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Running head: MHT and breast cancer according to timing of treatment initiation

The work proposed here is original and has not been presented previously in any form.

ABSTRACT

PURPOSE

To investigate whether the relation between estrogen-progestagen menopausal hormone therapy (EP-MHT) and breast cancer risk varies according to the delay between menopause onset and treatment initiation.

MATERIALS AND METHODS

Between 1992 and 2005, 1,726 invasive breast cancers were identified among 53,310 postmenopausal women from the French E3N cohort (mean duration of follow-up: 8.1 years). Hazard ratios (HRs) and confidence intervals (CI) were estimated using Cox models, with MHT never-users as the reference.

RESULTS

Among recent users of EP-MHT, the risk of breast cancer varied according to the timing of treatment initiation. This variation was confined to short durations of use (≤2 years): the HR was 1.54 (95% CI 1.28-1.86) for short treatments initiated in the 3-year period following menopause onset and 1.00 (95% CI 0.68-1.47) for short treatments initiated later (p=0.04 for homogeneity). This pattern of risks was however not observed in users of EP-MHT containing progesterone, among whom there was no significantly increased risk associated with short durations of use (HR 0.87, 95% CI 0.57-1.32 for treatments initiated ≤3 years after menopause and 0.90, 95% CI 0.45-1.81 for treatments initiated later). Longer durations of EP-MHT use were generally associated with increases in breast cancer risk, whatever the gap time.

CONCLUSION

Our results suggest that, for some EP-MHT, the timing of treatment initiation transiently modulates the risk of breast cancer; and that, when initiated close to menopause, even short durations of use are associated with an increased breast

cancer risk. Estrogen-progesterone combinations might be an exception in this regard.

INTRODUCTION

Up to the end of the 1990's, menopausal hormone therapy (MHT) was largely considered beneficial, especially regarding relief of climacteric symptoms and prevention of osteoporosis and coronary heart disease (CHD); however, concerns regarding risks of breast cancer and thromboembolic disease had been raised.[1] In 1998, a secondary prevention trial challenged the belief in a protective effect of MHT regarding CHD.[2] This doubt extended to primary prevention after the release of the Women's Health Initiative (WHI) trial in 2002.[3] These cardiovascular safety concerns (later supported by the WISDOM trial findings[4]), together with the WHI and Million Women Study reports of an increased breast cancer risk among estrogen-progestagen (EP) MHT users,[3,5,6] led some health agencies to restrict indications of MHT to the treatment of climacteric symptoms,[7] considering that, otherwise, risks outweigh benefits.

It has been recently hypothesized that the benefit/risk ratio of MHT varies according to the delay between menopause onset and treatment initiation: the CHD risk might be lessened when MHT is started soon after menopause rather than a few years later.[8-10] This "timing hypothesis" has been largely spread and discussed. However, it only concerns the risk of heart disease and is not currently definitively confirmed.

Especially around menopause, the burden of breast cancer is important. In American women aged 45 to 59 years, it is comparable to that of CHD.[11] It is thus of utmost importance to know also to what extent MHT use close to menopause onset impacts breast cancer risk. Recent analyses of the WHI data suggest that women who initiate MHT soon after menopause have a higher breast cancer risk than those who start treatment later.[12,13]

We therefore investigated the relation between MHT use and breast cancer risk according to the timing of treatment initiation. We used data from the French E3N cohort study, with biennial information on menopausal status, MHT use and occurrence of breast cancer. As unopposed estrogen therapy has been shown to be associated with a lower breast cancer risk than EP-MHT,[14] and because we had limited numbers of unopposed estrogen users, we focused on EP-MHT.

MATERIALS AND METHODS

The E3N cohort

E3N is a prospective cohort 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 self-administered questionnaires (sent biennially between 1990 and 2005) that addressed 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.[15]

Identification of breast cancer cases

Occurrence of cancer was self-reported in each questionnaire, and a small number of cases were further identified from the insurance files or information on causes of death, obtained from the National Service on Causes of Deaths.

The pathology report, used to confirm the diagnosis of invasive breast cancer (our primary outcome), was obtained for 93% of declared breast cancers. We also included in our analyses the cases for which pathology reports had not been obtained, as the proportion of false positive self-reports was very low (<5%).

