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EPIDEMIOLOGY

Unequal risks for breast cancer associated with different hormone replacement therapies: results from the E3N cohort study

Agnès Fournier · Franco Berrino · Françoise Clavel-Chapelon

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Abstract Large numbers of hormone replacement therapies (HRTs) are available for the treatment of menopausal symptoms. It is still unclear whether some are more deleterious than others regarding breast cancer risk. The goal of this study was to assess and compare the association between different HRTs and breast cancer risk, using data from the French E3N cohort study. Invasive breast cancer cases were identified through biennial self-administered questionnaires completed from 1990 to 2002. During follow-up (mean duration 8.1 postmenopausal years), 2,354 cases of invasive breast cancer occurred among 80,377 postmenopausal women. Compared with HRT never-use, use of estrogen alone was associated with a significant 1.29-fold increased risk (95% confidence interval 1.02–1.65). The association of estrogen–progestagen combinations with breast cancer risk varied significantly according to the type of progestagen: the relative risk was 1.00 (0.83–1.22) for estrogen–progesterone, 1.16 (0.94–1.43) for estrogen–dydrogesterone, and 1.69 (1.50–1.91) for estrogen combined with other progestagens. This latter category involves progestins with different physiologic activities (androgenic, non-androgenic, antiandrogenic), but their associations with breast cancer risk did not differ significantly from one another. This study found no evidence of an association with risk according to the route of estrogen administration (oral or transdermal/percutaneous). These findings suggest that the choice of the progestagen component in combined HRT is of importance regarding breast cancer risk; it could be preferable to use progesterone or dydrogesterone.

Keywords Breast cancer · Cohort · Dydrogesterone · Estrogen · Hormone replacement therapy · Menopause · Progestagens · Progesterone

A. Fournier · F. Clavel-Chapelon (✉)
Institut National de la Santé et de la Recherche Médicale
ERI 20, Institut Gustave Roussy, 94805 Villejuif, France
e-mail: clavel@igr.fr

F. Berrino
Department of Preventive and Predictive Medicine, Istituto Nazionale Tumori, 20138 Milan, Italy

Introduction

Estrogen–progestagen postmenopausal hormone replacement therapy (HRT) has been classified as carcinogenic to humans with respect to breast cancer, on the basis of both observational studies and randomized controlled trials [1]. However, small structural changes in progestagens may considerably alter their effects [2, 3]. Until now, most studies have evaluated estrogen associated with medroxyprogesterone acetate or 19-nortestosterone derivatives [4, 5], but other combined estrogen–progestagen therapies are used around the world and it is still unclear whether some are more hazardous than others. The relationship between estrogen-only therapy and breast cancer risk is also the subject of intense debate: unopposed estrogen use was associated with a decreased risk of breast cancer in the Women's Health Initiative (WHI) trial [6], but not in some observational studies [7–14].

Millions of women are still using HRTs, as estrogen remains the most effective treatment to alleviate menopausal symptoms [15]. It is therefore crucial to evaluate the effect of different HRTs on breast cancer risk and identify the safest preparations.

In France, estrogen, mostly estradiol administered through the skin, is used alone or combined with a variety of progestagens. Further to our earlier report (2005), in which we discussed the breast cancer risk associated with three broad categories of HRTs (estrogens alone, combined with progesterone, or with synthetic progestins) [16], we now report on the association between various other HRTs and breast cancer risk in 80,377 postmenopausal women after up to 12 years of follow-up. This longer follow-up has more than doubled the number of cases analyzed, allowing us to move towards our objective of evaluating and comparing more precisely the impact of different HRTs on breast cancer risk.

Materials and methods

The E3N cohort

E3N is a prospective cohort initiated in 1990, the aim of which is to investigate risk factors for cancer in women. At that date, half a million women, aged between 40 and 65 years, living in metropolitan France and insured by the Mutuelle Générale de l'Education Nationale (MGEN), a health insurance plan covering mostly teachers, were invited to participate. A total of 98,995 women agreed to volunteer, by filling in the first questionnaire and an informed consent form. Participants regularly completed self-administered questionnaires addressing medical history, menopausal status, and a variety of lifestyle characteristics. The first questionnaire was sent in 1990, with follow-up questionnaires in 1992, 1993, 1995, 1997, 2000 and 2002. The study was approved by the French National Commission for Data Protection and Privacy. E3N is the French component of the European Prospective Investigation into Cancer and Nutrition (EPIC) [17].

