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Postmenopausal breast cancer risk and interactions between body mass index, menopausal hormone therapy use, and vitamin D supplementation: Evidence from the E3N cohort

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Experimental studies suggest protective effects of vitamin D on breast carcinogenesis, but epidemiological evidence is not conclusive. Body mass index (BMI) has been shown to modulate the effect of supplementation on the vitamin D status, but its potential influence on the relationship with breast cancer risk has been little studied. We investigated a potential interaction between BMI and vitamin D supplementation on breast cancer risk while considering an already reported interaction between vitamin D supplementation and menopausal hormone therapy (MHT) use. Vitamin D supplementation was prospectively investigated in 57,403 postmenopausal women from the French E3N cohort including 2,482 incident breast cancer cases diagnosed between 1995 and 2008. Multivariable hazard ratios (HR) for primary invasive breast cancer and 95% confidence intervals (CI) were estimated using Cox models. Among MHT ever users, vitamin D supplementation was associated with decreased breast cancer risk, similarly across BMI strata ($P_{homogeneity} = 0.83$). Among MHT never users, ever vitamin D supplementation was associated with increased postmenopausal breast cancer risk in women with baseline BMI <25 kg/m^2 (HR = 1.51, 95% CI: 1.13, 2.02), but not in women with higher BMI (0.98, 95% CI: 0.62, 1.56), $P_{homogeneity} = 0.12$. In conclusion, our findings suggest that vitamin D supplementation may reduce the excess breast cancer risk in MHT users, but draw attention on a potential risk in postmenopausal women not exposed to high exogenous or endogenous hormones, *i.e.* non-overweight MHT-non users, especially in the present context of increasing vitamin D supplement use and decreasing MHT use.

Introduction

Although experimental studies suggest protective effects of vitamin D on breast carcinogenesis,[1] epidemiological evidence is not conclusive.[2,3] Few studies investigated potential interactions on the relationship between vitamin D supplement intake and breast cancer risk. Randomized trials showed that a higher vitamin D dose intake was necessary to achieve similar 25-hydroxyvitamin D concentrations in subjects with BMI $\geq$25 kg/m^2.[4–7] Since vitamin D is stored in the fat tissue and in muscles, it has been suggested that it may be due to volume dilution.[5]

In a previous analysis, we reported an interaction between current vitamin D supplementation and menopausal hormone therapy (MHT) use on postmenopausal breast cancer risk, with a decreased risk in MHT ever users, and no association in MHT never users.[8] Past vitamin D supplementation was even associated with a borderline increased risk in MHT never users. Considering the known interactions between hormones and BMI, and our previous findings, we further investigated whether the associations between vitamin D supplementation, MHT, and postmenopausal breast cancer risk were influenced by BMI.

Key words: vitamin D, supplements, BMI, breast cancer, cohort
Abbreviations: BMI: body mass index; MHT: menopausal hormone therapy
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Material and 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 years old at recruitment in 1990. Information about their lifestyle and health status has been collected by self-administered questionnaires every 2–3 years. All study subjects signed an informed consent form in compliance with the rules of the French National Commission for Computed Data and

What's new?

Experimental studies suggest protective effects of vitamin D on breast carcinogenesis, but epidemiological evidence is inconclusive. Body mass index (BMI) modulates the relationship between vitamin D intake and status. However, while BMI is a risk factor for postmenopausal breast cancer, its influence on the relationship between vitamin D supplementation and breast cancer risk has been little investigated. This study suggests that vitamin D supplementation could decrease breast cancer risk in menopausal hormone therapy (MHT) users whatever their BMI, while highlighting a potential higher risk in non-overweight MHT-non users, especially given the popularity of vitamin D supplements and decreasing MHT use.

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, requesting addresses of physicians and permission to contact them. 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 D supplementation

Ever vitamin D supplementation was assessed by using data from self-administered questionnaires and the database of all reimbursements for health expenditures available since January 1, 2004. Information on current vitamin D supplementation was collected in the 1995, 2000, 2002, and 2005 questionnaires. We also identified vitamin D supplements among self-reported names of medications used currently or since last questionnaire in the 1993, 1995, 1997, 2000, 2002, and 2005 questionnaires. Finally, we considered participants as users at the 2005 questionnaire if they had a reimbursement for vitamin D supplements in the 3 months preceding the date the questionnaire was completed.

