

**Title: Estrogen-progestagen menopausal hormone therapy and breast cancer: does delay from menopause onset to treatment initiation influence risks?**

Agnès Fournier, Sylvie Mesrine, Marie-Christine Boutron-Ruault, Françoise Clavel-Chapelon

► **To cite this version:**

Agnès Fournier, Sylvie Mesrine, Marie-Christine Boutron-Ruault, Françoise Clavel-Chapelon. Title: Estrogen-progestagen menopausal hormone therapy and breast cancer: does delay from menopause onset to treatment initiation influence risks?: MHT and breast cancer according to timing of treatment initiation. *Journal of Clinical Oncology, American Society of Clinical Oncology*, 2009, 27 (31), pp.5138-43. <10.1200/JCO.2008.21.6432>. <inserm-01319999>

**HAL Id: inserm-01319999**

**<http://www.hal.inserm.fr/inserm-01319999>**

Submitted on 23 May 2016

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

**Title:** Estrogen-progestagen menopausal hormone therapy and breast cancer: does delay from menopause onset to treatment initiation influence risks?

**Authors:** Agnès Fournier, Sylvie Mesrine, Marie-Christine Boutron-Ruault, and Françoise Clavel-Chapelon

**Affiliations:** Inserm (Institut National de la Santé et de la Recherche Médicale), ERI 20 / Université Paris-Sud, EA 4045, IFR 69 / Institut Gustave-Roussy, Villejuif, France

**Acknowledgments of research support**

Supported by Mutuelle Générale de l'Education Nationale; European Community; French League against Cancer; Gustave Roussy Institute; Institut National de la Santé et de la Recherche Médicale; 3M Company; several General Councils of France; Direction Générale de la Santé; Agence Française de Sécurité Sanitaire des Produits de Santé.

**Corresponding author:** Françoise Clavel-Chapelon, Inserm ERI 20 – Equipe E3N, Institut Gustave Roussy, 39 rue Camille Desmoulins, F-94805 Villejuif Cedex, France; Tel: +33 1 42 11 41 48; Fax: +33 1 42 11 40 00; E-mail: clavel@igr.fr.

**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.

1 **ABSTRACT**

2 **PURPOSE**

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

6 **MATERIALS AND METHODS**

7 Between 1992 and 2005, 1,726 invasive breast cancers were identified among  
8 53,310 postmenopausal women from the French E3N cohort (mean duration of  
9 follow-up: 8.1 years). Hazard ratios (HRs) and confidence intervals (CI) were  
10 estimated using Cox models, with MHT never-users as the reference.

11 **RESULTS**

12 Among recent users of EP-MHT, the risk of breast cancer varied according to the  
13 timing of treatment initiation. This variation was confined to short durations of use ( $\leq 2$   
14 years): the HR was 1.54 (95% CI 1.28-1.86) for short treatments initiated in the 3-  
15 year period following menopause onset and 1.00 (95% CI 0.68-1.47) for short  
16 treatments initiated later ( $p=0.04$  for homogeneity). This pattern of risks was however  
17 not observed in users of EP-MHT containing progesterone, among whom there was  
18 no significantly increased risk associated with short durations of use (HR 0.87, 95%  
19 CI 0.57-1.32 for treatments initiated  $\leq 3$  years after menopause and 0.90, 95% CI  
20 0.45-1.81 for treatments initiated later). Longer durations of EP-MHT use were  
21 generally associated with increases in breast cancer risk, whatever the gap time.

22 **CONCLUSION**

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

- 1 cancer risk. Estrogen-progesterone combinations might be an exception in this
- 2 regard.
- 3

## 1 INTRODUCTION

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

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

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

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

7

## 1 **MATERIALS AND METHODS**

### 2 **The E3N cohort**

3 E3N is a prospective cohort of 98,995 French women born between 1925 and  
4 1950 and insured by a health insurance plan covering mostly teachers. Participants,  
5 who gave written informed consent, completed self-administered questionnaires  
6 (sent biennially between 1990 and 2005) that addressed medical history,  
7 menopausal status, and a variety of lifestyle characteristics. The study was approved  
8 by the French National Commission for Data Protection and Privacy. E3N is the  
9 French component of the European Prospective Investigation into Cancer and  
10 Nutrition.<sup>15</sup>