Identification of breast cancer cases

We identified most breast cancer cases from self reports in the questionnaires and a small amount from the MGEN files or information on deaths. Deaths were detected from reports by family members and by searches in the MGEN files and causes of death were obtained from the National Service on Causes of Deaths. We obtained pathology reports for 96% of the incident cases identified in the entire cohort, and we included the cases for which pathology reports were unobtainable in our analysis, as the proportion of false-positive self-reports was very low (<5%).

Identification of HRT use

Information on lifetime use of hormonal treatments was first recorded in the 1992 questionnaire. It requested the start date and duration of each episode of hormone use, together with the corresponding brand names. To help women remember what preparation they had taken, they were given a booklet listing the hormonal treatments marketed in France, complete with color photographs of the products. The information was updated for each of the subsequent questionnaires. The complete history of HRT use was established using data from all the questionnaires.

Population for analysis and follow-up

Analysis was limited to postmenopausal women. Women were considered postmenopausal if they had had 12 consecutive months without menstrual periods (unless due to hysterectomy), had undergone bilateral oophorectomy, had ever used HRT, or self-reported that they were postmenopausal. Age at menopause was defined as age at last menstrual period, at bilateral oophorectomy, at start of HRT, self-reported age at menopause, or age at start of menopausal symptoms if no other information was available. Women for whom age at menopause could not be determined (e.g., who reported a hysterectomy but gave no other information) were considered menopausal at age 47 if menopause was artificial, and at age 51 otherwise, ages which corresponded to the median ages for artificial and natural menopause in the cohort, respectively.

Follow-up started either at the date of return of the baseline questionnaire for the women who were already postmenopausal, or at the date of menopause. Subjects contributed person-time until the date of diagnosis of cancer, date of the last completed questionnaire or July 2002, whichever occurred first.

Among the postmenopausal women (n = 87,936), we excluded those who had reported a cancer other than a basal cell carcinoma before the start of follow-up (n = 5,849). We further excluded women for whom no age at first HRT use was available (n = 1,710). This left us with 80,377 postmenopausal women for analysis. They were followed for an average of 8.1 postmenopausal years (standard deviation [SD] 3.9). The last follow-up questionnaire (July 2002) was completed by 88.7% of the 80,377 women; of the 9,095 non-respondents, 892 had been diagnosed with a cancer, 866 had died, and 7,337 were lost to follow-up (of them, 3,979 had replied to the previous questionnaire).

The average age at start of follow-up was 53.1 years (SD 4.5; range 40.0–66.1 years). A total of 652,972

Table 1 Selected baseline characteristics of participants overall and according to whether or not they had used HRT as recorded at the end of follow-up