Assessment of dietary intakes

A validated 208-item food frequency questionnaire administered between 1993 and 1995 assessed the previous year usual diet.[9] Daily nutrient intakes were estimated using a food composition table derived from the updated French national database.[10]

Assessment of BMI, menopause, and MHT use

Height and weight were recorded at each follow-up questionnaire. BMI was computed as weight/(height × height) in kg/m^2.

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. Lifetime MHT use was recorded in the 1992 questionnaire and 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 at this date, or at the date of completion of the questionnaire where menopause was first reported. 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 June 30, 2008, whichever occurred first. We excluded women who were still premenopausal at the 2005 questionnaire ($n = 6{,}237$), were diagnosed with a cancer (other than basal cell carcinoma) before baseline ($n = 9{,}190$), did not answer the 1993 dietary questionnaire ($n = 17{,}676$) or the 1995 questionnaire ($n = 6{,}740$), or did not answer any further questionnaire ($n = 669$), or with extreme values (top and bottom 1%) of the ratio of energy intake to required energy computed on the basis of age, weight, and height ($n = 1{,}080$). Thus, 57,403 postmenopausal women were available for the study.

Statistical analyses

Hazard ratios (HRs) of breast cancer risk and 95% confidence intervals (CIs) were estimated using Cox proportional hazards models, with age as the time scale.

Vitamin D supplementation was assessed prospectively and analyzed as a time-depend variable: the information collected at the time questionnaire n and earlier was used to asses vitamin D supplementation (ever *vs.* never) for the period between completions of questionnaires n and $n + 1$. A potential influence of BMI on the relationship between vitamin D supplementation and postmenopausal breast cancer risk was investigated by stratifying models on BMI at baseline (<25, ≥25 kg/m^2) and performing Wald χ^2 tests for homogeneity of risk across BMI strata. Three models stratified on BMI were performed: crude, adjusted for known risk factors for breast cancer and potential confounders, and further adjusted for the interaction term between vitamin D supplementation (ever, never) and MHT use (ever, never). To control for a potential cohort effect, models were stratified by 5-year interval birth cohorts. Known risk factors for breast cancer included in multivariable models were age at menarche, age at menopause, parity and age at first full-term pregnancy, use of oral contraceptives, MHT use, personal history of benign breast disease, family history of breast cancer, alcohol consumption, and physical activity (see legend of Table 2 for further details). Potential confounders included factors that may affect vitamin D status, *i.e.* dietary vitamin D intake, skin complexion, UV radiation dose exposure at the place of residence, and other factors: total daily energy intake, dietary calcium intake or supplements, other supplementations, smoking status, recent mammography,

Table 1. Characteristics of participants at the end of follow-up according to vitamin D supplementation, MHT use and BMI at baseline in the E3N cohort ($n = 57,403$)

