

**Menopausal hormone replacement therapy and risk of ovarian cancer in a prospective
study**

James V. Lacey, Jr., Ph.D.

Pamela J. Mink, Ph.D.

Jay H. Lubin, Ph.D.

Mark E. Sherman, M.D.

Rebecca Troisi, Sc.D.

Patricia Hartge, Sc.D.

Arthur Schatzkin, M.D., Dr.P.H.

Catherine Schairer, Ph.D.

Author Affiliations: Division of Cancer Epidemiology and Genetics, National Cancer Institute.

Correspondence to: James V. Lacey Jr., Ph.D., National Cancer Institute, Division of
Epidemiology and Genetics, 6120 Executive Blvd. MSC 7234, Rockville, MD 20852-7234;
Telephone: 301-435-3985, FAX: 301-402-0916, E-mail: jimlacey@nih.gov.

Note: Portions of this article were presented at the 92nd Annual Meeting of the American
Association for Cancer Research, March 24-28, 2001, New Orleans, LA.

Keywords: ovarian carcinoma; estrogen replacement therapy; estrogens; progesterone.

Running title: HRT and ovarian cancer risk.

Text word count: 3,661 (Limit: 4,000, but count includes in-text references and section titles).

ABSTRACT

Context. The association between menopausal hormone replacement therapy and ovarian cancer is unclear.

Objective. To determine whether estrogen replacement therapy and estrogen-progestin replacement therapy increase the risk of ovarian cancer.

Design. A 1980-1998 cohort study of former participants in the Breast Cancer Detection Demonstration Project, a nationwide breast cancer screening program.

Setting. Twenty-nine U.S. clinical centers.

Participants. A total of 44,241 postmenopausal women (mean age at start of follow-up, 57 years).

Main Outcome Measure. Incident ovarian cancer.

Results. We identified 329 cases of ovarian cancer. In time-dependent analyses adjusted for age, type of menopause, and oral contraceptive use, ever-use of estrogen-only was significantly associated with ovarian cancer (relative risk [RR] 1.6, 95% confidence interval [CI], 1.2-2.0). Neither estrogen-progestin-only use (RR 1.1, 95% CI 0.64-1.7) nor estrogen-progestin use following estrogen-only use (RR=0.96, 95% CI, 0.39-.24) was associated with ovarian cancer. Increasing duration of estrogen-only use was positively associated with ovarian cancer. The RRs for 10-19 years and for 20 or more years were 1.8 (95% CI, 1.1-3.0) and 3.2 (95% CI, 1.7-5.7), respectively, with a P-value for trend of 0.001 and a 7% (95% CI, 2% to 13%) increase in RR per year of estrogen-only use. There was no association between duration of estrogen-progestin use and ovarian cancer (RR for four or more years of use=1.1, 95% CI, 0.51-2.3). No consistent associations emerged for time since last estrogen-only use. We observed significantly elevated

RRs with increasing duration of estrogen-only use across all strata of other ovarian cancer risk factors and among women who no longer had a uterus.

Conclusion. Women who used estrogen-only replacement therapy, particularly for ten or more years, were at significantly increased risk of ovarian cancer in this study. Women who used estrogen-progestin replacement therapy were not at increased risk, but risk associated with long-term estrogen-progestin replacement therapy warrants further investigation.

Abstract word count: 293 (limit is 300).

INTRODUCTION

Despite case-control studies (1), pooled analyses (2;3), and meta-analyses (4;5), the potential association between menopausal hormone replacement therapy (HRT) and ovarian cancer remains unresolved. Most retrospective studies found no association, and the studies that showed increased risks predominantly included weak, non-significant associations and an absence of dose-response (6). Small size (7) and incomplete information about other ovarian cancer risk factors (8) limited the few available prospective studies. A large, prospective study recently reported a statistically significant two-fold increased risk of ovarian cancer mortality among long-term users of estrogen-only replacement therapy (ERT) (9). Comparable investigations of incident ovarian cancer have not been published. In addition, although use of combined estrogen-progestin replacement therapy (EPRT) has increased recently (10), epidemiologic data on EPRT are limited (8;11); most publications to date assessed only ERT use. To explore these issues, we analyzed data from the Breast Cancer Detection Demonstration Project (BCDDP) Follow-Up Study, a large prospective cohort. Multiple data collections between 1980 and 1998 included specific information on ERT and EPRT.