	All (n = 80,377) n (%) or mean (SD)	HRT never-users (n = 23,703) n (%) or mean (SD)	HRT never-users (n = 23,703) n (%) or mean (SD)
Year of birth			
1925–1930	6,617 (8.2%)	4,003 (16.9%)	2,614 (4.6%)
1930–1935	11,066 (13.8%)	4,601 (19.4%)	6,465 (11.4%)
1935–1940	16,377 (20.4%)	4,052 (17.1%)	12,325 (21.7%)
1940–1945	20,673 (25.7%)	3,960 (16.7%)	16,713 (29.5%)
≥1945	25,644 (31.9%)	7,087 (29.9%)	18,557 (32.7%)
Age at start of follow-up, years	53.1 (4.5)	55.0 (4.8)	52.3 (4.1)
Age at menarche, years			
<13	37,498 (46.7%)	11,116 (46.9%)	26,382 (46.6%)
≥13	42,879 (53.3%)	12,587 (53.1%)	30,292 (53.4%)
Parity			
Nulliparous	9,747 (12.1%)	3,400 (14.3%)	6,347 (11.2%)
Parous, first child before 30, 1 or 2 children	39,892 (49.6%)	10,615 (44.8%)	29,277 (51.7%)
Parous, first child before 30, 3 + children	22,594 (28.1%)	7,071 (29.8%)	15,523 (27.4%)
Parous, first child after 30	8,144 (10.1%)	2,617 (11.0%)	5,527 (9.8%)
Breastfeeding, months[a]			
Never	20,682 (29.3%)	5,711 (28.1%)	14,971 (29.7%)
<12	38,539 (54.6%)	10,178 (50.1%)	28,361 (56.4%)
≥12	3,906 (5.5%)	1,549 (7.6%)	2,357 (4.7%)
Unknown	7,503 (10.6%)	2,865 (14.1%)	4,638 (9.2%)
Age at menopause, years	50.2 (3.7)	50.7 (3.9)	50.1 (3.6)
Type of menopause			
Artificial	6,611 (8.2%)	1,831 (7.7%)	4,780 (8.4%)
Natural/Unknown	73,766 (91.8%)	21,872 (92.3%)	51,894 (91.6%)
Personal history of benign breast disease			
Yes	21,259 (26.4%)	5,561 (23.5%)	15,698 (27.7%)
No	59,118 (73.6%)	18,142 (76.5%)	40,976 (72.3%)
Family history of breast cancer in first degree relatives			
Yes	9,256 (11.5%)	2,970 (12.5%)	6,286 (11.1%)
No	71,121 (88.5%)	20,733 (87.5%)	50,388 (88.9%)
Body Mass Index, kg/m^2			
≤20	11,231 (13.4%)	2,697 (11.4%)	8,534 (15.1%)
20–25	50,912 (63.3%)	13,382 (56.5%)	37,530 (66.2%)
25–30	14,649 (18.2%)	5,730 (24.2%)	8,919 (15.7%)
>30	3,585 (4.5%)	1,894 (8.0%)	1,691 (3.0%)
Total physical activity, MET-h/wk[b]			
<34	19,536 (24.3%)	5,984 (25.2%)	13,552 (23.9%)
34–47	20,935 (26.1%)	5,868 (24.8%)	15,067 (26.6%)
47–62	19,957 (24.8%)	5,818 (24.5%)	14,139 (24.9%)
≥62	19,949 (24.8%)	6,033 (25.5%)	13,916 (24.6%)

[a] Among parous women

[b] Metabolic equivalent cost-hour/week

person-years were accumulated and 2,354 cases of invasive breast cancer were identified, 2,243 (95.3%) of which were confirmed by pathology reports.

Statistical analysis

We used the Cox proportional hazards model for left-truncated and right-censored data in the modeling of the time to postmenopausal cancer outcome, and we chose time since menopause as the time scale. Potential confounding variables included in the models are indicated in the footnotes to Table 2. Fewer than 5% of the values of the covariates were missing and were imputed with the mode or the median observed for subjects with complete data, except for duration of breastfeeding in parous women, for which a separate category for missing data was created.

HRT use was included as a time-dependent variable, and the ''healthy screenee'' bias, due to mammograms usually being performed before HRT is started, was dealt with by not considering women as exposed to HRT until 1 year following the start of treatment; from

the start of treatment and until 1 year had elapsed, they therefore contributed person-years to a separate category [18]. Separate estimates were computed for each type of HRT, defined by (i) the type of estrogen and its route of administration, and (ii) the associated progestagen molecule that was orally administered. Conjugated equine estrogens were only marginally used by women in our cohort (1.3%), so separate estimates for conjugated equine estrogens and estradiol compounds are not provided. Women who did not use the same class of HRT throughout follow-up contributed person-years to the appropriate category until they changed class; thereafter they contributed person-years to a ''mixed use'' category.

Tests for trend in duration of use were computed by adding ordinal variables corresponding to four duration of use strata (<2 years, [2–4[years, [4–6[years, 6+ years) in the models. All tests of statistical significance were two sided, and significance was set at the 0.05 level. We performed all analyses using the SAS software, version 9.1 (SAS Institute, Inc., Cary, North Carolina).

Results

Table 1 shows selected characteristics of women at start of postmenopausal follow-up, overall and according to whether or not they had used HRT as recorded at the end of follow-up. Seventy percent of women had used HRT, for a mean duration of 7.0 years (SD 5.2); the mean age at treatment start was 52.4 years (SD 4.6).

