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Breast cancer risk in relation to different types of hormone replacement therapy in the E3N-EPIC cohort

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Most epidemiological studies have shown an increase in breast cancer risk related to hormone replacement therapy (HRT) use. A recent large cohort study showed effects of similar magnitude for different types of progestogens and for different routes of administration of estrogens evaluated. Further investigation of these issues is of importance. We assessed the risk of breast cancer associated with HRT use in 54,548 postmenopausal women, who had never taken any HRT 1 year before entering the E3N-EPIC cohort study (mean age at inclusion: 52.8 years); 948 primary invasive breast cancers were diagnosed during follow-up (mean duration: 5.8 years). Data were analyzed using multivariate Cox proportional hazards models. In this cohort where the mean duration of HRT use was 2.8 years, an increased risk in HRT users compared to nonusers was found (relative risk (RR) 1.2 [95% confidence interval 1.1–1.4]). The RR was 1.1 [0.8–1.6] for estrogens used alone and 1.3 [1.1–1.5] when used in combination with oral progestogens. The risk was significantly greater ($p < 0.001$) with HRT containing synthetic progestins than with HRT containing micronized progesterone, the RRs being 1.4 [1.2–1.7] and 0.9 [0.7–1.2], respectively. When combined with synthetic progestins, both oral and transdermal/percutaneous estrogens use were associated with a significantly increased risk; for transdermal/percutaneous estrogens, this was the case even when exposure was less than 2 years. Our results suggest that, when combined with synthetic progestins, even short-term use of estrogens may increase breast cancer risk. Micronized progesterone may be preferred to synthetic progestins in short-term HRT. This finding needs further investigation.

Key words: hormone replacement therapy; estrogens; progestogens; progesterone; risk factors: menopause; breast cancer; cohort study

The results of the American WHI study published in July 2002[1] caused considerable concern among hormone replacement therapy (HRT) users and prescribers in many countries. This placebo-controlled trial of an oral continuous combined conjugated equine estrogens (CEE) plus medroxyprogesterone acetate (MPA) regimen was prematurely discontinued because the overall health risks exceeded the benefits. In particular, it showed an increased breast cancer risk in the CEE plus MPA arm.[2] More recently, the Million Women Study, a large cohort study conducted in the United Kingdom, has suggested that this result may also apply to other types of components, to sequential regimens and to other routes of estrogen administration.[3] This makes the safety of HRT, used worldwide by millions of women, highly questionable with regard to breast cancer risk. Following the publication of the results of the CEE+MPA *vs.* placebo component of the WHI trial, prescriptions of Prempro (the combined HRT tested in that study) considerably declined in the USA.[4] In contrast, the results of the CEE alone *vs.* placebo component of this trial were reassuring with regard to breast cancer risk.[5] However, these HRTs are 2 amongst a variety of treatments prescribed all over the world. Apart from the Million Women Study, few epidemiological studies have had sufficient sample sizes or accurate information to assess the breast cancer risk related to different types and route of administration of estrogens, and to different types of progestins. Moreover, micronized progesterone in combined HRT has never been evaluated. It might be then premature to definitively advise against any HRT as the risk of breast cancer (and other conditions) has not been yet properly studied for certain types of HRT. Furthermore, we lack accurate data on the impact of short-term use of HRT, which is now crucial since several agencies or administrations recently advised that hormones should be used for the shortest possible duration. It is therefore of paramount importance to bring new data on these issues.

E3N (Etude Epidémiologique de femmes de la Mutuelle Générale de l'Education Nationale) is a large cohort study offering the opportunity to investigate the breast cancer risk associated with various types and routes of HRT administration, using very detailed and updated information on hormonal treatments and menopausal status recorded prospectively every 2 years.

Material and methods

E3N is a French prospective study investigating cancer risk factors in 98,997 women born between 1925 and 1950.[6] All women belong to the MGEN, a health insurance scheme primarily covering teachers. Part of the E3N cohort (*i.e.*, women who replied to a dietary questionnaire) is also included in the European Prospective Investigation into Cancer and Nutrition (EPIC).[7] Since June 1990, after having given informed consent, participants have been asked at approximately 24-month intervals to complete self-administered questionnaires including a variety of lifestyle characteristics. For each questionnaire, up to 2 reminders were sent to nonrespondents. Information on lifetime use of hormonal treatments was first recorded in the January 1992 questionnaire. In order to facilitate accurate recall, a booklet presenting an extensive list and color photographs of the hormonal treatments marketed in France was mailed to all study participants. Brand name, age at first use and duration of use were recorded for up to 24 periods of treatment. Information on HRT use was updated in each of the subsequent questionnaires. Information on the doses of the treatments used was not requested. We categorized HRT use according to i) the type of estrogens and the route of administration: weak estrogens (oral estriol compounds or vaginally administered low-dose estrogens), oral estradiol compounds, transdermal or percutaneous estradiol compounds and CEE, and ii) the type of oral progestogens used in association with the estrogens: none, micronized progesterone, progesterone derivatives (retroprogesterone, pregnane or norpregnane derivatives, such as MPA, chlormadinone acetate, medrogestone, nomegestrol acetate or promegestone)