METHODS

Study participants were selected from the Breast Cancer Detection Demonstration Project (BCDDP), a mammography screening program conducted at 29 U.S. screening centers between 1973 and 1980 by the American Cancer Society and the U.S. National Cancer Institute (NCI) (12). In 1979, NCI initiated a follow-up study of 64,182 of the original 283,222 participants: (i) all 4,275 women diagnosed with breast cancer during the Project, (ii) all 25,114 women who underwent breast surgery during the Project but had no evidence of malignant disease, (iii) all

9,628 women who were recommended by the Project for surgical consultation, but for whom neither biopsy nor aspiration was performed, and (iv) 25,165 women sampled from participants who had neither surgery nor recommendation for surgical consultation during screening (13). The NCI Institutional Review Board approved the study. All participants provided informed consent.

The BCDDP Follow-Up Study consisted of four phases. Phase I (1979-1986) involved a baseline telephone interview (completed by 61,431 women, or 96%) and up to six (usually four) annual telephone follow-up interviews through 1986. Phases 2, 3, and 4 each used single, self-administered, mailed questionnaires between 1987 and 1989, 1993 and 1995, and 1995 and 1998, respectively. Respondents who were not known to be deceased at the end of the previous phase were sent each subsequent questionnaire. Nonrespondents to mailed questionnaires were interviewed by telephone, if possible.

Phase 1 interviews collected age at first use and duration of use of female hormones (excluding creams), but did not distinguish ERT from EPRT. The Phase 2 questionnaire included use of menopausal hormones in the form of shots, creams, patches, or pills since the last interview. This questionnaire queried menopausal ERT and EPRT, duration of ERT and EPRT, and number of days in the month progestins were used. Phases 3 and 4 updated these data and collected pill names and doses. Each phase included questions about menopausal status, reproductive surgeries, and other risk factors. Interviews during the screening phase collected demographic data (e.g., education level and ethnicity) and measured height and weight, which were updated in Phase 2.

Analytic Data Set.

We excluded 12,581 women who reported a bilateral oophorectomy, 4 women who died, 30 women diagnosed with ovarian cancer, and 4,086 women diagnosed with breast cancer before the start of follow-up. We limited analysis to the remaining women who were menopausal before the start of follow-up or who became menopausal during follow-up. We defined menopause as no menstrual period for at least three months or as a result of hysterectomy with at least one ovary retained. Women who stopped menstruating because of hysterectomy but who retained at least one ovary or whose ovarian status was uncertain were considered to have reached menopause at age 57 years (the 75th percentile for age at menopause in the study population) or their age at hysterectomy, whichever was later. They maintained an unknown value for analysis of age at menopause. We excluded 483 women whose menopausal status remained unknown throughout the follow-up study. Analysis therefore included 44,247 participants who completed a Phase 1 interview. The numbers who subsequently completed Phase 2, 3, and 4 questionnaires were 37,657 (85%), 32,891 (74%), and 31,354 (71%), respectively. Death (2%), refusal (4%), and illness or inability to contact before the end of the questionnaire period (9%) accounted for missing Phase 2 questionnaires. Respective proportions for missing Phase 3 and Phase 4 questionnaires were 8%, 3%, and 15%; and 12%, 4%, and 13%.

Case ascertainment.

Personal history of ovarian cancer was first ascertained in Phase 2. Phases 3 and 4 ascertained ovarian cancer diagnoses since the previous interview. We verified reported ovarian cancer diagnoses through medical record review. Trained personnel completed standardized abstract forms when records were retrieved, and two of us (JVL Jr. and MES) reviewed those original records for this analysis. We linked the cohort to state cancer registries to identify

additional cancer diagnoses and to the National Death Index (NDI) to identify deaths during follow-up (with death certificate retrieval for study deaths). A total of 71% of the women who completed a baseline interview and 85% of the women who completed a Phase 2 questionnaire were linked against state cancer registries.