	MHT never users ($n = 16,933$)				MHT ever users ($n = 40,470$)			
	Women with BMI <25 kg/m^2 ($n = 11,291$)		Women with BMI ≥25 kg/m^2 ($n = 5,642$)		Women with BMI <25 kg/m^2 ($n = 31,347$)		Women with BMI ≥25 kg/m^2 ($n = 9,123$)	
	Vitamin D supplementation		Vitamin D supplementation		Vitamin D supplementation		Vitamin D supplementation	
	Never ($n = 8,632$)	Ever ($n = 2,609$)	Never ($n = 4,831$)	Ever ($n = 811$)	Never ($n = 23,845$)	Ever ($n = 7,502$)	Never ($n = 7,589$)	Ever ($n = 1,534$)
Age at end of follow-up, years	67.0 ± 7.80[1]	69.9 ± 7.63	68.1 ± 7.66	70.6 ± 7.40	65.2 ± 5.80	67.8 ± 6.09	65.7 ± 5.92	68.6 ± 6.33
Age at menarche, years	12.9 ± 1.40	13.0 ± 1.43	12.6 ± 1.41	12.7 ± 1.41	12.9 ± 1.38	12.9 ± 1.38	12.5 ± 1.37	12.6 ± 1.39
Age at menopause, years	51.4 ± 3.70	51.0 ± 3.84	51.2 ± 4.07	50.8 ± 4.34	50.3 ± 3.54	50.0 ± 3.77	50.3 ± 3.82	49.9 ± 4.38
Education level, %								
Undergraduate	12.8	10.9	18.5	16.0	9.3	8.0	13.7	12.3
Graduate from high school or postgraduate	87.3	89.2	81.5	84.0	90.7	92.0	86.3	87.7
BMI at baseline, kg/m^2	21.9 ± 1.89	21.6 ± 1.98	28.6 ± 3.48	27.9 ± 2.97	21.9 ± 1.78	21.5 ± 1.87	27.7 ± 2.73	27.4 ± 2.41
Alcohol consumption, g of ethanol/day	10.3 ± 13.1	9.6 ± 12.13	11.3 ± 14.9	11.2 ± 14.1	12.0 ± 13.6	10.8 ± 13.0	12.3 ± 15.0	12.0 ± 14.2
Total energy intake without alcohol, kcal/day	2,093 ± 531	2,083 ± 533	2,166 ± 577	2,156 ± 593	2,133 ± 525	2,099 ± 517	2,171 ± 579	2,161 ± 559
Dietary calcium intake, mg/day	1,037 ± 361	1,053 ± 373	1,106 ± 384	1,104 ± 392	1,056 ± 354	1,054 ± 364	1,111 ± 382	1,121 ± 378
Ever calcium supplementation, %	15.6	86.6	12.1	81.5	14.2	85.9	11.4	81.3
Ever supplement use in other micronutrients, %	41.0	75.2	32.0	70.3	42.5	73.4	36.4	71.8
Physical activity, METs-hr/week	25.5 ± 18.6	25.3 ± 18.2	20.3 ± 16.5	20.2 ± 16.4	26.6 ± 18.6	26.8 ± 18.5	21.9 ± 17.1	23.0 ± 17.7
Smoking status								
Former or never	92.1	95.3	93.9	95.8	91.3	93.4	93.4	95.2
Current	7.9	4.7	6.1	4.2	8.7	6.6	6.6	4.8
Skin complexion, %								
Very fair to medium	57.7	58.0	61.0	58.2	58.0	60.4	61.6	63.8
Dark to very dark	42.3	42.1	39.0	41.8	42.0	39.6	38.4	36.1
Ultraviolet radiation dose exposure at place of residence, kJ/m^2/day	1.57 ± 0.19	1.57 ± 0.19	1.54 ± 0.19	1.54 ± 0.19	1.54 ± 0.19	1.55 ± 0.19	1.52 ± 0.18	1.52 ± 0.18

Table 1. Characteristics of participants at the end of follow-up according to vitamin D supplementation, MHT use and BMI at baseline in the E3N cohort ($n = 57,403$) (Continued)

	MHT never users ($n = 16,933$)				MHT ever users ($n = 40,470$)			
	Women with BMI <25 kg/m^2 ($n = 11,291$)		Women with BMI ≥25 kg/m^2 ($n = 5,642$)		Women with BMI <25 kg/m^2 ($n = 31,347$)		Women with BMI ≥25 kg/m^2 ($n = 9,123$)	
	Vitamin D supplementation		Vitamin D supplementation		Vitamin D supplementation		Vitamin D supplementation	
	Never ($n = 8,632$)	Ever ($n = 2,609$)	Never ($n = 4,831$)	Ever ($n = 811$)	Never ($n = 23,845$)	Ever ($n = 7,502$)	Never ($n = 7,589$)	Ever ($n = 1,534$)
Personal history of benign breast disease, %	34.3	38.1	25.5	30.5	38.6	42.9	31.6	36.1
History of breast cancer in first degree relatives, %	11.9	13.2	10.3	12.0	10.3	10.4	10.7	11.2
Mammography in the previous follow-up period, %	78.0	82.1	75.5	79.2	93.5	94.7	91.9	92.2
Parity, %								
Nulliparous	14.3	16.6	12.7	13.4	10.4	12.1	9.9	10.6
Parous, first child before age 30 years, 1 or 2 children	47.0	47.3	42.4	42.7	53.4	52.5	48.1	49.6
Parous, first child before age 30 years, 3+ children	27.6	25.5	34.2	32.1	26.1	25.1	32.7	31.4
Parous, first child after age 30 years	11.1	10.7	10.7	11.8	10.1	10.3	9.3	8.4
Ever use of oral contraceptives before menopause, %	49.6	44.4	44.6	42.2	68.2	61.8	61.7	53.7
Vitamin D intake from foods, μg/day	2.38 ± 1.16	2.39 ± 1.18	2.76 ± 1.34	2.78 ± 1.31	2.56 ± 1.19	2.54 ± 1.20	2.83 ± 1.30	2.86 ± 1.36

