|
| Never vitamin D supplementation and never use of MHT | 110,410 | 451 | 1 (ref) | 1 (ref) | 408 |
| Never vitamin D supplementation and ever use of MHT | 297,252 | 1668 | 1.63 (1.46, 1.81) | 1.67 (1.49, 1.86) | 682 |
| Current vitamin D supplementation and never use of MHT | 25,866 | 49 | 1.25 (0.93, 1.68) | 1.22 (0.91, 1.65) | 498 |
| Current vitamin D supplementation and ever use of MHT | 78,948 | 113 | 1.17 (0.95, 1.45) | 1.20 (0.96, 1.48) | 490 |
| Past vitamin D supplementation and never use of MHT | 10,193 | 40 | 1.47 (1.06, 2.04) | 1.46 (1.05, 2.02) | 596 |
| Past vitamin D supplementation and ever use of MHT | 24,083 | 97 | 1.63 (1.31, 2.03) | 1.67 (1.33, 2.09) | 682 |

[1]64 cases were diagnosed among women for whom current use of vitamin D supplementation was not known. Women with unknown exposure to current vitamin D supplementation at the end of follow-up contributed 34,333 person-years. MET, metabolic equivalent of task; MHT, menopausal hormone therapy; ref, reference.

[2]Model 1: adjusted for age (time scale) and stratified by year of birth (1925–1930, 1930–1935, 1935–1940, 1940–1945, 1945–1950).

[3]Model 2: adjusted for age (time scale), BMI (in kg/m$^2$; <18.5, 18.5–24.9, ≥25; time-dependent), use of oral contraceptives before menopause (ever, never), 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, 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 questionnaire), dietary vitamin D intake (tertiles: 1.95, 1.95–2.85, ≥2.85 $\mu$g/d; as recorded in the 1993 questionnaire), calcium intakes (2 categories: dietary intake as recorded in the 1993 questionnaire <1014 mg/d, and dietary intake as recorded in the 1993 questionnaire ≥1014 mg/d or calcium supplementation; time dependent), supplementation with micronutrients other than calcium and vitamin D (ever, never; time-dependent), skin complexion (very fair to medium, dark to very dark), ultraviolet radiation dose exposure at place of residence (quartiles: <1.40, 1.40–1.49, 1.50–1.68, ≥1.69 kJ · m$^{-2}$ · d$^{-1}$; as recorded in the 1995 questionnaire), alcohol consumption (median of quartiles of intake; as recorded in the 1993 questionnaire), smoking status (current smoker, former smoker, never smoker; time-dependent), physical activity (<12, ≥12 MET/d; time-dependent), personal history of benign breast disease (yes, no; time-dependent), mammography in the previous follow-up period (yes, no; time-dependent), family history of breast cancer in first-degree relatives (yes, no), and educational level (undergraduate, graduate from high school, postgraduate); further stratified by year of birth (1925–1930, 1930–1935, 1935–1940, 1940–1945, 1945–1950).

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### The estrogen pathway and other biological mechanisms

In vitro and animal studies suggest that vitamin D has anticarcinogenic properties through regulation of cell proliferation, differentiation, apoptosis, and growth factor signaling in breast cells (1, 2). In addition, some mechanisms support interactions between calcitriol, the hormonally active form of vitamin D, and estrogens. In vitro, calcitriol reduces the proliferative stimulus of estrogens on ER+ breast tumors by downregulating the expression of ER$\alpha$ expression in breast cancer cells (3, 4, 36). Calcitriol also decreases aromatase expression and, thus, estrogen synthesis in adipose tissue surrounding breast cancer cells (3). Other potential mechanisms include competition for cell membrane megalin receptors, which are involved in the endocytosis of sex hormones and vitamin D (37, 38), and increase in calbindin circulating concentrations with estrogen, resulting in an inhibition of vitamin D–induced apoptosis (37, 38).

We observed a decreased risk of ER+ tumors associated with current vitamin D supplementation and a borderline increased risk associated with past supplementation. This suggests that calcitriol may stop the progression of pre-existing ER+ tumors in the short-term only. Heterogeneity of risk estimates according to the ER status of the tumor was not significant, but power was limited by the low number of ER− tumors. However, we cannot exclude that there may be a longer-term effect of vitamin D supplementation on ER− tumors.

A short-term effect of calcitriol on ER+ tumors may particularly benefit women currently receiving MHT. Indeed, the proliferative stimulus of estrogens on ER+ tumors indicates that MHT may directly and rapidly promote pre-existing ER+ tumors (31). However, a potential interaction between vitamin D and the use of MHT has been little investigated in previous epidemiologic studies. Of the prospective studies that considered circulating 25-hydroxyvitamin D concentrations (13, 15, 17), only one (15) reported an interaction with MHT use, with an inverse association restricted to MHT users at the time of blood collection. Only the Women's Health Initiative randomized trial and the Nurse's Health Study cohort considered vitamin D supplementation; no interaction with MHT randomization or current use was observed (11, 26). We found no heterogeneity of the association between current vitamin D supplementation and breast cancer risk according to current or past MHT use (data not shown). The relation between past MHT use and breast cancer risk is complex and depends on the type of MHT and duration of use; we lacked the power to finely address this issue (39).

### Strengths and limitations

The strengths of our study included its prospective design, large size, long follow-up with minimal loss, and case ascertainment through pathology reports. The main strength of our study with regard to previous epidemiologic studies is that information on vitamin D supplementation was regularly updated. This allowed us to investigate a potential modification of the association between postmenopausal breast cancer risk and vitamin D supplementation by current or past use. We also had updated information on a large set of potential confounders, including factors that are known to influence vitamin D status. Although vitamin D supplements in our population were mostly medically prescribed (and most prescribed medications in France have doses ≥400 IU/d), we missed precise enough information to investigate associations between breast cancer risk and vitamin D supplement dose and duration. We could estimate that there was virtually no vitamin D intake from fortified foods, because such products have only been introduced recently on the French market and are still little used, except by children. Dietary vitamin D intake was low compared with other populations consuming fortified foods. In addition, there was no heterogeneity of association between vitamin D supplementation and breast cancer risk according to dietary vitamin D intake (data not shown). Vitamin D supplementation was mostly self-reported, which may have been responsible for nondifferential misclassification, and, therefore, may have resulted in underestimated risks. However, misclassification was limited for the "current supplementation" class by updating information from multiple sources and considering nonresponders in a missing class. Finally, we cannot rule out some residual confounding, prescription bias, or chance finding regarding the observed interaction between MHT and vitamin D supplement uses.

### Conclusion

In this observational study, current vitamin D supplementation—mostly taken daily and combined with calcium—was associated with decreased postmenopausal breast cancer risk in MHT users. These findings should be confirmed before considering vitamin D supplementation to partly balance the MHT-associated increased breast cancer risk.

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