Table 2 shows relative risks of invasive breast cancer associated with the most frequently used HRTs, compared with HRT never-use. For any given route of administration of the estrogen (oral or transdermal/percutaneous), relative risks varied significantly between the different progestagens. Estrogen–progesterone and estrogen–dydrogesterone combinations were associated with no or slight and non-significant increases in risk; all the other estrogen–progestagen combinations were associated with increased risks (most of them statistically significant)—these risks did not differ significantly between preparations. For estrogen-alone or any given estrogen–progestagen combination, the route of

Table 2 Relative risks for invasive breast cancer according to route of estrogen administration and type of progestagen, compared with HRT never-use

	Oral Estrogen		Transdermal/ Percutaneous estrogen		P-values for homogeneity tests between routes of estrogen administration
	Cases/PY[a]	Relative risk[b] (95% CI)	Cases/PY[a]	Relative risk[b] (95% CI)	
Estrogen alone	13/3,598	1.32 (0.76–2.29)	56/14,826	1.28 (0.98–1.69)	0.93
Estrogen combined with:					
Progesterone		–[c]	121/35,513	1.08 (0.89–1.31)	–
Dydrogesterone	7/3,217	0.77 (0.36–1.62)	90/25,405	1.18 (0.95–1.48)	0.27
Medrogestone	9/1,104	2.74 (1.42–5.29)	28/4,590	2.03 (1.39–2.97)	0.43
Chlormadinone acetate	8/1,431	2.02 (1.00–4.06)	35/7,774	1.48 (1.05–2.09)	0.43
Cyproterone acetate	34/4,779	2.57 (1.81–3.65)		– [c]	–
Promegestone	13/2,814	1.62 (0.94–2.82)	69/14,910	1.52 (1.19–1.96)	0.84
Nomegestrol acetate	8/2,623	1.10 (0.55–2.21)	91/18,826	1.60 (1.28–2.01)	0.30
Norethisterone acetate	46/7,401	2.11 (1.56–2.86)		–[c]	–
Medroxyprogesterone acetate	29/7,035	1.48 (1.02–2.16)		–[c]	–
P-value for homogeneity among all progestagens		0.03		0.01	
P-value for homogeneity among progestagens other than progesterone and dydrogesterone		0.16		0.59	

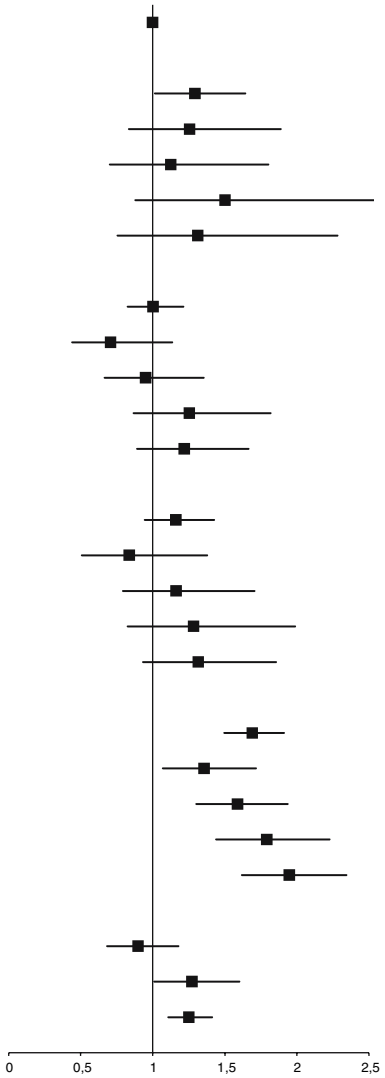
[a] PY = person-years. The numbers of cases and person-years do not add up to the totals (2,354 and 652,972, respectively) as data are only presented for the most frequently used HRTs

[b] Adjusted for: time since menopause (time scale), age at menarche (<13/≥13 years old), parity and age at first full-term pregnancy (nulliparous/first full-term pregnancy at age <30, 1 or 2 children/first full-term pregnancy at age <30, 3 or more children/first full-term pregnancy at age ≥30), breastfeeding (no/<12 months/≥12 months/unknown), age at menopause (continuous), type of menopause (artificial/natural or unknown), personal history of benign breast disease (yes/no), family history of breast cancer in first-degree relatives (yes/no), family history of breast cancer in other relatives (yes/no), BMI (≤20/[20–25]/[25–30]/>30 kg/m^2), physical activity (<34/[34–47]/[47–62]/≥62 MET-h/week), previous mammography (yes/no, time-dependant variable). Further stratified on year of birth ([1925–1930]/[1930–1935]/[1935–1940]/[1940–1945]/[1945–1950])

[c] Data are not presented as there are less than five cases in this HRT category

Table 3 Relative risks for invasive breast cancer by type of HRT and duration of exposure, compared with HRT never-use

HRT type and duration of exposure (years)	Cases/PY [a]	Relative risk [b] (95%CI)
None	**766/244,632**	**1 (ref)**
Estrogen alone	**76/20,347**	**1.29 (1.02-1.65)**
<2	24/6,747	1.26 (0.83-1.89)
[2-4[	18/5,705	1.13 (0.70-1.81)
[4-6[	14/3,172	1.50 (0.88-2.56)
6+	13/3,301	1.31 (0.76-2.28)
p for trend		0.73
Estrogen + progesterone	**129/40,537**	**1.00 (0.83-1.22)**
<2	18/8,697	0.71 (0.44-1.14)
[2-4[	33/11,647	0.95 (0.67-1.36)
[4-6[	30/7,619	1.26 (0.87-1.82)
6+	43/10,111	1.22 (0.89-1.67)
p for trend		0.04
Estrogen + dydrogesterone	**108/31,045**	**1.16 (0.94-1.43)**
<2	16/6,923	0.84 (0.51-1.38)
[2-4[	28/8,697	1.16 (0.79-1.71)
[4-6[	21/5,590	1.28 (0.83-1.99)
6+	35/7,876	1.32 (0.93-1.86)
p for trend		0.16
Estrogen + other progestagens	**527/104,243**	**1.69 (1.50-1.91)**
<2	86/22,792	1.36 (1.07-1.72)
[2-4[	134/30,189	1.59 (1.30-1.94)
[4-6[	106/19,942	1.79 (1.44-2.23)
6+	156/23,817	1.95 (1.62-2.35)
p for trend		0.01
Weak estrogens [c]	**56/17,091**	**0.90 (0.68-1.18)**
Others [d] / unknown HRT	**82/21,071**	**1.27 (1.01-1.60)**
Mixed [e]	**538/130,594**	**1.25 (1.11-1.41)**



[a] PY = person-years. There are a further 43,414 person-years (and 72 cases) in the first-year following HRT initiation. For each HRT type, the numbers of cases and person-years in the different duration of use strata do not add up to the totals because of missing information

[b] Adjusted for the same covariates as in Table 2

[c] Orally or vaginally administered promestriene or estriol

[d] Intramusculary administered estrogen or progestogen; androgen; nasally administered estrogen; transdermally administered progestagen; or tibolone

[e] Women who did not use the same class of HRT throughout follow-up contribute person-years to this ''Mixed'' category from the time they changed class

administration of the estrogen did not have a statistically significant effect on the association between HRT use and breast cancer. As a result of the above findings, we subsequently calculated separate estimates for HRTs containing progesterone or dydrogesterone, but grouped the other progestagens together. In addition, we did not distinguish between routes of estrogen administration. In what follows, ''other progestagens'' should be understood to mean ''progestagens other than progesterone and dydrogesterone''.

Relative risks of breast cancer by type of HRT and duration of exposure are shown in Table 3. Compared

with women who had never used HRT, women in the estrogen alone and estrogen–other progestagens groups had a significantly increased breast cancer risk (relative risks 1.29 (95% confidence interval 1.02–1.65), and 1.69 (1.50–1.91), respectively). Estrogen–progesterone was associated with a relative risk of 1.00 (0.83–1.22), and estrogen–dydrogesterone with a relative risk of 1.16 (0.94–1.43). Estrogen alone, estrogen–progesterone and estrogen–dydrogesterone were associated with breast cancer risks that did not differ significantly from one another, but that were all significantly lower than that of estrogen–other progestagens (P for homogeneity 0.03, <0.001, and <0.001, respectively). There were significant trends of increased risk with increased duration of use of estrogen–progesterone and estrogen–other progestagens. However, even short spells of estrogen–other progestagens use (<2 years) were associated with a significant 1.36-fold increase in breast cancer risk (Table 3).

Finally, relative risks of breast cancer were estimated by how recently the different types of HRT had been used (Table 4). Among recent users (current use or treatment stopped for less than 2 years), differences in effect estimates were still significant between estrogen–other progestagens and either estrogen alone, estrogen–progesterone, or estrogen–dydrogesterone. More than 2 years after treatment has been stopped, no significant differences were observed between effect estimates of the different HRTs, and there were no significant increased risks, except in estrogen alone users who had stopped their treatment 2–5 years previously.