[1]Mean ± SD (all such values).
Abbreviations: BMI, body mass index; MET, metabolic equivalent of task; MHT, menopausal hormone therapy.

Table 2. Hazard ratios of invasive postmenopausal breast cancer associated with vitamin D supplementation stratified on BMI at baseline ($N = 57,403$) (E3N cohort, 1995–2008)

	BMI <25 kg/m^2				BMI ≥25 kg/m^2			
	Nb of cases	HR ($CI_{95\%}$)[1]	HR ($CI_{95\%}$)[2]	HR ($CI_{95\%}$)[3]	Nb of cases	HR ($CI_{95\%}$)[1]	HR ($CI_{95\%}$)[2]	HR ($CI_{95\%}$)[3]
Vitamin D supplementation								
Never	1,609	1 (ref)	1 (ref)	1 (ref)	574	1 (ref)	1 (ref)	1 (ref)
Ever	238	0.98 (0.86–1.13)	0.96 (0.83–1.11)	1.55 (1.18–2.02)	61	0.97 (0.75–1.27)	0.91 (0.69–1.20)	1.07 (0.68–1.67)

[1]Adjusted for age (time scale) and stratified on year of birth (1925–1930, 1930–1935, 1935–1940, 1940–1945, and 1945–1950).
[2]Further adjusted for 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 and 1 or 2 children, first full-term pregnancy at age <30 and 3 or more children, first full-term pregnancy at age ≥30), age at menarche (years, continuous), age at menopause (years, continuous), total energy intake without alcohol (kcal/day, continuous, as recorded in the 1993 questionnaire), dietary vitamin D intake (tertiles: <1.95, 1.95–2.85, ≥2.85 μg/day, as recorded in the 1993 questionnaire), calcium intakes (dietary intake as recorded in the 1993 questionnaire <1,014, dietary intake as recorded in the 1993 questionnaire ≥1,014 mg/day or calcium supplementation, time-dependent), supplementation in other micronutrients than calcium and vitamin D (ever, never, time-dependent), skin complexion (very fair to medium, dark to very dark), UVR dose exposure at place of the residence (quartiles: <1.40, 1.40–1.49, 1.50–1.68, ≥1.69 kJ/m^2/day, as recorded in the 1995 questionnaire), alcohol consumption (median of quartiles of intake, as recorded in the 1993 questionnaire), smoking status (current, former or never smokers, time-dependent), physical activity (<12, ≥12 METs/day, 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), education level (undergraduate, graduate from high school, or postgraduate).
[3]Further adjusted for the interaction term between use of MHT and ever vitamin D supplementation.
Abbreviations: BMI, body mass index; MET, metabolic equivalent of task; MHT, menopausal hormone therapy.