Assessment of MHT Use

Information on lifetime use of MHT was recorded in the 1992 questionnaire, which formed the baseline of the current report. It requested the start date, brand names, and duration of each episode of hormone use. Women were given a booklet

with color photographs, which listed the hormonal treatments marketed in France. MHT information was updated in each of the subsequent questionnaires.

EP-MHTs were classified as i) estrogen combined with progesterone, ii) estrogen combined with dydrogesterone, or iii) estrogen combined with other progestagens, following our previous finding that associations with breast cancer risk varied significantly across these different treatments.[16] We did not evaluate in the present study MHTs consisting of unopposed estrogen or tibolone, those containing promestriene, estriol, or androgen, those vaginally, nasally or intramuscularly administered, those with transdermally administered progestagen, as well as MHT with no specified formulation. Women who used these MHTs were censored at the date of first report of use. “Recent” MHT use was defined as current use or use occurring within the previous 12-month period.

Assessment of menopause

Information on menopausal status was updated in each questionnaire. 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 MHT, or self-reported that they were postmenopausal. Age at menopause was defined as age at last menstrual period; age at bilateral oophorectomy; or self-reported age at menopause. We excluded women for whom age at menopause could not be determined accurately (e.g. in case of both no self-reported age at menopause and an unknown age at last menstrual period, or of no self-reported age at menopause and MHT use preceding the cessation of menstruation, or of cessation of menstruation due to hysterectomy).

Population for analysis and follow-up

Follow-up started either at the date of return of the baseline questionnaire for women who were already postmenopausal at that time, otherwise at the date of the first report of menopause. Participants contributed person-years of follow-up until the date of diagnosis of any type of malignancy, of the last completed questionnaire, of the first report of use of a MHT other than EP, or July 2005 (date at which the 8th questionnaire was sent to participants), whichever occurred first.

Among the women who reported menopause at the time of the 7th questionnaire or before (n = 90,297), we excluded those with no follow-up information (n = 4,906), those who had been diagnosed with a cancer other than a basal cell carcinoma before the start of follow-up (n = 6,172), those who did not answer the baseline questionnaire about lifetime use of MHT while being postmenopausal at the date this questionnaire was sent (n = 3,661), those for whom no age at first MHT use was available (n = 1,595), those for whom age at menopause could not be determined accurately (n = 12,942), and those who had used an MHT other than EP before the start of follow-up (n = 7,711). This left us with 53,310 women.

Statistical analysis

Hazard ratios (HRs) of breast cancer and 95% confidence intervals (CI) were estimated using Cox proportional hazards models, with age as the time scale. We retained potential confounding variables (Table 1) in the final model if they altered the risk estimates for at least one of the MHT-related parameters by 10 percent or more; only age at menopause fitted these requirements and was thus included in the parsimonious models. Models were additionally stratified on year of birth, using 5-year categories.

Regarding EP-MHT exposure status, the information reported in questionnaires n and earlier was used to prospectively categorize participants for the period between completion of questionnaires n and n+1. For women who did not answer questionnaire n, MHT exposure status was classified as missing for the period between the date at which questionnaire n was sent to the participants and the date of completion of the subsequent questionnaire. In analyses estimating HRs associated with different types of EP-MHT, estimates were computed for the type of treatment used the most recently, and indicators corresponding to previous use of other types of MHT were simultaneously entered in the models. Tests for trend with duration of use were computed by adding duration of use as a continuous variable in the models. Comparison of HRs was performed by Wald Chi square test of homogeneity.

Time from menopause onset to treatment initiation (hereafter referred to as "gap time") was initially planned to be categorized as ≤1 year (60% of MHT ever-users),]1-3] years (22%),]3-5] years (7%) and more than 5 years (11%). However, to gain statistical precision and simplicity in reporting results, and on the basis of the similarity of the estimates in each group, categories were collapsed into ≤3 years and >3 years (estimates in the initial four gap time categories are shown in online tables).

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

A total of 1,726 first primary invasive breast cancers were diagnosed during 433,647 person-years of follow-up (mean duration: 8.1 years). The main characteristics of the participants are shown in Table 1.