The final analytic cohort included 329 cases: 118 cases from medical records, 79 cases from registry data, 114 cases from death certificates, and 18 self-reported cases. Records were not available for those 18 cases because they were not received by the end of the study period, nonresponse of physicians or hospitals, or participants did not grant permission for record retrieval. We further classified tumors according to histology data from records or cancer registries: serous (65), endometrioid (41), mucinous (13), clear cell (8), other unclassified (71), or unavailable (132). We defined diagnosis date hierarchically from medical records, state cancer registry data, self-report, or, when no other date of diagnosis was available, death certificates (including time since cancer onset, when available). Fifty-one other participants reported an ovarian cancer, but medical record review revealed another primary tumor (N=43), benign or non-neoplastic lesions (N=6), or metastatic tumors (N=2). We excluded another 6 non-epithelial tumors based on medical records or state cancer registry data.

Analysis.

Follow-up began at the baseline interview date or menopause date, whichever was later. Person-years accrued until the earliest of the following dates: ovarian cancer diagnosis, bilateral (or 2nd) oophorectomy, death from any cause, Phase 4 questionnaire completion, or end of study date. For participants without a Phase 4 questionnaire but with whom we had some contact (e.g., telephone or notice of refusal) during Phase 4, the end of study date was that contact date.

Because NDI was likely to identify deaths among study participants, we assumed all other participants without a Phase 4 questionnaire had not died. For these participants, we calculated mean intervals between Phases 2 and 4 for all participants with completed questionnaires and added those mean intervals to the date of last completed questionnaire to generate an end of study date.

Poisson regression modelled the rate of developing ovarian cancer during follow-up and generated rate ratios (RRs) with 95 percent confidence intervals (CIs) for categorized variables using standard likelihood ratio methods (14). Likelihood-based methods produced CIs for the linear excess RR model (15). We assessed statistical significance of trends via score tests.

We modeled attained age and all HRT exposures as time-dependent variables. Participants could contribute person-time to multiple exposure categories during follow-up. When exposure status or duration became unknown, subsequent person-years were assigned to the “unknown” category. HRT use was calculated to one year prior to attained (or current) age to eliminate exposure that was most likely not causal. Because information on progestin use was not collected until the Phase 2 questionnaire, progestin use was unknown for the 6,586 participants who did not answer this interview (and for other participants who could not recall whether they had used progestins). For these participants, exposed person-time and cases associated with ERT were included in the “ERT, unknown progestins (PRT)” category if the participant reported a natural menopause; otherwise, they were included in the “ERT-only” category because women with a surgical menopause are less likely to use progestins.

We calculated body mass index (kg/m^2 ; BMI) from measurements obtained during the screening visit closest in time to the baseline follow-up interview. To assess potential confounding by BMI, parity, and other suspected risk factors, we assessed associations between

exposure and ovarian cancer and then evaluated parameter estimate changes in models before and after stratification by (i.e., adjustment for) confounding variables. Fully adjusted models included stratification on age, menopause type (natural, surgical, unknown type), and duration of oral contraceptive use (none, ≤ 2 years, > 2 years).

RESULTS

The 44,241 participants accrued 593,496 person-years of follow-up, with a mean follow-up of 13.4 years (range, 1 month to 19.8 years). The mean age at the start of follow-up was 56.6 years (range, 36-89 years).

Risk factors.

Ovarian cancer was inversely associated with parity, oral contraceptive use, and hysterectomy, and not associated with age at menopause or BMI, in our data. One quarter of respondents reported breast cancer or ovarian cancer in first-degree relatives, but family history of these cancers was not associated with ovarian cancer. Family history of ovarian cancer was not collected until the Phase 4 questionnaire and was therefore unavailable for 29% of the cohort (data not shown).

Participants who were older or had a surgical menopause were more likely to use ERT. Participants who had a natural menopause, had an older age at menopause, had a lower BMI, and used oral contraceptives for longer durations were more likely to use EPRT (Table 1).

Ever-use of HRT.