Table 3. Hazard ratios of invasive postmenopausal breast cancer associated with vitamin D supplementation stratified on BMI at baseline and MHT use ($N = 57,403$) (E3N cohort, 1995–2008)

	BMI <25 kg/m^2				BMI ≥25 kg/m^2			
	MHT never users		MHT ever users		MHT never users		MHT ever users	
	Nb of cases	HR ($CI_{95\%}$)[1]	Nb of cases	HR ($CI_{95\%}$)[1]	Nb of cases	HR ($CI_{95\%}$)[1]	Nb of cases	HR ($CI_{95\%}$)[1]
Vitamin D supplementation								
Never	280	1 (ref)	1,329	1 (ref)	191	1 (ref)	383	1 (ref)
Ever	69	1.51 (1.13–2.02)	169	0.84 (0.70–0.99)	22	0.98 (0.62–1.56)	39	0.87 (0.62–1.23)

[1]Adjusted for age (time scale), use of oral contraceptives before menopause (ever, never), parity and age at first full-term pregnancy (nulliparous, first full-term pregnancy at age <30 and 1 or 2 children, first full-term pregnancy at age <30 and 3 or more children, first full-term pregnancy at age ≥30), age at menarche (years, continuous), age at menopause (years, continuous), total energy intake without alcohol (kcal/day, continuous, as recorded in the 1993 questionnaire), dietary vitamin D intake (tertiles: <1.95, 1.95–2.85, ≥2.85 μg/day, as recorded in the 1993 questionnaire), calcium intakes (dietary intake as recorded in the 1993 questionnaire <1,014, dietary intake as recorded in the 1993 questionnaire ≥1,014 mg/day or calcium supplementation, time-dependent), supplementation in other micronutrients than calcium and vitamin D (ever, never, time-dependent), skin complexion (very fair to medium, dark to very dark), UVR dose exposure at place of the residence (quartiles: <1.40, 1.40–1.49, 1.50–1.68, ≥1.69 kJ/m^2/day, as recorded in the 1995 questionnaire), alcohol consumption (median of quartiles of intake, as recorded in the 1993 questionnaire), smoking status (current, former or never smokers, time-dependent), physical activity (<12, ≥12 METs/day, 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), education level (undergraduate, graduate from high school or postgraduate). Further stratified on year of birth (1925–1930, 1930–1935, 1935–1940, 1940–1945, and 1945–1950).
Abbreviations: BMI, body mass index; MET, metabolic equivalent of task; MHT, menopausal hormone therapy.

and education level. Absolute annual risks for postmenopausal breast cancer were calculated using a model fully adjusted. Since all 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 for qualitative variables or the median value for quantitative variables.

To investigate potential reverse causation, we performed a sensitivity analysis where cases diagnosed in the first 5 years of follow-up were excluded.

All statistical tests were two-sided. We performed all analyses using SAS software, version 9.3 (SAS Institute, Cary, NC).

Results

Characteristics of the study population

A total of 2,482 invasive postmenopausal breast cancers were diagnosed during 581,085 person-years of follow-up. At the end of follow-up, 22% of the study population was estimated to be ever exposed to vitamin D supplementation, mostly taken daily and combined with calcium. The estimated vitamin D intake from foods was low, *i.e.* <200 IU/day for 96% of participants. Mean baseline BMI was 23.4; 74% of participants had a BMI <25 kg/m^2. Characteristics of the participants are displayed in Table 1. Compared with never users,

ever users of vitamin D supplements were older at the end of follow-up, they were less frequently users of oral contraceptives before menopause and more frequently users of supplements with other micronutrients, and they had more frequently a personal history of benign breast disease. There was no major specific difference between vitamin D supplement users *vs.* nonusers across BMI and MHT use strata (see Table 1).

Influence of BMI

Overall, BMI did not influence the relationship between ever vitamin D supplementation and postmenopausal breast cancer risk when not adjusting for the interaction between vitamin D supplementation and MHT use (HR = 0.96, 95% CI: 0.83, 1.11 in women with BMI <25 kg/m^2, HR = 0.91, 95% CI: 0.69, 1.20 in women with BMI ≥25 kg/m^2, $P_{homogeneity} = 0.73$) (Table 2). When adjusting for the interaction term between vitamin D supplementation and MHT use, vitamin D supplementation was associated with increased postmenopausal breast cancer risk in women with BMI <25 kg/m^2 (HR = 1.55, 95% CI: 1.18, 2.02), while there was no association in women with higher BMI (HR =1.07, 95% CI: 0.68, 1.67) (Table 2), $P_{homogeneity} = 0.17$.