11

### 12 **Identification of breast cancer cases**

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

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

20

### 21 **Assessment of MHT Use**

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

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

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

13

#### 14 **Assessment of menopause**

15 Information on menopausal status was updated in each questionnaire. Women  
16 were considered postmenopausal if they had had 12 consecutive months without  
17 menstrual periods (unless due to hysterectomy), had undergone bilateral  
18 oophorectomy, had ever used MHT, or self-reported that they were postmenopausal.  
19 Age at menopause was defined as age at last menstrual period; age at bilateral  
20 oophorectomy; or self-reported age at menopause. We excluded women for whom  
21 age at menopause could not be determined accurately (e.g. in case of both no self-  
22 reported age at menopause and an unknown age at last menstrual period, or of no  
23 self-reported age at menopause and MHT use preceding the cessation of  
24 menstruation, or of cessation of menstruation due to hysterectomy).

25



## 1 **Population for analysis and follow-up**

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

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

17

## 18 **Statistical analysis**

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

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

13           Time from menopause onset to treatment initiation (hereafter referred to as  
14 “gap time”) was initially planned to be categorized as  $\leq 1$  year (60% of MHT ever-  
15 users), ]1-3] years (22%), ]3-5] years (7%) and more than 5 years (11%). However,  
16 to gain statistical precision and simplicity in reporting results, and on the basis of the  
17 similarity of the estimates in each group, categories were collapsed into  $\leq 3$  years and  
18  $>3$  years (estimates in the initial four gap time categories are shown in online tables).

19           All tests of statistical significance were two-sided, and significance was set at  
20 the .05 level. We performed all analyses using SAS software, version 9.1 (SAS  
21 Institute Inc, Cary, NC).

22

## 1 RESULTS

2 A total of 1,726 first primary invasive breast cancers were diagnosed during  
3 433,647 person-years of follow-up (mean duration: 8.1 years). The main  
4 characteristics of the participants are shown in Table 1.

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

13 In EP-MHT recent users, the variation in HRs according to gap time was  
14 confined to short durations ( $\leq 2$  years) of use (Table 3). The HR was 1.54 (95% CI  
15 1.28-1.86) for treatments of short duration initiated within 3 years of menopause and  
16 1.00 (95% CI 0.68-1.47) for treatments of short duration initiated later (p for  
17 homogeneity = 0.04). This pattern of risks was not observed in recent users of  
18 estrogen-progesterone: there was no significantly increased risk associated with  
19 short durations of use, whatever the gap time. In contrast, short durations of use of  
20 estrogen-dydrogesterone were associated with an increased risk of borderline  
21 statistical significance (HR 1.44, 95% CI 0.99-2.09; p = 0.055) for gap times  $\leq 3$  years  
22 and of 1.14 (95% CI 0.51-2.55) for longer gap times, but the variation in risks was not  
23 statistically significant (p for homogeneity = 0.60). Short durations of use of estrogens  
24 combined with other progestagens were associated with a HR of 1.89 (95% CI 1.53-  
25 2.34) for gap times  $\leq 3$  years and of 1.02 (95% CI 0.59-1.78) for longer gap times (p

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

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

7

## 1 **DISCUSSION**

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

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

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

1 settings. The present analysis adds to our previous results that different  
2 progestagens in EP-MHT may impact breast cells differently.<sup>16,17</sup>

3         Only the WHI group assessed the impact of the timing of MHT initiation on  
4 breast cancer risk.<sup>12,13</sup> In line with our results, this group found that HRs associated  
5 with EP-MHT use (consisting in conjugated equine estrogens combined with  
6 medroxyprogesterone acetate) decreased with increasing gap time. For women who  
7 initiated EP-MHT within 5 years of menopause, the HR estimate in the WHI was of  
8 1.32 during the first two years of treatment, but this estimate, based on only 7 cases,  
9 was imprecise. With more than 100 cases in women using EP-MHT (containing  
10 progestagens other than progesterone or dydrogesterone) for 2 years or less, we  
11 found a significant doubling in risk when MHT began within 3 years of menopause,  
12 as compared with MHT never-use. For the other durations of use, estimates of the  
13 WHI and ours are consistent.