EP-MHT ever-users were at significantly increased breast cancer risk compared with MHT never-users, whatever the gap time (≤3 years or >3 years) (Table 2). In EP-MHT recent users, there was a variation in HRs according to gap time (1.61, 95% CI 1.43-1.81 and 1.35, 95% CI 1.13-1.63 for gap time ≤3 years or >3 years, respectively), which however did not reach statistical significance (p for homogeneity = 0.07). In past users, i.e. those who had stopped using EP-MHT for more than 1 year, there were no significantly increased risks, whatever the gap time. Therefore, estimates were subsequently shown only for recent EP-MHT use.

In EP-MHT recent users, the variation in HRs according to gap time was confined to short durations (≤2 years) of use (Table 3). The HR was 1.54 (95% CI 1.28-1.86) for treatments of short duration initiated within 3 years of menopause and 1.00 (95% CI 0.68-1.47) for treatments of short duration initiated later (p for homogeneity = 0.04). This pattern of risks was not observed in recent users of estrogen-progesterone: there was no significantly increased risk associated with short durations of use, whatever the gap time. In contrast, short durations of use of estrogen-dydrogesterone were associated with an increased risk of borderline statistical significance (HR 1.44, 95% CI 0.99-2.09; p = 0.055) for gap times ≤3 years and of 1.14 (95% CI 0.51-2.55) for longer gap times, but the variation in risks was not statistically significant (p for homogeneity = 0.60). Short durations of use of estrogens combined with other progestagens were associated with a HR of 1.89 (95% CI 1.53-2.34) for gap times ≤3 years and of 1.02 (95% CI 0.59-1.78) for longer gap times (p

for homogeneity = 0.04). When the latter type of treatments was used for more than 2 years, HRs of the order of 2 were generally observed, whatever the gap time.

Finally, among women who initiated MHT soon after menopause, significant trends of increasing risk with increasing duration of use were seen with recent use of estrogen associated with progesterone, but not with estrogen associated with dydrogesterone or other progestagens (Table 3).

DISCUSSION

Our results suggest that, in recent users of some EP-MHT, the timing of treatment initiation modulates the risk of breast cancer: short durations (≤2 years) of use were associated with significant increases in risk – with the exception of MHT containing progesterone – when initiated in the 3-year period following menopause, but not when initiated later. This variation in risk according to the timing of treatment initiation appeared to be transient: longer durations (>2 years) of use were generally associated with increases in breast cancer risk, whatever the gap time.

As breast cancer risk may vary according to the characteristics of EP-MHT, we must specify that, in our cohort, the estrogenic component consisted almost exclusively in estradiol compounds, with progesterone and dydrogesterone as the most frequently associated progestagens; other progestagens were mostly nomegestrol acetate and promegestone (each accounting for approximately one quarter of the person-years associated with recent use of estrogen+other progestagens), followed by chlormadinone acetate, norethisterone acetate, medroxyprogesterone acetate, medrogestone, and cyproterone acetate (approximately one tenth of the person-years each).

In the present study, we found specific patterns of risks for estrogen+progesterone combinations: we did not observe elevated risks with short durations of use, even when initiated close to menopause. Significantly increased risks were seen with more than 5 years of use, which still appeared less elevated than those observed in the estrogen+other progestagen group. However, because widespread use of progesterone has been a French peculiarity, the relationship between estrogen+progesterone and breast cancer risk has not been studied in other

settings. The present analysis adds to our previous results that different progestagens in EP-MHT may impact breast cells differently.[16,17]

Only the WHI group assessed the impact of the timing of MHT initiation on breast cancer risk.[12,13] In line with our results, this group found that HRs associated with EP-MHT use (consisting in conjugated equine estrogens combined with medroxyprogesterone acetate) decreased with increasing gap time. For women who initiated EP-MHT within 5 years of menopause, the HR estimate in the WHI was of 1.32 during the first two years of treatment, but this estimate, based on only 7 cases, was imprecise. With more than 100 cases in women using EP-MHT (containing progestagens other than progesterone or dydrogesterone) for 2 years or less, we found a significant doubling in risk when MHT began within 3 years of menopause, as compared with MHT never-use. For the other durations of use, estimates of the WHI and ours are consistent.