Compared to no use, ERT-only use was positively and significantly associated with ovarian cancer in age-adjusted (RR=1.4) and fully adjusted models (RR=1.6, 95% CI, 1.2-2.0; Table 2). EPRT use was not associated with ovarian cancer in age-adjusted (RR=1.2) or fully adjusted models (RR=1.2, 95% CI, 0.86-1.8). We further classified EPRT use on the basis of prior ERT use. EPRT use following ERT-only use generated a positive but non-significant association in fully adjusted models (RR=1.5, 95% CI, 0.91-2.3). However, further adjustment for duration of prior ERT-only use among these participants (<10 years vs. ≥10 years) generated a null association (RR=0.96, 95% CI, 0.39-2.4). EPRT-only use was not associated with ovarian cancer (RR=1.1, 95% CI, 0.66-1.8). ERT-only use with unknown progestin use was also significantly associated with ovarian cancer (RR=2.6, 95% CI, 1.8-3.7), but unknown HRT use was not. Fully adjusted estimates were slightly higher than estimates adjusted for age only.

Duration of ERT-only use.

Increasing duration of ERT-only use was positively and significantly associated with ovarian cancer, with an increase in the fully adjusted RR of 0.07 (95% CI, 0.02 to 0.13) for each additional year of use (Table 3). Adjusted models produced stronger associations than unadjusted models. Risk estimates in Table 3 reflect ERT-only use, but models that also included duration of any ERT use (e.g., duration of ERT use among women had unknown progestin use) generated similar associations (e.g., RR=3.4 for 20 or more years of use; p=0.001).

Duration of any EPRT use.

Duration of EPRT use was not associated with ovarian cancer after adjustment for other risk factors and prior ERT use. The RR for four or more years of EPRT use was 1.1, and there was no evidence of a dose-response. The mean person-year-weighted duration of use among women who had used EPRT for 4 or more years was 7.3 years. Among participants who used EPRT following ERT, the RR for 4 or more years of use was 2.8 (95% CI, 1.4-5.9) before adjustment for duration of ERT use and 1.1 (95% CI, 0.49-2.7) after adjustment. Among participants who used EPRT only, 4 or more years of use was not associated with ovarian cancer (RR=0.64, 95% CI, 0.20-2.0).

Duration and hysterectomy status.

Women who had a hysterectomy accounted for most cases that occurred among long-term ERT users: the RRs for 10-19 years of use and 20 or more years of use were 2.0 (95% CI, 0.96-4.3) and 3.4 (95% CI 1.6-7.5; p-value for trend=0.001), respectively, and the RR increased 0.08 per year of use (95% CI, 0.02 to 0.18). Among women who had a natural menopause (i.e., an intact uterus), ERT for less than four years (RR=1.4, 95% CI, 0.92-2.0) and 4-9 years (RR=2.1, 95% CI, 1.3-3.5) were positively associated with ovarian cancer. Almost all cases among EPRT users had a natural menopause (data not shown).

Time since last ERT use.

Compared to never use, current use was not associated with ovarian cancer (RR=1.3, 95% CI, 0.80-2.2). Last ERT-only use less than two years ago was significantly associated with ovarian cancer (RR=3.9, 95% CI, 2.4-6.4). Associations with last use less than 10 years ago (RR=1.2) and 10 or more years ago (RR=1.2) were non-significant.

Because long-term use and recent use can be correlated, we assessed duration in recent vs. former users. Only 20 cases occurred among current users, and ERT-only use for 10-19 years (RR=1.6) and for 20 or more years (RR=1.4) were positively, but not significantly, associated with ovarian cancer. Among former users, ERT-only use for 10-19 years (RR=1.7) and for 20 or more years (RR=2.5) were significantly associated with ovarian cancer.

Current use of any EPRT was not associated with ovarian cancer (RR=0.97, 95% CI, 0.58-1.6). Last EPRT use less than two years ago was significantly associated with ovarian cancer (RR=2.3, 95% CI, 1.1-4.8), but there was no association with last use two or more years ago (RR=1.4, 95% CI 0.72-2.7). Too few women used EPRT to assess associations by duration and time since last use.

Other results.