Abbreviations: BMI, body mass index; CEE, conjugated equine estrogen; CI, confidence interval; E3N, Étude épidemiologique des femmes de la Mutuelle Générale de l'Education Nationale; EPIC, European prospective investigation into cancer and nutrition; GH, growth hormone; HRT, hormone replacement therapy; IGF-I, insulin-like growth factor-I; MPA, medroxyprogesterone acetate; RR, relative risk; SD, standard deviation; WHI, Women's Health Initiative.

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and testosterone derivatives (19-nortestosterone derivatives, such as norethisterone acetate or lynestrenol).

In each questionnaire (last one sent out in June 2000), participants were asked whether breast cancer had been diagnosed, requesting their physicians' addresses and permission to contact them. Deaths in the cohort were detected from reports by family members and by searches in the insurance company (MGEN) file, which contains information on vital status. Cause of death information was obtained from the National Service on Causes of Deaths (INSERM). Information on nonrespondents was obtained from the MGEN file on reimbursement of hospital fees for women who gave consent for external health follow-up by the health insurer. In the latter case, the subject's physician was then contacted for diagnostic information, enabling additional breast cancer cases to be found.

Follow-up started either at the date of return of the baseline questionnaire (sent out in June 1990) for women already postmenopausal at that time, or at the date of menopause as reported in the follow-up questionnaires. Women who only replied the baseline questionnaire were excluded. Follow-up continued for 1 year after return of the follow-up questionnaire sent out in January 1992, June 1993, January 1995 or April 1997, whichever was answered last. Person-years accrued until that date, diagnosis of cancer, death or June, 2000, whichever occurred first.

To ensure that the constructed menopause variables were as accurate as possible, date of menopause, type of menopause, date of last menstruation, date of start of menopausal symptoms and date of hysterectomy were updated on receipt of each new questionnaire. Women for whom age at menopause could not be determined (*e.g.*, women who reported a hysterectomy but gave no information on oophorectomy or menopausal symptoms or women who indicated they were postmenopausal without any other information) were considered as menopausal at age 46 if menopause was artificial, and at age 50 otherwise, ages that correspond in our cohort to the median age at menopause when artificial and natural, respectively. Among the postmenopausal women (n = 70,630), those who had reported a cancer other than a basal cell carcinoma before the start of follow-up were excluded from the analysis (n = 5,045), as were those reporting an *in situ* breast cancer during follow-up (n = 168). Moreover, to mimic trials where, optimally, patients have never been under treatment at baseline, women who had reported using HRT before the year preceding the start of follow-up (n = 10,869) were not considered, since the inclusion of prevalent users at baseline (either current or past users) causes a spurious selection into the study of exposed women who did not develop breast cancer, particularly after a short period of use (see Discussion). This left us with 54,548 postmenopausal women for the analysis. They were followed an average of 5.8 years [standard deviation (SD) 2.4; range: 0.1 to 10.6 years]. A total of 315,086 person-years accumulated for this group, which had an average age at start of follow-up of 52.8 years (SD 4.9; range: 40.0 to 66.1 years).

Statistical analysis

Relative risks for breast cancer were estimated using Cox proportional hazards models. Time since menopause was chosen as the time scale. Potential confounding variables were tested in the proportional hazard model and those retained if they improved model fit by the $p<0.1$ criterion are indicated in the footnotes of the tables. Missing data in adjustment factors were imputed to the modal value in the population with complete data. The baseline questionnaire asked if respondents ever underwent a mammogram. Each subsequent questionnaire then asked whether a mammogram had been performed during the last follow-up interval. In all models, mammography status was considered as a time-dependent variable according to respondent status at the start of each follow-up interval: no mammography reported in the latest questionnaire/at least 1 mammography reported in the latest questionnaire/not known (*e.g.*, no questionnaire returned for the interval concerned).