Our results of increased breast cancer risks even with relatively short durations of some EP-MHT are consistent with epidemiological studies which found significant increases in risk for current or recent short-term (<5 years) use of EP-MHT,[6,18-22] although some did not find significant increases.[23-28]

One potential mechanistic explanation of our findings regarding timing of treatment initiation lies on a recently proposed hypothesis that progestins can reactivate cancer stem cells in women with pre-existing breast cancers.[29] One might postulate that, in postmenopause, the reservoir of occult tumors that request progestagens to be reactivated becomes dormant without exposure to MHT; and that time is needed for it to awake after delayed introduction of EP-MHT. Adaptive changes of breast cancers after estrogen deprivation that would decrease their susceptibility to proliferative stimulation by estrogens may also explain our results.[30]

We previously found that short-term (<5 years) use of EP-MHT was associated with no significantly increased risk of lobular breast cancer when containing progesterone, a 1.5-fold increased risk when containing dydrogesterone, and a doubled risk when containing other progestagens;[17] interestingly, this pattern of lobular breast cancer risk closely parallels what we observed in the present study with short durations (≤2 years) of EP-MHT initiated close to menopause, i.e.: no significantly increased risk of invasive breast cancer when containing progesterone, an intermediate increase in risk when containing dydrogesterone, and a doubling in risk when containing other progestagens. In light of these results and following Milanese *et al*. suggestion that MHT use may delay the age-related lobular involution, which has been found to be associated with a decreased breast cancer risk and to accelerate at approximately age 50 years,[31] one might therefore make the following hypothesis: MHT use close to menopause allows a reservoir of epithelial lobular breast cells potentially influenced by EP-MHT to remain important, whereas later introduction of MHT allows time for it to decrease substantially. These are of course only hypotheses and other complex mechanisms may intervene.

The main strengths of our study include the large population and regular updating of both exposure and menopausal status during follow-up. In particular, we were able to include in our analyses a substantial number of recently postmenopausal women, and hence of recent MHT initiators, allowing relatively precise estimation of HRs associated with recent, short-term use of EP-MHT, which was not the case in the WHI analysis. Furthermore, around 20% of women in our cohort had initiated EP-MHT more than 3 years after menopause onset, which can be explained by, first, the fact that MHT gained much popularity in the 1990's, when some participants were already menopausal for several years, and, second, by the

fact that MHT was indicated until year 2003 as a first line treatment for prevention of osteoporosis. Finally, exposure was assessed in a prospective way, thus eliminating the possibility of a differential recall between cases and non-cases. This prospective assessment however leads to an underestimation of the real duration of use, but the frequency of exposure updates limits this bias.

One of the limitations of our study is that data on MHT use were self-reported, and thus affected by (non-differential) recall bias, which should however be limited since data on MHT use were updated in each follow-up questionnaire; thus, apart from the first assessment of MHT where women had to recall lifetime use, only treatments taken during the previous 2-year period had to be recalled. Good agreement between prescription data and self-reported use of MHT is generally found, especially for recent use.[32,33] Menopausal status was also regularly updated, which allowed menopause-related parameters to be as accurate as possible. When we restricted our analyses to women who reached menopause during follow-up (i.e. for whom menopause occurred soon before information on menopausal status was collected), or to women who did not use exogenous hormones in the year preceding menopause (i.e. those for whom menopause cannot have been masked by hormones use), estimates were consistent with the main results presented (data not shown). We therefore believe that data on menopause and exposure were reported in a satisfactory way by participants. A screening bias is possible because hormone users have mammograms more frequently than non-users. However, further adjustment for recent mammogram (i.e., “mammogram performed in the preceding follow-up period (yes/no)”, as a time-dependent variable) did not alter meaningfully our risk estimates; besides, analyses restricted to women who reported a recent mammogram yielded concordant results (data not shown). Finally, we must

acknowledge that the relatively small number of cases in some subgroups may have limited our ability to detect significant associations or variations in HRs.