Ever vitamin D supplementation was associated with decreased postmenopausal breast cancer risk in MHT ever users (HR = 0.84, 95% CI: 0.72, 0.98), while it was associated with increased risk in MHT never users (HR = 1.34, 95% CI: 1.05, 1.71). The association remained similar across BMI strata in MHT ever users (HR = 0.84, 95% CI: 0.70, 0.99 in women with BMI <25 kg/m^2 and HR = 0.87, 95% CI: 0.62, 1.23 in women with BMI ≥25 kg/m^2, $P_{homogeneity} = 0.83$) (Table 3). In MHT never users, the increased risk associated with vitamin D supplementation was restricted to women with BMI <25 kg/m^2 (HR = 1.51, 95% CI: 1.13, 2.02), while there was no association in women with higher BMI (HR = 0.98, 95% CI: 0.62, 1.56) (Table 3), $P_{homogeneity} = 0.12$. To investigate whether the higher risk increased with a lower BMI, we further divided the <25 BMI class into <22 and 22–24.9 kg/m^2 using the median BMI in MHT nonusers with BMI <25 as cut-off. The increased risk associated with ever vitamin D supplementation in MHT nonusers was stronger in women with BMI <22 kg/m^2 (HR = 1.62, 95% CI: 1.11, 2.35) than in women with BMI of 22–24.9 kg/m^2 (HR = 1.35, 95% CI: 0.84, 2.17).

Absolute risks

The lowest absolute annual rate of postmenopausal breast cancer was observed in women with BMI <25 kg/m^2 who took neither MHT nor vitamin D supplements, *i.e.* 363/100,000 person-years. The highest absolute annual rates were observed in MHT users with no vitamin D supplementation (671/100,000 person-years in women with BMI <25 kg/m^2 and 653/100,000 person-years in women with BMI ≥25 kg/m^2). Rates were intermediary in women with BMI ≥25 kg/m^2 who took neither MHT nor vitamin D supplements (500/100,000 person-years), and in vitamin D supplement users either with MHT (540/100,000 person-years in women with BMI <25 kg/m^2 and 602/100,000 person-years in women with BMI ≥25 kg/m^2) or without MHT (551/100,000 person-years in women with BMI <25 kg/m^2 and 566/100,000 person-years in women with BMI ≥25 kg/m^2).

Sensitivity analyses

When excluding cases diagnosed during the first 5 years of follow-up ($n = 1{,}060$), the increased postmenopausal breast cancer risk associated with vitamin D supplementation in MHT nonusers with BMI <25 kg/m^2 remained similar (HR = 1.51, 95% CI: 1.04, 2.18).

Discussion

In this study, BMI did not modify the previously described inverse association between vitamin D supplementation and postmenopausal breast cancer risk in MHT ever users,[8] which was similar across BMI strata. Among MHT never users, vitamin D supplementation was associated with increased postmenopausal breast cancer risk in women with BMI <25 kg/m^2, while there was no association in women with higher BMI.

Evidence from other epidemiological studies

Studies including mostly postmenopausal women did not report any influence of BMI on the relationship between baseline 25(OH)D plasma concentrations and breast cancer risk.[11–15] However, randomized trials have shown that plasma 25(OH)D concentrations in response to vitamin D supplementation were lower in overweight/obese subjects,[4–6,16,17] therefore higher vitamin D intake could be requested in overweight/obese subjects to reach the same plasma 25-(OH)D concentrations than non-overweight subjects. Few prospective studies considering vitamin D supplemental intake investigated a potential influence of BMI on the relationship between vitamin D intake and postmenopausal breast cancer risk. In one study, baseline BMI (<25, 25–29.9, ≥30 kg/m^2) did not influence the relationship between total or supplemental vitamin D intake and postmenopausal breast cancer risk[18] but MHT use was not investigated. In the Women's Health Initiative trial, 36,282 postmenopausal women were randomized to receive a daily supplementation of 400 IU vitamin D and 1,000 mg calcium during 7 years. Authors reported effect modification by personal intake of vitamin D at baseline, but not by randomization stratum on hormone therapy or by BMI (<25, 25–29.9, ≥30 kg/m^2).[19]

Biological hypotheses

Biological hypotheses support protective effects of vitamin D against breast cancer risk, in particular estrogen-receptor-positive (ER+) tumors.[1] Complex interactions may exist between vitamin D intake, BMI, and MHT use, and postmenopausal breast cancer risk.