It was decided that each woman should contribute person-years of exposure to the HRT category (according to the type and route of administration of estrogens and to the type of progestogens) corresponding to the hormones she had used for the greatest length of time since menopause. HRT use was included in the models as a time-dependent variable, exposure being lagged by 1 year (see Discussion). The referent group in each model therefore consisted of women who indicated that they had either never used any form of HRT or had started taking HRT less than 1 year before the end of follow-up. In Cox models estimating RRs according to duration of use, women were considered as exposed to HRT during the entire period from the start of exposure to the last reported HRT use at the end of follow-up. Tests for trend were calculated across categories of duration of use, excluding never-users.

The p values for assessing possible heterogeneity in effect estimates were computed from likelihood ratio tests. All tests of statistical significance were 2 sided. All analyses were performed using the SAS software, version 8.2.

Results

Characteristics of the study population

The main characteristics of the study population according to HRT exposure at the end of follow-up are shown in Table I. Users were more likely than nonusers to have had an early menarche, an early menopause, to be parous, to have a personal history of benign breast disease, to have no familial history of breast cancer in first degree relatives, to be lean, to have a higher level of education, to have used oral contraceptives and to have used oral progestogens before menopause.

A large majority of exposed women used estradiol delivered through the skin, of whom around 55% used percutaneous gels and 45% transdermal patches. The type of HRT most frequently used was a combination of transdermal or percutaneous estradiol compounds and progesterone derivatives (Table II). Transdermal/percutaneous estradiol compounds combined with micronized progesterone and oral estradiol compounds combined with progesterone derivatives were also widely used. There was only marginal use of CEE (alone or associated with a progestational agent) and of estradiol compounds combined with testosterone derivatives. In the subsequent tables, CEE was not distinguished from estradiol compounds, and progesterone- and testosterone-derivatives were considered as "synthetic progestins".

The mean duration of HRT use in this group of postmenopausal women who started treatment after baseline or in the preceding year, and during our study period, was 2.8 years (SD 1.9), ranging from 2.4 years (estradiol compounds used alone) to 3.1 years (transdermal/percutaneous estrogens combined with progesterone derivatives) for the types of HRT used the most frequently.

HRT use and breast cancer risk

During follow-up, 948 cases of new primary invasive breast cancer were identified among the 54,548 postmenopausal women who did not use HRT or started treatment after baseline or in the preceding year. Pathology reports were obtained for 96 % of cases.

The overall multivariate-adjusted RR of breast cancer was 1.2, 95% CI 1.1–1.4, for women ever exposed to HRT for the first time during the follow-up period or in the year preceding that period compared to never-users. Because of the possibility of effect modification by type of menopause, BMI, familial history of breast cancer, ever use of oral contraceptives or personal history of benign breast disease, interactions with these variables were studied. Differences in risk estimates were not significant, except with type of menopause (the RR being lower among women with an artificial menopause than among women with a natural menopause, p = 0.04) (data not shown).

Breast cancer RR according to exposure to various types of hormones is presented in Table III. No significant increase in risk was observed in users of weak estrogens (RR 0.7, 95% CI 0.4–1.2)