In conclusion, our results indicate that, contrary to what is currently hypothesized regarding the risk of heart disease, initiation of MHT close to menopause onset rather than later may not limit the increased risk of breast cancer. Instead, even short durations of use of some EP-MHTs were associated with substantially elevated risks of breast cancer when treatment was initiated close to menopause. Finally, our finding that, for short durations of use around menopause, progesterone in EP-MHT may be safer regarding breast cancer risk than other progestagens needs to be confirmed in other settings.

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Table 1. Selected characteristics of participants at start of follow-up, overall and according to MHT exposure status at the end of follow-up. E3N study 1992-2005.

	All (N = 53,310) n (%) or mean (SD)	**MHT never-users (N = 21,231) n (%) or mean (SD)**	**EP-MHT ever-users, gap time ≤3 years (N = 26,171) n (%) or mean (SD)**	**EP-MHT ever-users, gap time >3 years (N = 5,908) n (%) or mean (SD)**
Year of birth				
[1925-1930[	3944 (7.4%)	3320 (15.6%)	135 (0.5%)	489 (8.3%)
[1930-1935[	6598 (12.4%)	3943 (18.6%)	1173 (4.5%)	1482 (25.1%)
[1935-1940[	10406 (19.5%)	3643 (17.2%)	4831 (18.5%)	1932 (32.7%)
[1940-1945[	14121 (26.5%)	3719 (17.5%)	9060 (34.6%)	1342 (22.7%)
≥ 1945	18241 (34.2%)	6606 (31.1%)	10972 (41.9%)	663 (11.2%)
Age at start of follow-up, years	54.6 (4.5)	56.4 (4.9)	52.9 (3.3)	55.4 (4.9)
Age at menarche, years				
< 13	24472 (45.9%)	9788 (46.1%)	12074 (46.1%)	2610 (44.2%)
≥ 13	28838 (54.1%)	11443 (53.9%)	14097 (53.9%)	3298 (55.8%)
Parity				
Nulliparous	6313 (11.8%)	2932 (13.8%)	2682 (10.2%)	699 (11.8%)
Parous, first child before 30, 1 or 2 children	26347 (49.4%)	9526 (44.9%)	14082 (53.8%)	2739 (46.4%)
Parous, first child before 30, 3+ children	14988 (28.1%)	6386 (30.1%)	6731 (25.7%)	1871 (31.7%)
Parous, first child after 30	5662 (10.6%)	2387 (11.2%)	2676 (10.2%)	599 (10.1%)
Breastfeeding*				
No	14503 (30.9%)	5613 (30.7%)	7177 (30.6%)	1713 (32.9%)
<12 months	27042 (57.5%)	10117 (55.3%)	13997 (59.6%)	2928 (56.2%)
≥ 12 months	2836 (6.0%)	1535 (8.4%)	929 (4.0%)	372 (7.1%)
Unknown	2616 (5.6%)	1034 (5.7%)	1386 (5.9%)	196 (3.8%)
Age at menopause, years	50.6 (3.6)	50.8 (4.0)	50.8 (3.0)	49.1 (4.3)
Type of menopause				
Artificial	3583 (6.7%)	1802 (8.5%)	1293 (4.9%)	488 (8.3%)
Natural / Unknown	49727 (93.3%)	19429 (91.5%)	24878 (95.1%)	5420 (91.7%)
Personal history of benign breast disease				
Yes	14070 (26.4%)	5143 (24.2%)	7669 (29.3%)	1258 (21.3%)
No	39240 (73.6%)	16088 (75.8%)	18502 (70.7%)	4650 (78.7%)
Family history of breast cancer in first degree relatives				
Yes	6118 (11.5%)	2683 (12.6%)	2788 (10.7%)	647 (11.0%)
No	47192 (88.5%)	18548 (87.4%)	23383 (89.3%)	5261 (89.0%)
Family history of breast cancer in other relatives				
Yes	8087 (15.2%)	3211 (15.1%)	4062 (15.5%)	814 (13.8%)
No	45223 (84.8%)	18020 (84.9%)	22109 (84.5%)	5094 (86.2%)
Height, cm	161.4 (5.7)	161.1 (5.8)	161.7 (5.6)	161.0 (5.7)