In postmenopausal women, adipose tissue is the main source of endogenous estrogens through increased aromatase

enzyme conversion of androgenic precursors to estradiol, but the impact of adipose tissue estrogen production is minimized by MHT use. Accordingly, the positive association between BMI and postmenopausal breast cancer was shown to be weaker in MHT users than in MHT nonusers.[20,21] *In vitro*, calcitriol inhibits estrogen induced growth of ER+ breast cancer cells by downregulating ERα expression.[22–24] Thus, in agreement with our results, women with high estrogen levels such as MHT users may particularly benefit from vitamin D supplements, whatever their BMI.

Conversely, since vitamin D inhibits aromatase expression,[22] overweight/obese MHT nonusers might particularly benefit from vitamin D supplementation. However in our study, vitamin D supplementation was not associated with reduced postmenopausal breast cancer risk in overweight/obese MHT nonusers. Since BMI of participants was low compared with other studies, with only a small proportion of obese women, we cannot however exclude an inverse association of vitamin D supplementation and breast cancer risk in obese women.

Our results suggesting a potential adverse effect of vitamin D supplements on breast cancer risk in non-overweight women with no MHT are unexpected. However they agree with previously findings, suggesting potential interactions between the hormonal status, BMI and vitamin D. In the Nurses' Health Study II, in premenopausal women, higher plasma 25-hydroxyvitamin D concentrations was associated with an increased breast cancer risk in those with a high BMI,[25] *i.e.* those with lowest circulating hormonal levels.[20] In the Women's Health Initiative trial, breast cancer risk increased in the vitamin D plus calcium supplementation group among women with baseline vitamin D intake $\geq$600 IU/day. Of interest is that within the vitamin D supplemented arm, the mean BMI was lower in women with personal calcium or vitamin D use at baseline than in those with no personal use of calcium or vitamin D at baseline.[26] To our knowledge, no biological explanation to those findings has been provided thus far in the literature. Since high plasma 25-hydroxyvitamin D level has also been associated with an increased risk of pancreatic cancer, our results should prompt further research on the optimal vitamin D intake and/or status according to the hormonal or anthropometric background.

Strengths and limitations

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 and on potential confounders was regularly updated.

Our analyses in overweight and obese women may have lacked power to assess associations between vitamin D supplementation and breast cancer risk while considering MHT use, because of the relatively limited proportion of such women, especially of obese women taking vitamin D supplementation. On the opposite, few studies of postmenopausal women had the opportunity we had of investigating a potential effect of vitamin D supplementation in normal-weight women.

As previously reported, we did not have sufficient information on supplement doses but we were able to determine that vitamin D supplements used in our cohort were mostly daily supplements with doses $\geq$400 IU/day combined with calcium.[8] Non-differential misclassification of vitamin D supplementation may have occurred, although limited by updated information, and thus resulting in underestimating risks. Finally, further studies are needed to confirm that our results are not due to chance or bias. Residual confounding is unlikely because there was no major difference between vitamin D supplement users *vs.* nonusers across BMI and MHT strata for a large set of potential confounders.

In conclusion, our findings suggest that vitamin D supplementation may reduce the excess breast cancer risk in MHT users. In the opposite, they draw attention on a potential risk associated with vitamin D supplementation in postmenopausal women not exposed to high exogenous or endogenous hormones, *i.e.* non-overweight MHT-non users, especially in the present context of increasing use of vitamin D supplements and decreasing MHT use.

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Author contributions are as follows: F.C.-C. leads the E3N cohort and is responsible for data collection; C.C., A.F., G.F., and M.-C.B.-R. designed research; C.C. performed statistical analysis; C.C., A.F., S.M., and M.-C.B.-R. interpreted data; C.C. drafted the manuscript; A.F., S.M., F.C.-C., G.F., and M.-C.B.-R. critically reviewed the manuscript. M.-C.B.-R. had primary responsibility for final content. All authors read and approved the final manuscript.

Conflict of Interest

None of the authors declared a conflict of interest.

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