When analyses were restricted to women with the most accurate age at menopause (i.e., derived from information on age at last menstrual period—unless due to hysterectomy, and/or self-reported age at menopause), our main conclusions remained unchanged. This sensitivity analysis (n = 65,083, 1,955 invasive breast cancer cases) yielded relative risks of 1.2 (0.9–1.6), 1.0 (0.8–1.2), 1.2 (0.9–1.5), and 1.6 (1.4–1.9) for estrogen alone, estrogen–progesterone, estrogen–dydrogesterone, and estrogen–other progestagens, respectively, compared with HRT never-use.

Discussion

We found that the risk of invasive breast cancer was significantly lower with estrogen–progestagen HRTs containing progesterone or dydrogesterone than with HRTs containing other progestagens. The latter group involved a variety of progestins whose associations with breast cancer risk did not differ significantly from one another. We also observed a significantly increased risk of breast cancer with the use of estrogen alone.

The effect of progestagens on breast tissue is complex and not completely understood. The mechanisms by which they act on cell proliferation include interaction with steroid receptors, growth factors and oncogenes, and with the cell-cycle and estrogen metabolizing enzymes [3]. Because progestagens differ widely in their chemical structure, metabolism, pharmacokinetics and potency, it is reasonable to expect them to induce different responses in the breast [2]. However, the effects of progestagens generally differ according to the experimental conditions, the duration of treatment and the dose concentration [3, 19]. As a result it is impossible to establish, on the basis of the available and often conflicting in vitro data, whether the predominant effect of a given progestagen is to stimulate or inhibit breast cell proliferation. This complicated and unresolved situation makes the results of real life studies like ours particularly interesting.

Our study is the first epidemiological study conducted on women that we know of, that evaluated the association of the estrogen–progesterone and estrogen–dydrogesterone combinations with breast cancer risk. A major finding is that these combinations may be

Table 4 Relative risks for invasive breast cancer by type of HRT and recency of use, compared with HRT never-use

	Last use [0–2[years previously		Last use [2–5[years previously		Last use ≥5 years previously	
	Cases/PY [a]	Relative risk [b] (95% CI)	Cases/PY [a]	Relative risk [b] (95% CI)	Cases/PY [a]	Relative risk [b] (95% CI)
Estrogen alone	47/13,834	1.22 (0.90–1.65)	8/1,312	2.10 (1.04–4.21)	14/3,780	1.17 (0.69–1.99)
Estrogen combined with:						
Progesterone	115/35,804	1.03 (0.84–1.26)	9/1,369	1.93 (0.99–3.72)	0/902	–
Dydrogesterone	96/26,910	1.22 (0.98–1.52)	3/1,219	0.78 (0.25–2.44)	1/956	0.28 (0.04–1.97)
Other progestagens	461/90,478	1.75 (1.54–1.99)	13/3,720	1.07 (0.62–1.86)	8/2,542	0.85 (0.42–1.70)

[a] PY = person-years. For each HRT type, the numbers of cases and person-years in the different recency of use strata do not add up to the totals (cf. Table 3) because of missing information

[b] Adjusted for the same covariates as in Table 2

safer than others. Studies of the effect of progesterone on breast cells have demonstrated that the hormone can exert either growth-promoting, neutral, or antiproliferative effects on the breast tissue [20, 21]. Recently, Wood et al. [22] compared the effects of estradiol given with either medroxyprogesterone acetate or micronized progesterone on risk biomarkers for breast cancer in a postmenopausal primate model. In this randomized crossover trial, they found that, compared to placebo, estradiol + medroxyprogesterone acetate resulted in significantly greater proliferation (as measured by Ki67 expression) in lobular and ductal breast epithelium, while estradiol + micronized progesterone did not. This result supports our findings suggesting that, when combined with an estrogen, progesterone may have a safer risk profile in the breast compared with some other progestagens. The association of estrogen–dydrogesterone combinations with a nonsignificantly elevated relative risk in our study reinforces the plausibility of our finding since the retroprogesterone dydrogesterone is the progestin with the chemical structure and pharmacological effects closest to those of progesterone.