Body mass index, kg/m^2				
≤ 20	7670 (14.4%)	2582 (12.2%)	4214 (16.1%)	874 (14.8%)
]20-25]	33843 (63.5%)	12350 (58.2%)	17580 (67.2%)	3913 (66.2%)
]25-30]	9502 (17.8%)	4836 (22.8%)	3708 (14.2%)	958 (16.2%)
> 30	2295 (4.3%)	1463 (6.9%)	669 (2.6%)	163 (2.8%)
Total physical activity, MET-h/wk				
<27	13445 (25.2%)	5055 (23.8%)	6900 (26.4%)	1490 (25.2%)
[27-39[	13312 (25.0%)	4971 (23.4%)	6975 (26.7%)	1366 (23.1%)
[39-57[	13344 (25.0%)	5110 (24.1%)	6762 (25.8%)	1472 (24.9%)
≥ 57	13209 (24.8%)	6095 (28.7%)	5534 (21.1%)	1580 (26.7%)
Previous use of oral contraceptives				
Never	21513 (40.4%)	11202 (52.8%)	7358 (28.1%)	2953 (50.0%)
In the past 5 years	2861 (5.4%)	579 (2.7%)	2086 (8.0%)	196 (3.3%)
More than five years ago	16209 (30.4%)	5192 (24.5%)	9678 (37.0%)	1339 (22.7%)
Ever, but unknown recency	12727 (23.9%)	4258 (20.1%)	7049 (26.9%)	1420 (24.0%)
Use of oral progestagens alone in premenopause				
Never	31835 (59.7%)	15166 (71.4%)	12257 (46.8%)	4412 (74.7%)
In the past 5 years	15043 (28.2%)	3754 (17.7%)	10537 (40.3%)	752 (12.7%)
More than five years ago	4718 (8.9%)	1800 (8.5%)	2441 (9.3%)	477 (8.1%)
Ever, but unknown recency	1714 (3.2%)	511 (2.4%)	936 (3.6%)	267 (4.5%)
Mammography in the previous follow-up period				
Yes	34497 (64.7%)	12179 (57.4%)	18494 (70.7%)	3824 (64.7%)
No	14092 (26.4%)	8067 (38.0%)	4136 (15.8%)	1889 (32.0%)
Unknown	4721 (8.9%)	985 (4.6%)	3541 (13.5%)	195 (3.3%)

Abbreviations: EP-MHT, estrogen-progestagen menopausal hormone therapy; MET-h/wk, metabolic equivalent task-hour/week

* Among parous women

Table 2. Hazard ratios of invasive breast cancer for EP-MHT use compared with MHT never-use. E3N study 1992-2005.

	Ever use of EP-MHT		Recent use of EP-MHT		Past use of EP-MHT	
	No. of cases*	**Hazard ratio (95% CI)†**	**No. of cases**	**Hazard ratio (95% CI)†**	**No. of cases**	**Hazard ratio (95% CI)†**
Gap time						
≤ 3 years	885	1.54 (1.37-1.72)	786	1.61 (1.43-1.81)	85	1.09 (0.86-1.38)
> 3 years	182	1.31 (1.10-1.55)	151	1.35 (1.13-1.63)	28	1.04 (0.71-1.52)
P for homogeneity		*0.06*		*0.07*		*0.82*

Abbreviations: EP-MHT, estrogen-progestagen menopausal hormone therapy; CI, confidence interval

* 17 cases were diagnosed among women for whom recency of use was not known; 567 cases were further diagnosed among MHT never-users, and 92 among women with a missing MHT exposure status

† Adjusted for: age (time scale) and age at menopause (continuous). Further stratified on year of birth ([1925-1930[/[1930-1935[/[1935-1940[/[1940-1945[/[1945-1950])

Table 3. Hazard ratios of invasive breast cancer for EP-MHT recent use, according to gap time, type of progestagen and total duration of use, compared with MHT never-use. E3N study 1992-2005.