TABLE I – CHARACTERISTICS OF HRT USERS AND NONUSERS (n = 54,548). E3N COHORT STUDY

	Nonusers (n = 25,128)	Users (n = 29,420)	p value[1]
Year of birth			< 0.0001
[1925–1930]	4,335 (17.3%)	780 (2.7%)	
[1930–1935]	5,205 (20.7%)	2,504 (8.5%)	
[1935–1940]	4,845 (19.3%)	7,583 (25.8%)	
[1940–1945]	5,489 (21.8%)	11,940 (40.6%)	
≥ 1945	5,254 (20.9%)	6,613 (22.5%)	
Age at menarche, years[2]			< 0.0001
< 13	11,632 (46.3%)	13,941 (47.4%)	
[13–15]	10,785 (42.9%)	12,751 (43.3%)	
≥ 15	2,711 (10.8%)	2,728 (9.3%)	
Age at menopause, years			< 0.0001
< 48	5,142 (20.5%)	6,687 (22.7%)	
[48–52]	12,666 (50.4%)	14,943 (50.8%)	
≥ 52	7,320 (29.1%)	7,790 (26.5%)	
Parity[3]			< 0.0001
Nulliparous	3,481 (13.9%)	3,192 (10.9%)	
Parous, first child after 30, 1 child	1,085 (4.3%)	1,208 (4.1%)	
Parous, first child after 30, 2+ children	1,570 (6.3%)	1,551 (5.3%)	
Parous, first child before 30	18,992 (75.6%)	23,469 (79.8%)	
Personal history of benign breast disease[4]			<0.0001
Yes	5,457 (21.7%)	8,110 (27.6%)	
No	19,671 (78.3%)	21,310 (72.4%)	
Familial history of breast cancer in first degree relatives[4]			<0.0001
Yes	3,107 (12.4%)	3,307 (11.2%)	
No	22,021 (87.6%)	26,113 (88.8%)	
Body Mass index at baseline, kg/m^2[5]			<0.0001
≤ 22	9,457 (37.6%)	14,444 (49.1%)	
[22–25]	8,751 (34.8%)	10,281 (35.0%)	
[25–27]	3,039 (12.1%)	2,589 (8.8%)	
[27–30]	2,292 (9.1%)	1,458 (5.0%)	
≥ 30	1,589 (6.3%)	648 (2.2%)	
Educational level (years of education)[6]			< 0.0001
<13	4,609 (18.3%)	3,466 (11.8%)	
13–16	16,764 (66.7%)	20,813 (70.7%)	
17+	3,755 (14.9%)	5,141 (17.5%)	
Oral contraceptive use[7]			< 0.0001
Never	18,652 (74.2%)	17,368 (59.0%)	
Ever	6,476 (25.8%)	12,052 (41.0%)	
Use of oral progestogens before menopause[7]			< 0.0001
None or less than 2 years of use	22,996 (91.5%)	24,772 (84.2%)	
[2–5 years]	1,392 (5.5%)	3,182 (10.8%)	
≥ 5 years	740 (2.9%)	1,466 (5.0%)	

[1]Wilcoxon rank test for continuous variables and chi-square test for proportion.–[2]Values imputed to the modal value for 769 women with missing data.–[3]Values imputed to the modal value for 862 women with missing data.–[4]Values for missing data indistinguishable from "no" responses.–[5]Values imputed to the modal value for 16 women with missing data.–[6]Values imputed to the modal value for 2,823 women with missing data.–[7]Values for missing data indistinguishable from "never" responses.

or other estrogens used alone (RR 1.1, 95% CI 0.8–1.6), compared to nonexposed women.

We first investigated the impact of the route of administration of estrogens on breast cancer risk. The RRs for use of transdermal/percutaneous and oral estrogens did not differ significantly: when combined with synthetic progestins, they were 1.4 (95% CI 1.2–1.7) and 1.5 (95% CI 1.1–1.9), respectively, as compared to nonuse of HRT (p for heterogeneity 0.9). We did not compare the effect of the route of administration of estrogens when used alone or combined with micronized progesterone since too few women were exposed to oral estrogens in these groups.

We then investigated the impact of the type of progestogen used. Compared to nonexposed women, the risk increased significantly for users of estrogens combined with progestogens (RR 1.3, 95% CI 1.1–1.5) but this increase was limited to synthetic progestins (RR 1.4, 95% CI 1.2–1.7); there was no evidence of increased risk associated with the use of estrogens combined with micronized progesterone (RR 0.9, 95% CI 0.7–1.2). The test for heterogeneity between micronized progesterone and synthetic progestins was significant ($p < 0.001$). Different types of synthetic progestins were used, yielding similar risks for estrogens associated with progesterone-derivatives (RR 1.4, 95% CI 1.2–1.7) and for estrogens associated with testosterone-derivatives (RR 1.4, 95% CI 0.9–2.3) (p for heterogeneity 0.9).

The RR associated with estrogens used alone (RR 1.1, 95% CI 0.8–1.6) did not differ significantly from the RR associated with estrogens plus synthetic progestins (RR 1.4, 95% CI 1.2–1.7) (p for heterogeneity 0.14).

There was no evidence of increasing risk with increasing duration of HRT exposure, except for oral estrogens combined with synthetic progestins for which the trend was of borderline significance ($p = 0.07$) (Table IV). In the first tertile of exposure (< 2 years), the RRs varied according to the type of progestogen used: the risk was significantly increased with use of transdermal/percutaneous estrogens combined with synthetic progestins as compared to either no HRT use ($p < 0.0001$), or compared to transdermal/percutaneous estrogens combined with micronized progesterone ($p = 0.01$). This was also the case in the second tertile of exposure (2 to 4 years of exposure), the risk being significantly increased with use of transdermal/percutaneous estrogens combined with synthetic progestins as compared to either no HRT use ($p = 0.04$), or compared to transdermal/percutaneous estrogens combined with micronized progesterone ($p = 0.02$). No significant heterogeneity was seen across different types of HRT