Analyses further stratified by parity (nulliparous vs. parous) or median BMI (23.4 kg/m²) revealed no differences, although most cases that occurred among EPRT users, regardless of prior ERT-only use, had BMI below the median. Increasing duration of ERT use was positively and significantly associated with ovarian cancer among both serous (N=65) and endometrioid (N=41) tumors (data not shown).

Similar associations for duration of ERT emerged after excluding women whose age at menopause was unknown or assigned to 57 years, women whose menopause type was unknown, women whose ovarian cancer was based on self-report only, or women whose cancers were identified via death certificates only. Including the participants diagnosed with breast cancer before follow-up did not change the results. We saw identical results after restricting the analyses based on method of case ascertainment (questionnaires, death certificates, and cancer

registries) and after only considering ERT exposures that occurred two or more years before diagnosis in cases. EPRT data were too sparse for similar stratifications.

COMMENT

We observed significant associations between ERT use and incident ovarian cancer in this prospective study of 44,241 postmenopausal U.S. women followed for 18 years. In time-dependent analyses that adjusted for other ovarian cancer risk factors and included relatively large numbers of long-term ERT users, risk increased significantly and consistently with increasing duration of use.

A recent study of 944 fatal ovarian cancers among 211,581 postmenopausal women followed from 1982-1996 identified two-fold increased risks associated with 10 or more years of ERT use (9). The study adjusted for hysterectomy, oral contraceptive use, and other risk factors, but assessed exposure only through 1982 and lacked EPRT data. Other cohort (7) and case-control (2;16-25) studies report positive associations with ERT use, although numerous inverse (26-29) and null (1;8;30-33) associations have been published. One meta-analysis of 15 studies concluded ERT does not increase risk (5), but another meta-analysis of nine studies reported statistically significant summary odds ratios (OR) for ever-use of ERT (OR=1.15) and more than 10 years of ERT (OR=1.27) (34).

EPRT use was not associated with ovarian cancer in our data. A slightly increased risk among participants who used EPRT following ERT-only use disappeared after adjustment for prior ERT use, which was, on average, for six years' duration. Our analysis captured HRT use through 1998 but did not include many cases who had used EPRT for more than four years. Whether longer durations of EPRT are associated with ovarian cancer remains unclear.

One other report suggested risk associated with ERT exceeded risk associated with EPRT. In a case-control study of 327 non-mucinous cases and 564 controls, the adjusted OR per year of ERT (1.06, 95% CI, 1.01-1.10) and EPRT (1.02, 95% CI, 0.91-1.13) resembled our results, which were based on larger numbers of long-term ERT users but equally small numbers of EPRT users (21). A Swedish record linkage study—unadjusted by design—reported no association between ovarian cancer incidence and EPRT (8).

In our study, adjustment for confounding by hysterectomy and oral contraceptives had minimal effect on ever-use risk estimates but consistently increased duration risk estimates. Incomplete control for hysterectomy, oral contraceptives, and other risk factors may account for null or inverse associations in other studies. One meta-analysis (5) reported a summary OR of 1.1 from 15 heterogeneous studies and a statistically significant OR of 1.3 from four similarly designed U.S. studies (21;23-25) that used population-based controls and adjusted for hysterectomy and other risk factors. A pooled analysis (3) showed no association with ever-use (pooled OR=1.0, 95% CI 0.9-1.2) in five studies (1;26;29;35) that were unadjusted for hysterectomy but a significant positive association (pooled OR=1.3, 95% CI 1.1-1.5) in four studies (2;20;21;36) that included adjustment. Those four studies also reported positive, but not statistically significant, associations with increasing ERT duration. Similar re-analysis (2) of four European studies (17-19;22) generated a statistically significant OR for ever-use; here, too, control for confounders increased the OR.

Declining ERT use in the late 1970s (3) reduced the number of potential long-term users and may have prevented earlier studies from detecting an association with a rare outcome such as ovarian cancer. A pooled analysis of 12 case-control studies included only 51 incident cases who had used ERT for more than 10 years (1) and three other case-control studies included small

numbers of long-term ERT users (21;29;30). Compared to results published after seven years (36), follow-up for 14 years doubled the number of ovarian cancer deaths and produced stronger associations with ERT in the prospective mortality study (9). We also observed stronger associations with long-term ERT use after 18 years of follow-up than in analyses censored at 1986 (Phase 1), 1989 (Phase 2), or 1995 (Phase 3; data not shown). Small numbers of long-term users and the lower (i.e., than endometrial cancer) incidence rates might also explain why ovarian cancer incidence rates did not rise and fall—as did endometrial cancer rates—in response to the shifting population patterns of ERT use (37).