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

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

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

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

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

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



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

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

## REFERENCES

1. Barrett-Connor E: Hormone replacement therapy. *BMJ* 317:457-461, 1998
2. Hulley S, Grady D, Bush T, et al: Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/progestin Replacement Study (HERS) Research Group. *JAMA* 280:605-613, 1998
3. Rossouw JE, Anderson GL, Prentice RL, et al: Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *JAMA* 288:321-333, 2002
4. Vickers MR, MacLennan AH, Lawton B, et al: Main morbidities recorded in the women's international study of long duration oestrogen after menopause (WISDOM): a randomised controlled trial of hormone replacement therapy in postmenopausal women. *BMJ* 335:239, 2007
5. 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
6. Million Women Study Collaborators: Breast cancer and hormone-replacement therapy in the Million Women Study. *Lancet* 362:419-427, 2003
7. The European Agency for the Evaluation of Medicinal Products. EMEA public statement on recent publications regarding hormone replacement therapy; December 3, 2003.  
<http://www.emea.europa.eu/pdfs/human/press/pus/3306503en.pdf>

8. Phillips LS, Langer RD: Postmenopausal hormone therapy: critical reappraisal and a unified hypothesis. *Fertil Steril* 83:558-566, 2005
9. Barrett-Connor E: Hormones and heart disease in women: the timing hypothesis. *Am J Epidemiol* 166:506-510, 2007
10. Rossouw JE, Prentice RL, Manson JE, et al: Postmenopausal hormone therapy and risk of cardiovascular disease by age and years since menopause. *JAMA* 297:1465-1477, 2007
11. World Health Organization: The global burden of disease: 2004 update. Geneva, World Health Organization, 2008. <http://www.who.int/evidence/bod>
12. Prentice RL, Chlebowski RT, Stefanick ML, et al: Estrogen plus progestin therapy and breast cancer in recently postmenopausal women. *Am J Epidemiol* 167:1207-1216, 2008
13. Prentice RL, Chlebowski RT, Stefanick ML, et al: Conjugated equine estrogens and breast cancer risk in the Women's Health Initiative clinical trial and observational study. *Am J Epidemiol* 167:1407-1415, 2008
14. Collins JA, Blake JM, Crosignani PG: Breast cancer risk with postmenopausal hormonal treatment. *Hum Reprod Update* 11:545-560, 2005
15. 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
16. 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

17. Fournier A, Fabre A, Mesrine S, et al: Use of different postmenopausal hormone therapies and risk of histology- and hormone receptor-defined invasive breast cancer. *J Clin Oncol* 26:1260-1268, 2008
18. Persson I, Weiderpass E, Bergkvist L, et al: Risks of breast and endometrial cancer after estrogen and estrogen-progestin replacement. *Cancer Causes Control* 10:253-260, 1999
19. 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
20. Chen CL, Weiss NS, Newcomb P, et al: Hormone replacement therapy in relation to breast cancer. *JAMA* 287:734-741, 2002
21. 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
22. Brinton LA, Richesson D, Leitzmann MF, et al: Menopausal Hormone Therapy and Breast Cancer Risk in the NIH-AARP Diet and Health Study Cohort. *Cancer Epidemiol Biomarkers Prev* 17:3150-3160, 2008
23. Schairer C, Lubin J, Troisi R, et al: Menopausal estrogen and estrogen-progestin replacement therapy and breast cancer risk. *JAMA* 283:485-491, 2000
24. Porch JV, Lee IM, Cook NR, et al: Estrogen-progestin replacement therapy and breast cancer risk: the Women's Health Study (United States). *Cancer Causes Control* 13:847-854, 2002
25. Weiss LK, Burkman RT, Cushing-Haugen KL, et al: Hormone replacement therapy regimens and breast cancer risk(1). *Obstet Gynecol* 100:1148-1158, 2002