TABLE II – TYPES OF HORMONES USED (n = 29,420 WOMEN WITH INCIDENT HRT EXPOSURE[1]) E3N COHORT STUDY

Hormones	Any use (%)	Main use[2] (%)	Mean duration of use, years[3] (SD)
Weak estrogens[4]	7.1	4.5	2.1 (1.7)
Estradiol compounds used alone	22.1	9.9	2.4 (1.7)
Transdermal/percutaneous route	19.8	8.9	2.4 (1.8)
Oral route	2.9	1.2	2.3 (1.6)
Estradiol compounds combined with oral progestogens	88.6	83.3	2.9 (1.9)
Estradiol compounds combined with micronized progesterone	26.8	20.1	3.0 (1.9)
Transdermal/percutaneous route	25.3	18.9	3.0 (1.9)
Oral route	2.1	1.3	2.7 (1.8)
Estradiol compounds combined with progesterone derivatives[5]	67.9	58.3	2.9 (1.9)
Transdermal/percutaneous route	50.7	40.6	3.1 (2.0)
Oral route	23.5	17.6	2.5 (1.6)
Estradiol compounds combined with testosterone derivatives[6]	7.6	4.6	2.7 (1.9)
Transdermal/percutaneous route	0.8	0.4	2.8 (2.0)
Oral route	6.9	4.3	2.7 (1.9)
Conjugated equine estrogens[4]	1.9	1.0	3.3 (1.8)
Other[7]/not specified	—	1.3	2.9 (2.1)

[1]Had commenced HRT between 1 year before the start of and 1 year before the end of follow-up.–[2]Corresponding to the HRT used for the greatest length of time.–[3]Among main users.–[4]Used alone or with a progestogen.–[5]Mainly MPA or cyproterone acetate when combined with oral estrogens, retroprogesterone, nomegestrol acetate or promegestone when combined with transdermal estrogens.–[6]Almost exclusively norethisterone acetate when combined with oral estrogens, mainly lynestrenol or norethisterone acetate when combined with transdermal estrogens.–[7]HRT containing estrogens or progestogens administered intramuscularly, or androgens.

TABLE III – RELATIVE RISKS ASSOCIATED WITH USE OF DIFFERENT HORMONES BY WOMEN WITH INCIDENT HRT EXPOSURE[1] COMPARED WITH NONEXPOSED WOMEN[2] (n = 54,548) E3N COHORT STUDY

Exposure category[3]	Cases	Person-years	Age-adjusted RR [CI 95%]	Multivariate-adjusted RR [CI 95%][4]
Weak estrogens	13	5,802	0.7 [0.4–1.3]	0.7 [0.4–1.2]
Estrogens used alone	30	9,698	1.1 [0.8–1.6]	1.1 [0.8–1.6][6]
Transdermal/percutaneous route	29	8,691	1.2 [0.8–1.8]	1.2 [0.8–1.7]
Oral route	2	1,204	0.6 [0.2–2.4]	0.6 [0.2–2.4]
Estrogens combined with oral progestogens	323	89,148	1.3 [1.1–1.5]	1.3 [1.1–1.5]
Estrogens combined with micronized progesterone	55	21,994	0.9 [0.7–1.2]	0.9 [0.7–1.2][7]
Transdermal/percutaneous route	55	20,685	0.9 [0.7–1.2]	0.9 [0.7–1.2]
Oral route	0	1,385	—	—
Estrogens combined with synthetic progestins	268	66,925	1.4 [1.2–1.7]	1.4 [1.2–1.7][6,7]
Transdermal/percutaneous route	187	46,242	1.4 [1.2–1.7]	1.4 [1.2–1.7][8]
Oral route	80	20,504	1.4 [1.1–1.8]	1.5 [1.1–1.9][8]
Other[5]/not specified	6	1,426	1.5 [0.7–3.4]	1.5 [0.7–3.4]