In addition to the inconsistent epidemiologic data, lack of functional steroid receptors and demonstrable estrogen effects *in vitro* (38) raised question about biologic plausibility. Recent data, however, increasingly provide biologic support for a relationship. In a rabbit model, estrogen induced ovarian cancer cell line growth (39) and directly stimulated the ovarian surface epithelium—the suspected pathologic origin of most epithelial ovarian carcinomas (40) (41). Epithelial ovarian cancer cell lines expressed estrogen receptors (42), and recent work demonstrated estrogen receptor-alpha, estrogen receptor-beta, and androgen receptor expression in both normal and malignant ovarian epithelial cells (43). Evidence that progestins may reduce the risk of ovarian cancer (38) provides a biologic basis for weak or null associations with HRT formulations that include progestins.

For women who did not complete all mailed questionnaires, our analysis assumed no change in oophorectomy and hysterectomy status since their last questionnaire. Inaccurate oophorectomy reporting could be associated with HRT use and generate a spurious association, but a subset of BCDDP participants reported gynecologic surgery with reasonable accuracy in a previous study (44). Analyses excluding women with unknown oophorectomy or hysterectomy

produced similar results. ERT use that leads to side effects and hysterectomy could theoretically introduce a detection bias for ovarian cancers detected at hysterectomy. However, this seems unlikely in our study because, although 2% of non-cases and 7% of cases had a hysterectomy during follow-up, only four of those cases used ERT. Current HRT use was not associated with ovarian cancer, but the significantly increased risk among ERT-only users and EPRT users whose last use was less than two years ago may reflect a detection bias if participants stopped taking HRT—perhaps because of symptoms—shortly before diagnosis. Inclusion of women with unknown age at menopause can bias analyses of breast cancer and HRT (45;46). Age at menopause was not associated with ovarian cancer in our data, and our results were identical after excluding participants whose age at menopause was unknown or assigned to 57 years.

Although HRT preparations used today differ from the ERT used during this study's early years, our repeated exposure assessment through 1998 ensured generalizable HRT data. Some hormone exposures occurred before diagnosis but were reported after diagnosis; therefore, differential recall, in addition to exposure misclassification (47), by cases and non-cases was possible. However, only one case reported her first ERT use and ovarian cancer diagnosis on the same questionnaire. Mailed questionnaires included other potential ovarian cancer risk factors except tubal ligation. We queried family history of ovarian cancer only after 1995, but control for family history of breast cancer or ovarian cancer did not change the results.

Almost all cases who used ERT for 10 or more years in our study reported a hysterectomy. Long-term ERT, which significantly increases endometrial cancer risk (48), can be prescribed for women who no longer have a uterus because their endometrial cancer risk is negligible. However, a significantly increased ovarian cancer risk among women without a uterus suggests that consideration of ovarian cancer risk should accompany long-term ERT use.

In summary, women who used ERT, particularly for ten or more years, were at significantly increased risk of ovarian cancer in this study. Women who used EPRT were not at increased risk, but risk associated with long-term EPRT remains unclear. ERT and EPRT differentially affect both breast (13) and endometrial (48) cancer risk and may do the same for ovarian cancer. Additional data on long-term ERT and EPRT use, with particular attention to potential confounding by other ovarian cancer risk factors, will be necessary to confirm these observations.

Acknowledgements.

We thank the BCDDP participants; Susan Englehart, Catherine Ann Grundmayer, and the members of the BCDDP Staff at Westat Inc, Rockville, MD; and Leslie Carroll, Franklin Demuth, and Jennifer Boyd-Morin of IMS Inc, Silver Spring, MD, for computer support.

We acknowledge the California Department of Health Services, Cancer Surveillance Section; Florida Cancer Data System, under contract to the Florida Department of Health; