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Abstract

Purpose

We previously found that the risk of invasive breast cancer varied according to the progestagen component of combined postmenopausal hormone therapy (CHT): progesterone, dydrogesterone, or other progestagens. We conducted the present study to assess how these CHTs were associated with histology- and hormone receptor-defined breast cancer.

Patients and Methods

We used data from the French E3N cohort study, with 80,391 postmenopausal women followed for a mean duration of 8.1 years; 2,265 histologically confirmed invasive breast cancers were identified through biennial self-administered questionnaires completed from 1990 to 2002. The relative risks (RRs) were estimated using Cox proportional hazards models.

Results

Compared with postmenopausal hormone therapy (HT) never-use, ever-use of estrogen+progesterone was not significantly associated with the risk of any breast cancer subtype, but increasing duration of estrogen+progesterone was associated with increasing risks of lobular (P=.06) and estrogen receptor–positive/progesterone receptor–negative (ER+/PR-; P=.02). Estrogen+dydrogesterone was associated with a significant increase in risk of lobular carcinoma (RR, 1.7; 95% CI, 1.1 to 2.6). Estrogen+other progestagens was associated with significant increases in risk of ductal and lobular carcinomas (RR, 1.6; 95% CI, 1.3 to 1.8; and 2.0; 95% CI, 1.5 to 2.7, respectively), of ER+/PR+ and ER+/PR- carcinomas (RR, 1.8; 95% CI, 1.5 to 2.1; and 2.6; 95% CI, 1.9 to 3.5, respectively), but not of ER-/PR+ or ER-/PR- carcinomas (RR, 1.0; 95% CI, 0.5 to 2.1; and 1.4; 95% CI, 0.9 to 2.0, respectively).

Conclusion

The increase in risk of breast cancer observed with the use of CHTs other than estrogen+progesterone and estrogen+dydrogesterone seems to apply preferentially to ER+ carcinomas, especially those ER+/PR-, and to affect both ductal and lobular carcinomas.

Introduction

The relationship between postmenopausal hormone therapy (HT) use and breast cancer risk has been investigated in many epidemiological studies whose results have led to the conclusion that estrogen-progestagen menopausal treatments (combined HTs [CHTs]) are carcinogenic to the human breast.[1] However, first, breast cancer is not a single entity, and it has been suggested that tumors with different histological or hormone receptor (estrogen receptor/progesterone receptor [ER/PR]) profiles are etiologically distinct.[2-4] Second, CHT is also not a single entity, since various doses, routes of administration, regimens and molecules used throughout the world may differentially affect breast cancer risk.[5,6]

The mechanisms underlying the link observed between use of some HTs and breast cancer risk are not clear. Knowing how different HTs affect the risk of different types of breast cancer would provide a useful insight into the mechanisms by whichHTs act in the carcinogenic process.

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The particular interest in studying the relation between HT use and the risk of different histological types of breast cancer was stimulated by the observations that, in the United States, as the number of CHT users steadily increased, there was a marked increase in lobular but not ductal breast cancer incidence in women older than 50 years.[7] Later ecological studies from the United States and Switzerland confirmed these observations.[8-11] Improved diagnostic techniques, increased diagnostic activity, and changes in criteria for pathological classification of lobular, ductal, as well as mixed ductal-lobular carcinomas may have played a role. However, epidemiological studies showed that CHT was associated with more elevated relative risks for lobular than for ductal cancer,[3,12-24] with only two exceptions.[19,20]

Studies of the relationship between HT use and the risk of different receptor-defined breast cancers began to be carried out earlier[13,14,18-20,24-36] with the assumption that if HTs act through hormonal mechanisms, they should differentially affect the risk of cancers with different hormone receptor profiles. However, their results have been mixed.[37]

In an earlier report we examined the relationship between different types of HT and breast cancer risk, considered as a single disease, in the French E3N cohort.[6] We found that the risk was significantly lower with CHTs containing progesterone or dydrogesterone rather than other progestagens.We also observed a significant increase in risk with unopposed estrogens. We now examine whether the associations of these four types of HTs with breast cancer risk vary across different types of carcinomas, characterized by histological type and hormone receptor status.

Patients and Methods

The E3N Cohort

E3N is a prospective cohort initiated in 1990 that consists of 98,995 French women born between 1925 and 1950 and insured by a health insurance plan covering mostly teachers. Participants, who gave written informed consent, completed biennial self-administered questionnaires addressing medical history, menopausal status, and a variety of lifestyle characteristics. 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.[38]

Identification of Breast Cancer Cases

Occurrence of cancer was self-reported, and a small number of cases were further identified from the insurance files or information on deaths. Pathology reports were obtained for 96% of the identified incident cases. Information on ER and PR status and histological type was extracted from these reports, and the invasive breast cancer cases were classified by histological type into ductal, lobular (including mixed ductal-lobular), or other; and by receptor status into ER+/ PR+, ER+/PR-, ER-/PR+, ER-/PR-, or missing.

Identification of HT Use

Information on lifetime use of hormonal treatments was first recorded in the 1992 questionnaire. It requested the start date, brand names, and duration of each episode of hormone use. Women were given a booklet with color photographs listing the HTs marketed in France. The information was updated in each of the subsequent questionnaires sent in 1993, 1995, 1997, 2000, and 2002. The complete history of HT use was established using data from all the questionnaires. Unopposed HT consisted almost exclusively in estradiol compounds (1.3% of women ever-used conjugated equine estrogens). CHTs were classified as estrogen+progesterone, estrogen+ dydrogesterone, or estrogen+other progestagens, following our previous finding that associations with breast cancer risk varied significantly across these different treatments.[6]

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 HT, or self-reported that they were postmenopausal. Age at menopause was defined as age at last menstrual period (unless due to hysterectomy and if the last menstrual period occurred before HT use); age at bilateral oophorectomy; or, in decreasing order of priority, self-reported age at menopause, age at start of HT, age at start of menopausal symptoms; or, if no information was available, age 47

years if menopause was artificial, and age 51 years 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. Participants contributed person-years of follow-up until the date of cancer diagnosis, the 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), and women for whom no age at first HT use was available (n = 1,696). This left us with 80,391 women for analysis.