	Total duration of EP-MHT use								
	≤2 years		**]2-5] years**		**]5-10] years**		**>10 years**		***P for trend with duration of use***
	No. of cases	**Hazard ratio* (95% CI)**	**No. of cases**	**Hazard ratio* (95% CI)**	**No. of cases**	**Hazard ratio* (95% CI)**	**No. of cases**	**Hazard ratio* (95% CI)**	
Recent use of any type of EP-MHT									
Gap time ≤ 3 years	178	1.54 (1.28-1.86)	229	1.49 (1.26-1.76)	260	1.60 (1.37-1.88)	119	1.89 (1.53-2.34)	*0.09*
Gap time > 3 years	27	1.00 (0.68-1.47)	52	1.52 (1.14-2.03)	60	1.59 (1.21-2.09)	12	1.14 (0.64-2.04)	*0.68*
P for homogeneity		*0.04*		*0.89*		*0.96*		*0.10*	
Recent use of estrogen + progesterone									
Gap time ≤ 3 years	23	0.87 (0.57-1.32)	39	1.01 (0.72-1.41)	67	1.47 (1.11-1.95)	39	1.92 (1.34-2.74)	*0.002*
Gap time > 3 years	8	0.90 (0.45-1.81)	18	1.55 (0.96-2.48)	12	0.89 (0.50-1.59)	4	0.97 (0.36-2.62)	*0.54*
P for homogeneity		*0.93*		*0.14*		*0.11*		*0.20*	
Recent use of estrogen + dydrogesterone									
Gap time ≤ 3 years	31	1.44 (0.99-2.09)	34	1.21 (0.85-1.72)	40	1.38 (0.98-1.93)	16	1.35 (0.81-2.26)	*0.92*
Gap time > 3 years	6	1.14 (0.51-2.55)	6	0.95 (0.42-2.13)	13	1.77 (1.02-3.09)	4	1.83 (0.68-4.93)	*0.61*
P for homogeneity		*0.60*		*0.59*		*0.44*		*0.59*	
Recent use of estrogen + other progestagen									
Gap time ≤ 3 years	124	1.89 (1.53-2.34)	156	1.88 (1.56-2.27)	153	1.87 (1.54-2.27)	64	2.32 (1.76-3.06)	*0.18*
Gap time > 3 years	13	1.02 (0.59-1.78)	28	1.79 (1.22-2.63)	35	2.21 (1.56-3.14)	4	1.07 (0.40-2.87)	*0.27*
P for homogeneity		*0.04*		*0.81*		*0.38*		*0.13*	

Abbreviations: EP-MHT, estrogen-progestagen menopausal hormone therapy; CI, confidence interval

* Adjusted for the same covariates as in Table 2. In analyses according to type of recent EP-MHT, models are additionally adjusted for past use of estrogen combined with progesterone, estrogen combined with dydrogesterone, estrogen combined with other progestogens

Table 2bis. Hazard ratios of invasive breast cancer for EP-MHT use compared with MHT never-use.

	Ever use of EP-MHT		Recent use of EP-MHT		Past use of EP-MHT	
	No. of cases*	**Hazard ratio (95% CI)†**	**No. of cases**	**Hazard ratio (95% CI)†**	**No. of cases**	**Hazard ratio (95% CI)†**
Gap time						
≤ 1 year	660	1.56 (1.38-1.76)	586	1.63 (1.43-1.84)	64	1.16 (0.89-1.51)
1-3 years	225	1.45 (1.24-1.71)	200	1.54 (1.30-1.82)	21	0.91 (0.59-1.41)
3-5 years	68	1.20 (0.93-1.55)	61	1.33 (1.02-1.74)	7	0.69 (0.33-1.46)
> 5 years	114	1.37 (1.12-1.69)	90	1.37 (1.09-1.72)	21	1.24 (0.80-1.93)

Abbreviations: EP-MHT, estrogen-progestagen menopausal hormone therapy; CI, confidence interval

* 17 cases were diagnosed among women for whom recency of use was not known; 567 cases were further diagnosed among MHT never-users; and 92 among women with a missing MHT exposure status

† Adjusted for: age (time scale) and age at menopause (continuous). Further stratified on year of birth ([1925-1930[/[1930-1935[/[1935-1940[/[1940-1945[/[1945-1950])

Table 3bis. Hazard ratios of invasive breast cancer for EP-MHT recent use, according to gap time, type of progestagen and total duration of use, compared with MHT never-use. E3N study 1992-2005.