[1]Had commenced HRT between 1 year before the start of and 1 year before the end of follow-up.–[2]Had never used any form of HRT or had started taking HRT less than 1 year before the end of follow-up.–[3]Corresponding to the HRT used for the greatest length of time.–[4]Adjusted for time since menopause, BMI (continuous), age at menopause (continuous), parity and age at first full-term pregnancy (nulliparous/first full-term pregnancy at age <30/first full-term pregnancy at age ≥30, 1 child/first full-term pregnancy at age ≥30, 2 or more children), familial history of breast cancer in sisters, mother, children (no/1/more than 1), familial history of breast cancer in other relatives (yes/no), personal history of benign breast disease (yes/no), use of oral progestogens before menopause (none or less than 2 years of use/2 to 5 years of use/more than 5 years of use), ever use of oral contraceptives and previous mammography (as a time-dependent variable).–[5]HRT containing estrogens or progestogens administered intramuscularly, or androgens.–[6]Test for heterogeneity between estrogens used alone and associated with synthetic progestins: p = 0.14.–[7]Test for heterogeneity between estrogens associated with micronized progesterone and associated with synthetic progestins: $p < 0.001$.–[8]Test for heterogeneity between transdermal/percutaneous estrogens associated with synthetic progestins and oral estrogens associated with synthetic progestins : $p = 0.9$.

for longer durations of exposure. We also estimated RRs associated with less than 1 year of exposure, which yielded a significant increase in risk for transdermal/percutaneous estrogens combined with synthetic progestins (RR 1.7, 95% CI 1.3–2.3).

Discussion

Our study shows an increased risk of breast cancer associated with HRT use. It indicates that the association between HRT use and breast cancer risk most likely varies according to the type of progestogen used. There was no or little increase in risk with estrogens used alone or combined with micronized progesterone, at least when used for short periods. The increase in risk reached significance when estrogens were combined with synthetic progestins and was significantly greater than when combined with micronized progesterone. Overall, the RRs did not vary according to the route of administration of estrogens. Even short durations of exposure were associated with significantly increased risks when estrogens were combined with synthetic progestins : < 2 years and 2–4 years for transdermal/percutaneous estrogens, 2–4 years for oral estrogens.

Most epidemiological data on HRT available up to 2002 have come from studies performed in the USA and have thus concerned oral CEE alone or associated with MPA, whereas CEEs were used by only 2% of the postmenopausal women in our cohort. Some studies have also been performed in Northern Europe, where estradiol is usually associated with testosterone-derived progestogens. Recently, the Million Women Study conducted in the UK has compared the breast cancer risk associated with several types of estrogens, progestogens and routes of administration.[3] However, there were no results for micronized progesterone in combined HRT. Using the data from the E3N cohort study, we inves-

TABLE IV – DURATION OF EXPOSURE AND BREAST CANCER RISK ACROSS MAIN TYPES OF HRT AMONG WOMEN WITH INCIDENT HRT EXPOSURE[1] COMPARED WITH NON-EXPOSED WOMEN[2] (n = 54,548), E3N COHORT STUDY

Exposure category[5]	Duration of exposure[3]						p for trend
	< 2 years		[2–4 years[		≥ 4 years		
	Cases	RR [CI 95%][4]	Cases	RR [CI 95%][4]	Cases	RR [CI 95%][4]	
Any HRT use	185	1.2 [1.0–1.5]	115	1.2 [1.0–1.5]	72	1.2 [0.9–1.6]	0.7
Transdermal/percutaneous estrogens							
Used alone	18	1.4 [0.8–2.2]	10	1.4 [0.7–2.6]	1	0.3 [0.1–1.8]	0.4
Combined with oral micronized progesterone	26	0.9 [0.6–1.4]	13	0.7 [0.4–1.2]	16	1.2 [0.7–2.0]	0.9
Combined with oral synthetic progestins	95	1.6 [1.3–2.0]	57	1.4 [1.0–1.8]	35	1.2 [0.8–1.7]	0.3
Oral estrogens							
Combined with oral synthetic progestins	36	1.2 [0.9–1.8]	27	1.6 [1.1–2.3]	17	1.9 [1.2–3.2]	0.07

[1]Had commenced HRT between 1 year before the start of and 1 year before the end of follow-up.–[2]Had never used any form of HRT or had started taking HRT less than 1 year before the end of follow-up.–[3]Disregarding exposure in the year before the end of follow-up.–[4]Adjusted for the same covariates as in Table III.–[5]Corresponding to the type of HRT used for the greatest length of time. Duration of exposure is categorized according to tertiles.

tigated a variety of hormones available in France, where the most widely used types of HRT are transdermal/percutaneous estradiol associated with either micronized progesterone or progesterone derivatives. Most users of transdermal estrogens receive preparations delivering 50 μg per day or less. Orally administered estrogens are mostly 1.0 to 2.0 mg of estradiol per day.

Our study confirms previous findings of an increase in invasive breast cancer risk with estrogens combined with synthetic progestins compared to no HRT use. The carcinogenic effect of the CEE plus MPA association in continuous administration was proved by the WHI trial[2] and recent observational studies performed in the USA.[8–13] Studies performed in Sweden or in Denmark, where testosterone-derivatives are widely used, found a positive association with breast cancer risk for combined HRT.[14–18] In the Million Women Study, progesterone- and testosterone-derived progestins were associated with an increase in breast cancer risk, and the RR showed little variation according to the progestogen constituent.[3] Compared to estrogens used alone, adding synthetic progestins was found to further increase breast cancer risk in several studies,[3,8–10] as in our study, though the test of heterogeneity between estrogens used alone and estrogens associated with synthetic progestins did not reach significance.

So far, reports on the effect of progesterone on breast cells have been contradictory,[19] some studies supporting an increase in the proliferation of human breast epithelial cells[20–22] and others a decrease.[23–26] The only epidemiological study comparing the impact of progesterone and synthetic progestins on the breast was the PEPI trial,[27] in which the authors assessed differences between placebo and several HRTs on the change in mammographic percent density. Our result of breast cancer risk significantly greater with HRT containing synthetic progestins than with HRT containing micronized progesterone, at least for short durations of use (< 4 years), is therefore new. Additional follow-up time in our cohort will allow us to investigate whether this differential impact of micronized progesterone and synthetic progestins on breast cancer risk persists for longer durations of use.

Previous cohort studies[3,9,11,28–30] and a meta-analysis[31] have shown an increase in risk with increased duration of HRT use. In our study, there was a significant increase in risk with very short exposure to transdermal/percutaneous estrogens combined with oral synthetic progestins (< 2 years) that was not more pronounced with longer durations of use. In contrast, a trend, of borderline significance, of increasing risk with increasing duration of exposure was found with use of oral estrogens combined with oral synthetic progestins, with a significant increase in risk in the 2–4 years and ≥ 4 years of exposure stratum. To what extent the type, the route of estrogens, and the type of progestogens may contribute to this deleterious impact of short-term use is difficult to determine. Interestingly, only studies performed in Europe, where estrogens used in HRT often consist in estradiol rather than CEEs, found such a deleterious impact of short-term HRT.[3,14,16–18] Some experimental findings suggest that components of CEEs, the 17 alpha-dihydroderivatives of equilenin and equilin, have a nonestrogenic or even an anti-estrogenic effect on breast tissue.[32] Physiological studies have also shown that the route of administration has a major impact on the growth hormone/insulin-like growth factor-I axis (GH/IGF-I): estrogen administration by oral route (but not by transdermal) has been found to reduce IGF-I and consequently to increase GH levels in postmenopausal women.[33,34] Several prospective studies have supported the association of circulating levels of IGF-I, a potent mitogen that stimulates breast cancer cells in synergy with estrogens,[33] with the subsequent breast cancer risk, particularly in premenopausal, *i.e.*, estrogenised women.[35–37] Our results do not contradict this mechanism since, when combined with synthetic progestins, transdermal/percutaneous estrogens seemed to impact breast cancer risk with shorter exposures than oral estrogens. However, no significant heterogeneity was seen across these 2 types of HRT in any strata of duration of exposure (<2 years, [2–4 years[, ≥ 4 years) and therefore the possibility of a different impact of HRT according to the route of administration of estrogens should be further explored.

In our study, the effect of hormone use on breast cancer appeared to be similar across categories of BMI (data not shown), contradicting previous findings that the increase in risk associated with HRTs primarily concerns underweight women.[3,9,31] The French women in our cohort are lean compared to participants in cohort studies in other countries[38] and the period of time since menopause may be too short to have modified their body shape into a more androgenic one. They may thus be more sensitive to exogenous hormones than women with abdominal obesity, which produces endogenous estrogens and androgens synthesis.

We adjusted our analyses as carefully as possible for known potential confounders, so as to minimize any bias due to confounding by treatment- and outcome-related factors. Uncontrolled residual bias may however remain. The effect of errors in menopausal age on the estimation of the RRs[39,40] was minimized by reassessing age at menopause every 2 years. Women whose age at menopause could however not be determined were kept in our analyses by considering them as menopausal at age 46 if menopause was artificial, and at age 50 otherwise. Excluding those women from the analyses did not alter our results.

A "surveillance bias" is possible because hormone users are more likely to have repeated mammograms after initiation of HRT. However, these mammograms may also be less likely to aid in the diagnosis of breast cancer because of possible decreased sensitivity.[41,42] In our analyses, we chose to control for previous mammograms. This in fact had little impact on the estimates of the relative risks associated with HRT use.

As Schairer *et al.* in a study on HRT of a similar design,[9] we chose to lag exposure by 1 year, that is i) to disregard exposure during the year before the end of follow-up and ii) to consider the year following treatment initiation as a nonexposed period. This allowed us to eliminate exposure that was unlikely to be causal.

This also aimed at minimizing any "healthy screenee" bias corresponding to a lower risk during the first months of HRT use. Indeed, before initiating HRT, women usually undergo a mammogram and are therefore not likely to have breast cancer diagnosed during the following months; as expected, in our cohort, HRT users were at significantly decreased risk of breast cancer in the first year following treatment initiation, compared to nonusers. Lagging exposure by 1 year thus allowed us to take into account this minimum time for pathogenesis and detection. Lagging the exposure by 6 months instead of 1 year led to slightly diluted HRT effects estimates, without affecting our conclusions.

We used regularly updated data on HRT use during follow-up, thus diminishing "classification bias", especially for treatment duration. No cohort studies published to date have excluded women who had started using HRT before the baseline study questionnaire ("prevalent users", *i.e.*, past and current users at baseline), which generally corresponds to the start of the follow-up period. As subjects with a prevalent cancer are usually excluded, only users who have not developed breast cancer before enrollment are kept in the analyses. As a result, only "healthy" women who have already started HRT before enrollment are included in the analysis, leading to an underestimation of the breast cancer risk if breast cancers occur at increased frequency early in therapy.[43] Moreover, a "treatment length bias" is likely in these circumstances, corresponding to differential selection of cases by duration of use: women who had started HRT before enrollment and developed breast cancer shortly afterwards are likely to be excluded as prevalent cases, whereas those developing breast cancer after a longer duration of use are more likely to be included as incident cases, biasing RRs according to duration of use.

To assess the magnitude of these potential biases in our study, we ran an additional model including nonusers, and both incident (*i.e.*, those who had commenced HRT after the year preceding the start of follow-up) and prevalent (*i.e.*, those, excluded from our main analysis, who had commenced HRT before the year preceding the start of follow-up) users. We found that the global RR associated with HRT use was lower among prevalent users than among incident users. Whereas estimates associated with estrogens used alone or associated with micronized progesterone were quite similar, RRs for HRT containing synthetic progestins were lower among prevalent users than among incident users (p for heterogeneity <0.05 for estrogens combined with synthetic progestins, as well as for transdermal/percutaneous estrogens combined with synthetic progestins). Among prevalent users, all these RRs were close to unity and none reached significance. This result comforts our view of a selective inclusion of less susceptible women among prevalent users. An additional sensitivity analysis on duration of exposure showed that, as expected, this difference in magnitude between incident and prevalent users was especially marked in short term users, with estimates for exposure of less than 2 years and 2–4 years systematically lower among prevalent users than among incident users, heterogeneity between prevalent and incident users being significant among users of transdermal/percutaneous estrogens combined with oral synthetic progestins.

Our study has the best observational study design to avoid the above potential biases: analysis is based on regularly updated data on HRT use, and women who had already started HRT before the year preceding baseline are excluded. It suggests that breast cancer risk increases with increasing duration of HRT use of oral but not of transdermal/percutaneous estrogens. The sample size for long duration of use, however, is too small for any firm conclusion to be reached.

The authors of the Million Women Study underline that there may be little advantage in using estrogen-progestogen in preference to estrogen-only HRT for women who still have a uterus, given the respective effects of these 2 treatments on breast and endometrial cancer.[3] This conclusion may in fact be premature as, in our study, combinations containing micronized progesterone appeared to be associated with a significantly lower breast cancer risk than those containing synthetic progestins.

We acknowledge limited power to detect a small effect of estrogens used alone or associated with micronized progesterone on breast cancer risk in our study.

Given the major medical and public health implications of HRT use, it seems of major importance to further investigate to what extent estrogens combined with micronized progesterone are indeed associated with no or little excess in breast cancer risk. An evaluation of the impact of this association on other life-threatening diseases such as coronary heart disease, stroke or venous thromboembolic disease is also needed.

Our relatively short period of follow-up did not allow us to study the effect of HRTs on breast cancer risk by time since last use. Nor was it possible to study the impact of sequential *vs.* continuous combined therapy, as information on regimen was not recorded.

The E3N study is still continuing, with regular update of data on hormone use. It will thus be possible at a future date to assess the risks of breast cancer associated with longer HRT use and according to recency of use.

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