Statistical Analysis
Relative risks (RRs) for breast cancer were estimated using Cox proportional hazards models, with time since menopause as the time scale. For each specific type of breast cancer, separate models were used, and cases with an invasive cancer other than that under study were censored at the date of diagnosis. Cases with missing information on histological type or hormone receptor status were excluded from the corresponding analyses. Potential confounding variables included in the models are indicated in the footnotes of the tables. When fewer than 5% of the values of a covariate were missing, they were replaced with the mode or the median values observed among the subjects with complete data.
HT use was included as a time-dependent variable, and the "healthy screenee" bias (due to mammograms usually being performed before HT is started) was dealt with by not considering women as exposed to HT until 1 year following the start of treatment; from the start of treatment and until one year had elapsed, they contributed person-years of follow-up to a separate category.[39] Women who changed HT during follow-up contributed person-years to the appropriate category until they changed, and thereafter to a "mixed use" category. Tests of homogeneity in the effect of a given HT on the risk of different types of breast cancer were based on Wald χ^2 statistics.[40] All tests of statistical significance were two-sided, and significance was set at the .05 level. We performed all analyses using SAS software, version 9.1 (SAS Institute Inc, Cary, NC).

Results
The average age at start of follow-up was 53.1 years (standard deviation [SD], 4.5; range, 40.0 to 66.1 years). A total of 2,355 primary invasive breast cancers were diagnosed during 653,125 person-years of follow-up (mean duration: 8.1 years; SD, 3.9). Those confirmed by pathology reports (n = 2,265) were included in the analyses of the risk of histology-defined cancer. Among them, 473 (20.9%) had missing information on combined ER and PR status, so that 1,792 cases were included in the analyses of the risk of receptor-defined breast cancer. Table 1 presents the distribution of joint ER and PR status and histological types of the cases.

Table 1. **Distribution of Histologic Types and Hormone Receptors Found in Invasive Breast Cancers: E3N Study 1990-2002**

Hormone Receptors	Ductal (n = 1,560)		Lobular (n = 448)		Other (n = 257)		All Histologies (n = 2,265)	
	No.	%*	No.	%*	No.	%*	No.	%*
ER+/PR+	727	58.3	230	63.9	97	52.2	1054	58.8
ER+/PR-	250	20.1	78	21.7	44	23.7	372	20.8
ER-/PR+	43	3.5	15	4.2	6	3.2	64	3.6
ER-/PR-	226	18.1	37	10.3	39	21.0	302	16.9
Unknown	314		88		71		473	

Abbreviations: ER, estrogen receptor; PR, progesterone receptor.
*Among cases with a known joint ER/PR status.

The main characteristics of the women included in the analysis, overall and according to HT use, are shown in Table 2.
At the end of follow-up, few women were past users of HT, so we chose to group past and current users together after verifying that results did not change substantially when studying current users specifically (data not shown).

Table 2. **Selected Baseline Characteristics of Participants Overall and According to Whether or Not They Had Used Postmenopausal Hormone Therapy As Recorded at the End of Follow-Up: E3N Study 1990-2002**

Characteristic	All (n = 80,391)		HT Never-Users (n = 23,725)		HT Ever-Users (n = 56,666)	
	No.	%	No.	%	No.	%
Year of birth						
[1925-1930]	6,617	8.2	4,004	16.9	2,613	4.6
[1930-1935]	11,071	13.8	4,603	19.4	6,468	11.4
[1935-1940]	16,383	20.4	4,057	17.1	12,326	21.8
[1940-1945]	20,675	25.7	3,964	16.7	16,711	29.5
≥ 1945	25,645	31.9	7,097	29.9	18,548	32.7
Age at start of follow-up, years						
Mean	53.1		55.0		52.3	
Standard deviation	4.5		4.8		4.1	
Age at menarche, years						
< 13	37,502	46.7	11,128	46.9	26,374	46.5
≥ 13	42,889	53.3	12,597	53.1	30,292	53.5
Parity						
Nulliparous	9,748	12.1	3,404	14.3	6,344	11.2
Parous, first child before 30, 1 or 2 children	39,897	49.6	10,623	44.8	29,274	51.7
Parous, first child before 30, 3+ children	22,600	28.1	7,077	29.8	15,523	27.4
Parous, first child after 30	8,146	10.1	2,621	11.0	5,525	9.8
*Breastfeeding, months**						
Never	20,686	29.3	5,718	28.1	14,968	29.7
< 12	38,548	54.6	10,189	50.1	28,359	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						
Mean	50.2		50.7		50.1	
Standard deviation	3.7		3.9		3.6	
Type of menopause						
Artificial	6,612	8.2	1,832	7.7	4,780	8.4
Natural/unknown	73,779	91.8	21,893	92.3	51,886	91.6
Personal history of benign breast disease						
Yes	21,264	26.4	5,571	23.5	15,693	27.7
No	59,127	73.6	18,154	76.5	40,973	72.3
Family history of breast cancer in first degree relatives						
Yes	9,260	11.5	2,973	12.5	6,287	11.1
No	71,131	88.5	20,752	87.5	50,379	88.9
Body Mass Index, kg/m^2						
≤ 20	11,242	13.4	2,703	11.4	8,539	15.1
20-25	50,895	63.3	13,385	56.4	37,510	66.2
25-30	14,665	18.2	5,741	24.2	8,924	15.7
> 30	3,589	4.5	1,896	8.0	1,693	3.0
Total physical activity, MET-h/wk						
< 34	19,540	24.3	5,987	25.2	13,553	23.9
34-47	20,940	26.1	5,875	24.8	15,065	26.6
47-62	19,960	24.8	5,825	24.5	14,135	24.9
≥ 62	19,951	24.8	6,038	25.5	13,913	24.6
Previous use of oral contraceptives						
Yes	46,705	58.1	10,677	45.0	36,028	63.6
No	33,686	41.9	13,048	55.0	20,638	36.4
Use of oral progestagens alone in premenopause						
Yes	31,018	38.6	5,918	24.9	25,100	44.3
No	49,373	61.4	17,812	75.1	31,566	55.7

Abbreviations: MET-h/wk, metabolic equivalent cost-hour/week; HT, postmenopausal hormone therapy.

*Among parous women.

The RRs of invasive breast cancer associated with HT ever-use did not vary significantly according to histological type (Table 3). Lobular breast cancer risk was significantly increased in women in the estrogen+dydrogesterone and estrogen+other progestagens groups, and the risk of ductal carcinoma was significantly increased in women in the estrogen+other progestagens group. When analyses were conducted separately for pure lobular and mixed ductal-lobular carcinomas, risks associated with HT use were still stronger for pure lobular than for ductal carcinomas, and even stronger for mixed ductal-lobular carcinomas (Table 3).

Table 3. **Relative Risks of Histology-Defined Breast Cancers for HT Ever-Use Compared With HT Never Use: E3N Study 1990-2002**

Type of HT	Ductal (n = 1,560)			Lobular (n = 448)			*P*†	Pure Lobular (n = 387)			Mixed Ductal/Lobular (n = 61)		
	No. of Cases	Relative Risk*	95% CI	No. of Cases	Relative Risk*	95% CI		No. of Cases	Relative Risk*	95% CI	No. of Cases	Relative Risk*	95% CI
Any HT	998	1.3	1.1 to 1.4	317	1.5	1.2 to 1.9	.20	268	1.4	1.1 to 1.8	49	2.7	1.2 to 5.9
Estrogen alone	52	1.3	0.9 to 1.7	12	1.2	0.7 to 2.3	.89	10	1.1	0.6 to 2.1	2	3.0	0.6 to 14
Estrogen+progesterone	87	1.0	0.8 to 1.3	24	1.1	0.7 to 1.7	.78	22	1.1	0.7 to 1.8	2	1.0	0.2 to 5.0
Estrogen+dydrogesterone	70	1.1	0.9 to 1.4	28	1.7	1.1 to 2.6	.09	23	1.6	0.9 to 2.5	5	3.8	1.2 to 12
Estrogen+other progestagens	334	1.6	1.3 to 1.8	113	2.0	1.5 to 2.7	.11	95	1.9	1.4 to 2.6	18	3.9	1.6 to 9.5
Others/unknown‡	101	1.2	0.9 to 1.5	25	1.1	0.7 to 1.8	.80	22	1.1	0.7 to 1.8	3	1.6	0.4 to 6.0
Mixed use§	354	1.2	1.0 to 1.4	115	1.5	1.1 to 2.0	.24	96	1.4	1.0 to 1.9	19	2.7	1.1 to 6.5
P‖			<.001			.02				.07			.36

Abbreviation: HT, postmenopausal hormone therapy.

*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), height (continuous), 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), geographic area at baseline, period of time (before 1994/1994-1996/1997 or later), previous use of oral contraceptives (yes/no), use of oral progestagens alone in premenopause (yes/no). Further stratified on year of birth ([1925-1930[/[1930-1935[/[1935-1940[/[1940-1945[/[1945-1950]).

†*P* value for assessing homogeneity in relative risks of ductal and lobular subtypes of invasive breast cancer.

‡Orally or vaginally administered promestriene or estriol; intramuscularly administered estrogen or progestogen; androgen; nasally administered estrogen; transdermally administered progestagen; tibolone.

§Women who did not use the same class of HT throughout follow-up contributed person-years to this "Mixed" category from the time they changed class

‖*P* value for assessing homogeneity in relative risks associated with estrogen alone, estrogen+progesterone, estrogen+dydrogesterone, and estrogen+other progestagens.

We observed a trend of borderline significance of increased risk of ductal carcinomas with increased duration of estrogen+other progestagens use (Table 4). For lobular carcinomas, the same was observed with estrogen+progesterone. For any given duration of HT use, there was no significant difference in the association of each HT with the risk of ductal and lobular carcinomas, except for estrogen+dydrogesterone used for 5 or more years ($P = .05$).

No significant increases in risk of receptor-defined breast cancers were observed for women in the estrogen+progesterone or estrogen+dydrogesterone groups (Table 5). There were significant variations ($P = .02$) in the association of estrogen+other progestagens with different receptor-defined carcinomas; the RR of ER+/PR-was significantly higher than that of the other breast cancer types. Use of estrogen alone was associated with a significant increase in risk of ER+/PR+ breast cancer and with a nonsignificant increase in risk of ER+/PR- breast cancer (Table 5); the RR of ER+ breast cancer was 1.4; 95% CI, 1.1 to 2.0 (data not shown). No increase in risk of ER-/PR+ carcinoma was seen for any type of HT, but the numbers were small.

We investigated whether the risk of receptor-defined breast cancers increased with increasing duration of use of the different HTs (Table 6). We observed a significant trend for the risk of ER+/PR- carcinomas in estrogen+progesterone users.

Finally, as lobular tumors are more likely to be hormone receptor-positive than ductal tumors[41] (which was also observed in the present study, as presented in Table 1), we investigated the associations between HT use and breast cancer risk according to combined histological type and hormone receptor status. The increases in risk observed in the estrogen+other progestagens group were still more pronounced for lobular than ductal breast cancer, if they were ER+ (and whatever the PR status); there were no apparent differences among ER- breast cancers between ductal and lobular histological types, but the numbers of cases were small (data not shown).

As age at menopause may be an important confounder in the analyses of the relationship between postmenopausal HT use and breast cancer risk,[42,43] we performed a sensitivity analysis restricted to women with the most precise age at menopause (ie, derived from information on age at last menstrual period, and/or self-reported age at menopause, n = 65,096). Our conclusions remained unchanged except that the differences

between ductal and lobular breast cancer risks appeared more marked and reached statistical significance for estrogen+dydrogesterone (P = .03) and estrogen+other progestagens (P = .02).

Table 4. **Relative Risks of Histology-Defined Breast Cancers for HT Ever-Use Compared With HT Never-Use, According to Duration of Use: E3N Study 1990-2002**

Type of HT	Ductal (n = 1,560)			Lobular (n = 448)			*P*‡
	No. of Cases*	Relative Risk†	95% CI	No. of Cases*	Relative Risk†	95% CI	
Any HT							
< 5 years	411	1.2	1.0 to 1.4	126	1.4	1.1 to 1.9	.24
5+ years	433	1.4	1.2 to 1.6	145	1.7	1.3 to 2.3	.24
P for trend§		.02			.20		
Estrogen alone							
< 5 years	38	1.4	0.9 to 1.9	7	1.1	0.5 to 2.3	.57
5+ years	8	0.9	0.4 to 1.7	5	2.1	0.8 to 5.1	.13
P for trend§		.23			.26		
Estrogen+progesterone							
< 5 years	45	0.9	0.7 to 1.2	9	0.7	0.4 to 1.5	.58
5+ years	38	1.2	0.9 to 1.7	14	1.7	0.9 to 3.0	.35
P for trend§		.17			.06		
Estrogen+dydrogesterone							
< 5 years	39	1.1	0.8 to 1.5	13	1.5	0.8 to 2.7	.21
5+ years	26	1.1	0.8 to 1.7	13	2.1	1.2 to 3.8	.05
P for trend§		.93			.39		
Estrogen+other progestagens							
< 5 years	176	1.4	1.2 to 1.7	62	2.0	1.4 to 2.8	.08
5+ years	130	1.8	1.5 to 2.2	45	2.3	1.5 to 3.3	.31
P for trend§		.06			.59		

Abbreviation: HT, postmenopausal hormone therapy.

*For each HT type, the numbers of cases in the different duration of use strata do not add up to the totals (as presented in Table 3) because of missing information.

†Adjusted for the same covariates as in Table 3.

‡*P* value for assessing homogeneity in relative risks of ductal and lobular subtypes of invasive breast cancer.

§*P* value for assessing homogeneity in relative risks associated with less than 5 years and 5+ years of HT use.

Table 5. **Relative Risks of Receptor-Defined Breast Cancers for HT Ever-Use Compared With HT Never-Use: E3N Study 1990-2002**

Type of HT	ER+/PR+ (n = 1,054)			ER+/PR- (n = 372)			ER-/PR+ (n = 64)			ER-/PR- (n = 302)			*P*†
	No. of Cases	Relative Risk*	95% CI	No. of Cases	Relative Risk*	95% CI	No. of Cases	Relative Risk*	95% CI	No. of Cases	Relative Risk*	95% CI	
Any HT	711	1.4	1.2 to 1.6	262	1.7	1.3 to 2.2	35	0.9	0.5 to 1.6	193	1.2	0.9 to 1.5	.11
Estrogen alone	38	1.5	1.0 to 2.1	10	1.4	0.7 to 2.7	1	0.5	0.1 to 3.8	13	1.6	0.9 to 2.8	.77
Estrogen+progesterone	65	1.2	0.9 to 1.5	14	0.8	0.5 to 1.5	4	0.9	0.3 to 2.6	18	1.0	0.6 to 1.7	.73
Estrogen+dydrogesterone	47	1.2	0.9 to 1.6	15	1.3	0.7 to 2.2	3	0.9	0.3 to 3.0	19	1.4	0.8 to 2.3	.90
Estrogen+other progestagens	237	1.8	1.5 to 2.1	107	2.6	1.9 to 3.5	12	1.0	0.5 to 2.1	66	1.4	0.9 to 2.0	.02
Others/unknown‡	60	1.1	0.8 to 1.5	26	1.6	1.0 to 2.5	2	0.6	0.1 to 2.7	16	0.9	0.6 to 1.6	.37
Mixed use§	264	1.4	1.1 to 1.6	90	1.6	1.1 to 2.2	13	1.0	0.5 to 2.1	61	1.0	0.7 to 1.4	.23
P‖		.005			<.0001			.93			.56		

Abbreviations: HT, postmenopausal hormone therapy; ER, estrogen receptor; PR, progesterone receptor.

*Adjusted for the same covariates as in Table 3.

†*P* value for assessing homogeneity in relative risks of ER+/PR+, ER+/PR-, ER-/PR+ and ER-/PR- invasive breast cancers.

‡Orally or vaginally administered promestriene or estriol; intramuscularly administered estrogen or progestogen; androgen; nasally administered estrogen; transdermally administered progestagen; tibolone.

§Women who did not use the same class of HT throughout follow-up contributed person-years to this "Mixed" category from the time they changed class.

‖*P* value for assessing homogeneity in relative risks associated with estrogen alone, estrogen+progesterone, estrogen+dydrogesterone, and estrogen+other progestagens.

Table 6. **Relative Risks of Receptor-Defined Breast Cancers for HT Ever-Use Compared With HT Never-Use, According to Duration of Use: E3N Study 1990-2002**

	ER+/PR+ (n = 1,054)			ER+/PR- (n = 372)			ER-/PR- (n = 302)			
Type of HT	**No. of Cases***	**Relative Risk†**	**95% CI**	**No. of Cases***	**Relative Risk†**	**95% CI**	**No. of Cases***	**Relative Risk†**	**95% CI**	***P*‡**
Any HT										
< 5 years	269	1.3	1.1 to 1.5	96	1.4	1.0 to 2.0	85	1.1	0.8 to 1.5	.11
5 + years	345	1.6	1.4 to 1.9	125	2.1	1.5 to 2.8	70	1.1	0.8 to 1.6	.11
P for trend§			.01			.03			.72	
Estrogen alone										
< 5 years	23	1.3	0.9 to 2.0	7	1.4	0.7 to 3.2	10	1.7	0.9 to 3.2	.81
5 + years	11	1.6	0.9 to 3.0	3	1.8	0.6 to 5.8	2	1.1	0.3 to 4.6	.96
P for trend§			.54			.74			.60	
Estrogen+progesterone										
< 5 years	33	1.1	0.7 to 1.5	4	0.4	0.2 to 1.2	11	1.0	0.5 to 1.9	.28
5 + years	29	1.4	0.9 to 2.0	10	1.6	0.8 to 3.2	5	0.8	0.3 to 2.1	.55
P for trend§			.35			.02			.75	
Estrogen+dydrogesterone										
< 5 years	22	1.1	0.7 to 1.6	8	1.3	0.6 to 2.7	8	1.0	0.5 to 2.1	.95
5 + years	25	1.6	1.0 to 2.4	5	1.1	0.4 to 2.6	7	1.5	0.7 to 3.4	.86
P for trend§			.18			.72			.41	
Estrogen+other progestagens										
< 5 years	116	1.6	1.3 to 2.0	52	2.2	1.5 to 3.3	35	1.2	0.8 to 1.8	.05
5 + years	101	2.0	1.6 to 2.6	46	3.2	2.2 to 4.7	26	1.8	1.1 to 2.9	.08
P for trend§			.11			.12			.15	

Abbreviations: HT, postmenopausal hormone therapy; ER, estrogen receptor; PR, progesterone receptor.
NOTE. Estimates for ER-/PR+ breast carcinoma are not shown because the number of cases in duration strata were too small to allow any interpretation.
*For each HT type, the numbers of cases in the different duration of use strata do not add up to the totals (as presented in Table 5) because of missing information.
†Adjusted for the same covariates as in Table 3.
‡*P* value for assessing homogeneity in relative risks of ER+/PR+, ER+/PR-, ER-/PR+ and ER-/PR- invasive breast cancers.
§*P* value for assessing homogeneity in relative risks associated with less than 5 years and 5+years of HT use.

Discussion

We previously reported that the risk of invasive breast cancer, considered as a single disease, was significantly lower among users of estrogen+progesterone or users of estrogen+dydrogesterone than in users of estrogen+other progestagens.[6] In the present analysis, the use of estrogen+progesterone was not significantly associated with the risk of any breast cancer subtype, though we found trends of increasing risks with increasing duration of use for lobular and ER+/PR- carcinomas. The RR associated with estrogen+dydrogesterone was significantly above one for lobular breast cancer. Use of estrogen+other progestagens was associated with increases in risk of both ductal and lobular carcinomas, and of ER+/PR+ and ER+/PR- carcinomas.

Widespread use of progesterone is a French peculiarity.[44] In our analyses, the "other progestagens" category encompasses a variety of progestins, the most used being promegestone and nomegestrol acetate.[6]

Progestagens may act on breast tissue through their interactions with steroid receptors, growth factors, and oncogenes, and with the cell-cycle and estrogen-metabolizing enzymes.[45] Because they differ in their chemical structure, metabolism, pharmacokinetics, and potency, it is reasonable to expect them to induce different responses in the breast.[46] However, in vitro data are conflicting, possibly because of variations in the experimental conditions.[45,47] Therefore, in vivo studies are of particular interest. Some studies found that the proliferation of breast epithelium increased during the luteal phase of the menstrual cycle.[48,49] However, in vivo, progesterone has been found to oppose the proliferative effects of estradiol on breast tissue of pre- and postmenopausal women.[50,51] The contrary has been found for medroxyprogesterone acetate (MPA) in postmenopausal women[52] or surgically postmenopausal macaques.[53] In such a study on macaques, compared to placebo, estradiol+MPA resulted in significantly greater proliferation in lobular and ductal breast epithelium, while estradiol+micronized progesterone did not.[54] These studies support our findings suggesting that, when combined with an estrogen, progesterone may have a safer risk profile in the breast than some other progestagens. Our results regarding estrogen+dydrogesterone combinations are also plausible since the retroprogesterone dydrogesterone is the progestin with the chemical structure and pharmacologic effects closest to those of progesterone.

There is a strong suggestion in the literature that CHTs are more markedly related to risk of lobular than ductal carcinoma.[23] Our results do not contradict this observation, which is biologically plausible, as studies on PR-knockedout mice suggest that progesterone induces lobuloalveolar development, whereas estradiol stimulates ductal elongation and PR expression.[55] The lack of significant difference between ductal and lobular breast cancer risk in the estrogen+progesterone category may be due to a lack of statistical power.

Our findings that some CHTs primarily increase ER+ breast cancer risk is consistent with that of other epidemiological studies, [13,18,20,30,33] with two exceptions.[24,36] In the Women's Health Initiative trial, the increase in risk in the CHT group did not appear to be limited to ER+ breast cancer,[19] but the number of cases was quite small. Recently, parallel to the drop in HT use, incidence of breast cancer decreased in the United States in women who were 50 years of age or older; this decrease was confined to ER+cancers.[56] In human breast ER+ tumorigenesis, estrogens directly drive cell proliferation.[57] Biologic and epidemiological data therefore suggest that some HTs exert direct and rapid hormonal effects on pre-existing ER+ breast cancers; this does not exclude that there may be a longer-term impact on ER- tumors. In our study, the low number of ER- tumors may have limited our power to detect moderate increases in ER- breast cancer risk.

We found that the use of some CHTs was more markedly associated with the risk of ER+/PR- than with the risk of ER+/PR+ carcinomas. However, in other studies that have investigated the relationship between CHTs and different receptor-defined breast cancers, two found increases in risk that tended to be more marked for ER+/PR+ than for ER+/PR- carcinomas,[18,20] and one found comparable increases in risk for both types of carcinoma.[36] Technical issues are unlikely to explain our results. Indeed, PR expression decreases after withdrawal of HT, and surgery is often performed several days after HT has been stopped; this decrease is however too weak to fully explain our results.[58] Progestins also induce a PR down-regulation,[59] but this down-regulation disappears 48 hours after the progestin withdrawal.[60] Absence of PR while ER is present may be due to overexpression of human epidermal growth factor receptor 2 (HER-2).[61] One study, based on very small numbers, found that CHT was markedly associated with HER-2–amplified tumors.[24] Three other studies failed to find a significantly more frequent HER-2 overexpression in breast cancers diagnosed in HT users versus nonusers, but they too were based on small numbers of cases.[62-64] Absence of PR may also indicate high insulin-like growth factor (IGF), epidermal growth factor (EGF), and heregulin activities, which downregulate PR independently of ER status.[61] Progestins such as MPA and promegestone upregulate IGF and EGF receptors[65]; progesterone may also potentiate EGF pathway signaling in breast cancer cell lines,[66] perhaps to a lesser extent than other progestagens.[67] Progestagens might thus increase the potency of growth factors and hence preferentially affect the risk of ER+/ PR-tumors.

The main strengths of our study have been discussed previously.[6] They include the large population and regular updating of exposure during follow-up. Also, careful adjustment for various potential confounders decreased the probability that the differences we found on risk between different CHTs are explained by confounding. Lastly, there was no marked difference between users of the different types of CHTs regarding established breast cancer risk factors (data not shown).

Our study had several limitations. Firstly, data on hormone receptors were taken from various laboratories; ER and PR results were scored as positive or negative using techniques and cutoffs that may not have been standardized. Histological classification may have varied over time or between laboratories. However, any resulting outcome misclassification was unlikely to be related to the HT exposure, and would have tended to weaken and obscure any real differences in the association of HTs with different types of breast cancer. Another potential limitation is that the joint ER and PR status was not available for 20.9% of the histologically confirmed cases. However, we verified that HT use was not associated with hormone receptor status measurement, when the period of diagnosis was taken into account (before 1994, 1994 to 1996, 1997 or later, as introduced in the multivariate models presented in the current analysis; data not shown). Therefore, the lack of data on hormone receptor status for some breast cancer cases is unlikely to bias the estimates substantially. Finally, the relatively small numbers of cases in some subgroups (especially ER-/ PR+ carcinomas, lobular carcinomas, or estrogen alone users) may have limited our ability to detect significant associations. We also had insufficient power to further split the "estrogen+other progestagens" category and present meaningful data according to the exact progestagen molecule used, which are numerous in France. Longer follow-up and additional cases will make it possible.

In conclusion, the present study suggests that CHTs, when related to breast cancer risk, preferentially affect the risk of ER+ carcinomas, and especially those ER+/PR-. Our study also suggests that the progestagen component

of CHT may be of importance regarding breast cancer risk. Given the major public health implications associated with the use of postmenopausal HT, further research is needed on CHTs containing progesterone or dydrogesterone, which might be less harmful regarding breast cancer risk than those containing other progestagens.

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Acknowledgment
We thank R. Chaït, M. Fangon, L. Hoang and M. Niravong for their technical assistance; the E3N group; and C. Holmes for assistance in English. The authors are also grateful to all participants for providing the data and to practitioners for providing pathology reports.
Supported by Mutuelle Générale de l'Education Nationale; European Community; French League against Cancer; Gustave Roussy Institute; French Institute of Health and Medical Research; 3M Company; several General Councils of France; Fondation pour la Recherche Médicale; Cancéropôle Région Ile-de-France; Direction Générale de la Santé ; Agence Française de Sécurité Sanitaire des Produits de Santé.

References

1. Cogliano V, Grosse Y, Baan R, et al: Carcinogenicity of combined oestrogen-progestagen contraceptives and menopausal treatment. Lancet Oncol 6:552-553, 2005
2. Anderson WF, Chu KC, Chang S, et al: Comparison of age-specific incidence rate patterns for different histopathologic types of breast carcinoma. Cancer Epidemiol Biomarkers Prev 13:1128-1135, 2004
3. Li CI, Daling JR, Malone KE, et al: Relationship between established breast cancer risk factors and risk of seven different histologic types of invasive breast cancer. Cancer Epidemiol Biomarkers Prev 15:946-954, 2006
4. Ma H, Bernstein L, Pike MC, et al: Reproductive factors and breast cancer risk according to joint estrogen and progesterone receptor status: A metaanalysis of epidemiological studies. Breast Cancer Res 8:R43, 2006
5. Lee SA, Ross RK, Pike MC: An overview of menopausal oestrogen-progestin hormone therapy and breast cancer risk. Br J Cancer 92:2049-2058, 2005
6. Fournier A, Berrino F, Clavel-Chapelon F: Unequal risks for breast cancer associated with different hormone replacement therapies: Results from the E3N cohort study. Breast Cancer Res Treat 107:103-111, 2008
7. Li CI, Anderson BO, Porter P, et al: Changing incidence rate of invasive lobular breast carcinoma among older women. Cancer 88:2561-2569, 2000
8. Li CI, Anderson BO, Daling JR, et al: Trends in incidence rates of invasive lobular and ductal breast carcinoma. JAMA 289:1421-1424, 2003
9. Levi F, Te VC, Randimbison L, et al: Increase in lobular breast cancer incidence in Switzerland. Int J Cancer 107:164-165, 2003
10. Verkooijen HM, Fioretta G, Vlastos G, et al: Important increase of invasive lobular breast cancer incidence in Geneva, Switzerland. Int J Cancer 104: 778-781, 2003
11. Verkooijen HM, Koot VC, Fioretta G, et al: Hormone replacement therapy, mammography screening and changing age-specific incidence rates of breast cancer: An ecological study comparing two European populations. Breast Cancer Res Treat 107: 389-395, 2008
12. Li CI, Weiss NS, Stanford JL, et al: Hormone replacement therapy in relation to risk of lobular and ductal breast carcinoma in middle-aged women. Cancer 88:2570-2577, 2000
13. Ursin G, Tseng CC, Paganini-Hill A, et al: Does menopausal hormone replacement therapy interact with known factors to increase risk of breast cancer? J Clin Oncol 20:699-706, 2002
14. Chen CL, Weiss NS, Newcomb P, et al: Hormone replacement therapy in relation to breast cancer. JAMA 287:734-741, 2002

15. Daling JR, Malone KE, Doody DR, et al: Relation of regimens of combined hormone replacement therapy to lobular, ductal, and other histologic types of breast carcinoma. Cancer 95:2455-2464, 2002
16. Newcomb PA, Titus-Ernstoff L, Egan KM, et al: Postmenopausal estrogen and progestin use in relation to breast cancer risk. Cancer Epidemiol Biomarkers Prev 11:593-600, 2002
17. Newcomer LM, Newcomb PA, Potter JD, et al: Postmenopausal hormone therapy and risk of breast cancer by histologic type (United States). Cancer Causes Control 14:225-233, 2003
18. Li CI, Malone KE, Porter PL, et al: Relationship between long durations and different regimens of hormone therapy and risk of breast cancer. JAMA 289:3254-3263, 2003
19. Chlebowski RT, Hendrix SL, Langer RD, et al: Influence of estrogen plus progestin on breast cancer and mammography in healthy postmenopausal women: The Women's Health Initiative Randomized Trial. JAMA 289:3243-3253, 2003
20. Lee S, Kolonel L, Wilkens L, et al: Postmenopausal hormone therapy and breast cancer risk: The Multiethnic Cohort. Int J Cancer 118:1285-1291, 2006
21. Garcia-Closas M, Brinton LA, Lissowska J, et al: Established breast cancer risk factors by clinically important tumour characteristics. Br J Cancer 95:123-129, 2006
22. Rosenberg LU, Magnusson C, Lindstrom E, et al: Menopausal hormone therapy and other breast cancer risk factors in relation to the risk of different histological subtypes of breast cancer: A casecontrol study. Breast Cancer Res 8:R11, 2006
23. Reeves GK, Beral V, Green J, et al: Hormonal therapy for menopause and breast-cancer risk by histological type: A cohort study and meta-analysis. Lancet Oncol 7:910-918, 2006
24. Borgquist S, Anagnostaki L, Jirstrom K, et al: Breast tumours following combined hormone replacement therapy express favourable prognostic factors. Int J Cancer 120:2202-2207, 2007
25. Hildreth NG, Kelsey JL, Eisenfeld AJ, et al: Differences in breast cancer risk factors according to the estrogen receptor level of the tumor. J Natl Cancer Inst 70:1027-1031, 1983
26. Stanford JL, Szklo M, Boring CC, et al: A case-control study of breast cancer stratified by estrogen receptor status. Am J Epidemiol 125:184-194, 1987
27. Cooper JA, Rohan TE, Cant EL, et al: Risk factors for breast cancer by oestrogen receptor status: A population-based case-control study. Br J Cancer 59:119-125, 1989
28. Potter JD, Cerhan JR, Sellers TA, et al: Progesterone and estrogen receptors and mammary neoplasia in the Iowa Women's Health Study: How many kinds of breast cancer are there? Cancer Epidemiol Biomarkers Prev 4:319-326, 1995
29. Huang WY, Newman B, Millikan RC, et al: Hormone-related factors and risk of breast cancer in relation to estrogen receptor and progesterone receptor status. Am J Epidemiol 151:703-714, 2000
30. Kerlikowske K, Miglioretti DL, Ballard-Barbash R, et al: Prognostic characteristics of breast cancer among postmenopausal hormone users in a screened population. J Clin Oncol 21:4314-4321, 2003
31. Cotterchio M, Kreiger N, Theis B, et al: Hormonal factors and the risk of breast cancer according to estrogen- and progesterone-receptor subgroup. Cancer Epidemiol Biomarkers Prev 12:1053-1060, 2003
32. Stahlberg C, Pedersen AT, Andersen ZJ, et al: Breast cancer with different prognostic characteristics developing in Danish women using hormone replacement therapy. Br J Cancer 91:644-650, 2004
33. Chen WY, Hankinson SE, Schnitt SJ, et al: Association of hormone replacement therapy to estrogen and progesterone receptor status in invasive breast carcinoma. Cancer 101:1490-1500, 2004
34. Tjonneland A, Christensen J, Thomsen BL, et al: Hormone replacement therapy in relation to breast carcinoma incidence rate ratios: A prospective Danish cohort study. Cancer 100:2328-2337, 2004
35. Stefanick ML, Anderson GL, Margolis KL, et al: Effects of conjugated equine estrogens on breast cancer and mammography screening in postmenopausal women with hysterectomy. JAMA 295:1647-1657, 2006
36. Rosenberg LU, Einarsdottir K, Friman EI, et al: Risk factors for hormone receptor-defined breast cancer in postmenopausal women. Cancer Epidemiol Biomarkers Prev 15:2482-2488, 2006
37. Antoine C, Liebens F, Carly B, et al: Influence of HRT on prognostic factors for breast cancer: A systematic review after the Women's Health Initiative trial. Hum Reprod 19:741-756, 2004
38. Riboli E, Hunt KJ, Slimani N, et al: European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr 5:1113-1124, 2002
39. Weiss NS, Rossing MA: Healthy screened bias in epidemiologic studies of cancer incidence. Epidemiology 7:319-322, 1996

40. Lagakos SW: A covariate model for partially censored data subject to competing causes of failure. Applied Statistics 27:235-241, 1978
41. Li CI, Uribe DJ, Daling JR: Clinical characteristics of different histologic types of breast cancer. Br J Cancer 93:1046-1052, 2005
42. Rockhill B, Colditz GA, Rosner B: Bias in breast cancer analyses due to error in age at menopause. Am J Epidemiol 151:404-408, 2000
43. Pike MC, Ross RK, Spicer DV: Problems involved in including women with simple hysterectomy in epidemiologic studies measuring the effects of hormone replacement therapy on breast cancer risk. Am J Epidemiol 147:718-721, 1998
44. Campagnoli C, Clavel-Chapelon F, Kaaks R, et al: Progestins and progesterone in hormone replacement therapy and the risk of breast cancer. J Steroid Biochem Mol Biol 96:95-108, 2005
45. Pasqualini JR, Paris J, Sitruk-Ware R, et al: Progestins and breast cancer. J Steroid Biochem Mol Biol 65:225-235, 1998
46. Stanczyk FZ: All progestins are not created equal. Steroids 68:879-890, 2003
47. Santen RJ: Risk of breast cancer with progestins: Critical assessment of current data. Steroids 68:953-964, 2003
48. Ferguson DJ, Anderson TJ: Morphological evaluation of cell turnover in relation to the menstrual cycle in the "resting" human breast. Br J Cancer 44:177-181, 1981
49. Longacre TA, Bartow SA: A correlative morphologic study of human breast and endometrium in the menstrual cycle. Am J Surg Pathol 10:382-393, 1986
50. Chang KJ, Lee TT, Linares-Cruz G, et al: Influences of percutaneous administration of estradiol and progesterone on human breast epithelial cell cycle in vivo. Fertil Steril 63:785-791, 1995
51. Foidart JM, Colin C, Denoo X, et al: Estradiol and progesterone regulate the proliferation of human breast epithelial cells. Fertil Steril 69:963-969, 1998
52. Hofseth LJ, Raafat AM, Osuch JR, et al: Hormone replacement therapy with estrogen or estrogen plus medroxyprogesterone acetate is associated with increased epithelial proliferation in the normal postmenopausal breast. J Clin Endocrinol Metab 84:4559-4565, 1999
53. Cline JM, Soderqvist G, von Schoultz E, et al: Effects of hormone replacement therapy on the mammary gland of surgically postmenopausal cynomolgus macaques. Am J Obstet Gynecol 174:93-100, 1996
54. Wood CE, Register TC, Lees CJ, et al: Effects of estradiol with micronized progesterone or medroxyprogesterone acetate on risk markers for breast cancer in postmenopausal monkeys. Breast Cancer Res Treat 101:125-134, 2007
55. Anderson E: The role of oestrogen and progesterone receptors in human mammary development and tumorigenesis. Breast Cancer Res 4:197-201, 2002
56. Ravdin PM, Cronin KA, Howlader N, et al: The decrease in breast-cancer incidence in 2003 in the United States. N Engl J Med 356:1670-1674, 2007
57. Clarke RB: Ovarian steroids and the human breast: Regulation of stem cells and cell proliferation. Maturitas 54:327-334, 2006
58. Prasad R, Boland GP, Cramer A, et al: Shortterm biologic response to withdrawal of hormone replacement therapy in patients with invasive breast carcinoma. Cancer 98:2539-2546, 2003
59. Botella J, Duranti E, Duc I, et al: Inhibition by nomegestrol acetate and other synthetic progestins on proliferation and progesterone receptor content of T47-D human breast cancer cells. J Steroid Biochem Mol Biol 50:41-47, 1994
60. Wei LL, Krett NL, Francis MD, et al: Multiple human progesterone receptor messenger ribonucleic acids and their autoregulation by progestin agonists and antagonists in breast cancer cells. Mol Endocrinol 2:62-72, 1988
61. Cui X, Zhang P, Deng W, et al: Insulin-like growth factor-I inhibits progesterone receptor expression in breast cancer cells via the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin pathway: Progesterone receptor as a potential indicator of growth factor activity in breast cancer. Mol Endocrinol 17:575-588, 2003
62. Holli K, Isola J, Cuzick J: Low biologic aggressiveness in breast cancer in women using hormone replacement therapy. J Clin Oncol 16:3115-3120, 1998

63. Pappo I, Meirshon I, Karni T, et al: The characteristics of malignant breast tumors in hormone replacement therapy users versus nonusers. Ann Surg Oncol 11:52-58, 2004
64. Biglia N, Sgro L, Defabiani E, et al: The influence of hormone replacement therapy on the pathology of breast cancer. Eur J Surg Oncol 31:467-472, 2005
65. Lange CA, Richer JK, Shen T, et al: Convergence of progesterone and epidermal growth factor signaling in breast cancer: Potentiation of mitogenactivated protein kinase pathways. J Biol Chem 273:31308-31316, 1998
66. Carvajal A, Espinoza N, Kato S, et al: Progesterone pre-treatment potentiates EGF pathway signaling in the breast cancer cell line ZR-75. Breast Cancer Res Treat 94:171-183, 2005
67. Murphy LJ, Sutherland RL, Stead B, et al: Progestin regulation of epidermal growth factor receptor in human mammary carcinoma cells. Cancer Res 46:728-734, 1986