26. 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
27. Stahlberg C, Pedersen AT, Lynge E, et al: Increased risk of breast cancer following different regimens of hormone replacement therapy frequently used in Europe. *Int J Cancer* 109:721-727, 2004
28. Wu AH, Yu MC, Tseng CC, et al: Body size, hormone therapy and risk of breast cancer in Asian-American women. *Int J Cancer* 120:844-852, 2007
29. Horwitz KB, Sartorius CA: Progestins in hormone replacement therapies reactivate cancer stem cells in women with preexisting breast cancers: a hypothesis. *J Clin Endocrinol Metab* 93:3295-3298, 2008
30. Jordan VC: The 38th David A. Karnofsky lecture: the paradoxical actions of estrogen in breast cancer--survival or death? *J Clin Oncol* 26:3073-3082, 2008
31. Milanese TR, Hartmann LC, Sellers TA, et al: Age-related lobular involution and risk of breast cancer. *J Natl Cancer Inst* 98:1600-1607, 2006
32. Banks E, Beral V, Cameron R, et al: Agreement between general practice prescription data and self-reported use of hormone replacement therapy and treatment for various illnesses. *J Epidemiol Biostat* 6:357-363, 2001
33. Sandini L, Pentti K, Tuppurainen M, et al: Agreement of self-reported estrogen use with prescription data: an analysis of women from the Kuopio Osteoporosis Risk Factor and Prevention Study. *Menopause* 15:282-289, 2008

## **Acknowledgments**

The authors are indebted to all participants for providing data and to practitioners for providing pathology reports. They thank R. Chaït, M. Fangon, L. Hoang, C. Kernaleguen and M. Niravong for technical assistance; the E3N group; and F. Berrino for its support and fruitful discussions.

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)
<b>Year of birth</b>				
[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%)
<b>Age at start of follow-up, years</b>	54.6 (4.5)	56.4 (4.9)	52.9 (3.3)	55.4 (4.9)
<b>Age at menarche, years</b>				
< 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%)
<b>Parity</b>				
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%)
<b>Breastfeeding*</b>				
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%)
<b>Age at menopause, years</b>	50.6 (3.6)	50.8 (4.0)	50.8 (3.0)	49.1 (4.3)
<b>Type of menopause</b>				
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%)
<b>Personal history of benign breast disease</b>				
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%)
<b>Family history of breast cancer in first degree relatives</b>				
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%)
<b>Family history of breast cancer in other relatives</b>				
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%)
<b>Height, cm</b>	161.4 (5.7)	161.1 (5.8)	161.7 (5.6)	161.0 (5.7)

<b>Body mass index, kg/m<sup>2</sup></b>				
≤ 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%)
<b>Total physical activity, MET-h/wk</b>				
<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%)
<b>Previous use of oral contraceptives</b>				
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%)
<b>Use of oral progestagens alone in premenopause</b>				
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%)
<b>Mammography in the previous follow-up period</b>				
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)†
<b>Gap time</b>						
≤ 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)
<i>P for homogeneity</i>		<i>0.06</i>		<i>0.07</i>		<i>0.82</i>

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								<i>P for trend with duration of use</i>
	≤2 years		]2-5] years		]5-10] years		>10 years		
	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)	
<b>Recent use of any type of EP-MHT</b>									
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
<i>P for homogeneity</i>		0.04		0.89		0.96		0.10	
<b>Recent use of estrogen + progesterone</b>									
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
<i>P for homogeneity</i>		0.93		0.14		0.11		0.20	
<b>Recent use of estrogen + dydrogesterone</b>									
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
<i>P for homogeneity</i>		0.60		0.59		0.44		0.59	
<b>Recent use of estrogen + other progestagen</b>									
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
<i>P for homogeneity</i>		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)†
<b>Gap time</b>						
≤ 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								<i>P for trend with duration of use</i>
	≤2 years		]2-5] years		]5-10] years		>10 years		
	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)	
<b>Recent use of any type of EP-MHT</b>									
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
<b>Recent use of estrogen + progesterone</b>									
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
<b>Recent use of estrogen + dydrogesterone</b>									
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
<b>Recent use of estrogen + other progestagen</b>									
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