The high degree of androgenicity of progestins used in certain HRTs has been hypothesized to play a role in the increased risk of breast cancer [5]. Our results do not support this hypothesis, as, when combined with an estrogen, neither promegestone, nomegestrol acetate, chlormadinone acetate or medrogestone (all nonandrogenic progestagens) nor cyproterone acetate (an antiandrogenic progestagen) had effects that differed significantly from that of norethisterone acetate (the most androgenic progestagen cited). These results are in line with those of two other European studies [10, 11], which found no difference between the effect of 19-nortestosterone derivatives and medroxyprogesterone acetate (a 17-hydroxyprogesterone derivative with lower androgenic potential than 19-nortestosterone derivatives), implying that other parameters must be involved. However, possible preferential prescribing of the nonandrogenic or antiandrogenic HRTs to women with signs of insulin resistance or hyperandrogenism, who are at higher risk of breast cancer [23], could partly explain our findings.

In our study, estrogen alone was associated with a significantly lower increase in breast cancer risk than estrogen opposed with a progestagen (with the exception of progesterone or dydrogesterone), in line with the growing evidence that adding certain progestins to estrogen has an adverse impact on breast cancer risk [24]. However, our finding of a 1.3-fold increased breast cancer risk associated with the use of estrogen alone (almost exclusively estradiol compounds, and mostly administered through the skin) differs with that of the WHI estrogen-alone trial which found a decreased risk when oral conjugated equine estrogens were used in a population of older and often overweight women [6].

We had limited power to examine the effect of HRTs among past users as most women were still using HRT at the end of follow-up. However, our results are compatible with those of previous studies suggesting that the excess in risk associated with HRT use diminishes after treatment stop [4, 7, 10].

The major strengths of our study are the range of HRTs evaluated and the fact that exposure was regularly updated during follow-up. This allowed us to (i) isolate the effects of each type of HRT, taking into account changes from one treatment to another by creating a separate ‘‘mixed’’ use category, and (ii) avoid the misclassification of users and nonusers, duration, or recency of use that can occur in prospective studies with a single baseline assessment of exposure.

Our results would have been only slightly changed if we had restricted our analyses to women who had not been using HRT before the baseline questionnaire (‘‘incident users’’). (We restricted analysis in this way in our previous study [16], to avoid potential biases described by Ray [25]).

Analyses were adjusted for various potential confounders, and participants in the E3N cohort belong to a homogeneous occupational group (the great majority being teachers or teacher’s wives). This decreased the probability that the differences we found on risk between different estrogen–progestagen combinations are explained by confounding; in addition, there was no marked difference between users of the different types of estrogen–progestagen combinations regarding classical breast cancer risk factors, and stratified analyses yielded relative risks that were quite stable whatever the characteristics of the women (data not shown).

We were aware of the possibility of differential recall by HRT users and nonusers. We therefore ran a sensitivity analysis where exposure was included in the models in a prospective manner (i.e., using only the information on exposure reported in questionnaire i for the follow-up period between questionnaire i and questionnaire i + 1). Relative risks obtained with this sensitivity analysis were not below those obtained with our main analysis, showing that differential recall bias was unlikely to have occurred.

Analyses were controlled for previous mammograms, but a detection bias remains possible as women who use HRT have mammograms more frequently

than nonusers. However, there is no reason why this bias should have been less marked for estrogen–progesterone or estrogen–dydrogesterone than for estrogen–other progestagens combinations. Of concern is the possibility that different estrogen–progestagen HRTs may influence breast density and hence alter mammographic sensitivity in a different way. However, in the PEPI trial, Greendale et al. found that, over 12 months, the adjusted absolute mean changes in mammographic percent density did not differ significantly between conjugated equine estrogens plus cyclic medroxyprogesterone acetate and with conjugated equine estrogens plus cyclic micronized progesterone [26].

Nondifferential misclassification of HRT exposure, which was based on self-reported information, may have affected our results, most likely by diluting the magnitude of the relationship between HRTs and breast cancer risk, and reducing any real differences in the effects of different HRTs.

E3N is the first epidemiological study that we know of to be providing results indicating that estrogen–progesterone and estrogen–dydrogesterone combinations may be the least harmful estrogen–progestagen HRTs regarding breast cancer risk. However, more evidence is required before these results can be translated into firm clinical recommendations for the management of menopausal symptoms. In addition, the effect of these combinations in other diseases (e.g., coronary heart disease, venous thromboembolism and colorectal cancer) has also to be evaluated. We therefore encourage further studies and reflection on the links between estrogen–progesterone and estrogen–dydrogesterone HRTs and breast cancer.

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