	Total duration of EP-MHT use								
	≤2 years		]2-5] years		]5-10] years		>10 years		*P for trend with duration of use*
	No. of cases	**Hazard ratio* (95% CI)**	**No. of cases**	**Hazard ratio* (95% CI)**	**No. of cases**	**Hazard ratio* (95% CI)**	**No. of cases**	**Hazard ratio* (95% CI)**	
Recent use of any type of EP-MHT									
Gap time ≤ 1 year	133	1.61 (1.30-1.98)	163	1.45 (1.20-1.74)	196	1.64 (1.38-1.95)	94	1.91 (1.51-2.41)	*0.10*
Gap time 1-3 years	45	1.38 (1.01-1.89)	66	1.60 (1.23-2.08)	64	1.48 (1.13-1.93)	25	1.83 (1.22-2.76)	*0.46*
Gap time 3-5 years	10	0.94 (0.50-1.77)	19	1.36 (0.86-2.16)	25	1.56 (1.04-2.34)	7	1.41 (0.67-3.00)	*0.36*
Gap time > 5 years	17	1.03 (0.64-1.68)	33	1.63 (1.14-2.33)	35	1.61 (1.13-2.29)	5	0.90 (0.37-2.19)	*0.81*
Recent use of estrogen + progesterone									
Gap time ≤ 1 year	17	0.95 (0.58-1.55)	27	0.99 (0.67-1.47)	55	1.67 (1.23-2.25)	31	1.93 (1.31-2.85)	*0.004*
Gap time 1-3 years	6	0.69 (0.31-1.55)	12	1.02 (0.57-1.82)	12	0.93 (0.52-1.66)	8	1.77 (0.87-3.60)	*0.28*
Gap time 3-5 years	2	0.64 (0.16-2.58)	6	1.37 (0.61-3.07)	5	0.92 (0.38-2.23)	3	1.62 (0.52-5.10)	*0.40*
Gap time > 5 years	6	1.04 (0.46-2.32)	12	1.65 (0.93-2.92)	7	0.86 (0.41-1.82)	1	0.43 (0.06-3.10)	*0.14*
Recent use of estrogen + dydrogesterone									
Gap time ≤ 1 year	22	1.40 (0.90-2.16)	24	1.14 (0.75-1.74)	30	1.42 (0.97-2.08)	11	1.18 (0.64-2.18)	*0.88*
Gap time 1-3 years	9	1.54 (0.79-2.99)	10	1.36 (0.72-2.54)	10	1.23 (0.65-2.31)	5	1.87 (0.77-4.56)	*0.98*
Gap time 3-5 years	2	0.93 (0.23-3.71)	4	1.61 (0.60-4.31)	4	1.28 (0.48-3.43)	2	1.71 (0.43-6.90)	*0.86*
Gap time > 5 years	4	1.29 (0.48-3.45)	2	0.52 (0.13-2.09)	9	2.12 (1.09-4.12)	2	1.92 (0.48-7.77)	*0.59*
Recent use of estrogen + other progestagen									
Gap time ≤ 1 year	94	1.96 (1.55-2.49)	112	1.81 (1.46-2.24)	111	1.82 (1.46-2.25)	52	2.37 (1.76-3.20)	*0.25*
Gap time 1-3 years	30	1.70 (1.17-2.47)	44	2.07 (1.51-2.83)	42	1.99 (1.44-2.74)	12	2.04 (1.14-3.65)	*0.42*
Gap time 3-5 years	6	1.15 (0.51-2.58)	9	1.32 (0.68-2.56)	16	2.27 (1.37-3.75)	2	1.14 (0.28-4.57)	*0.43*
Gap time > 5 years	7	0.94 (0.44-1.98)	19	2.15 (1.35-3.40)	19	2.15 (1.35-3.42)	2	0.99 (0.25-4.01)	*0.43*

Abbreviations: EP-MHT, estrogen-progestagen menopausal hormone therapy; CI, confidence interval

* Adjusted for the same covariates as in Table 2bis. In analyses according to type of recent EP-MHT, models are additionally adjusted for past use of estrogen combined with progesterone, estrogen combined with dydrogesterone, estrogen combined with other progestagens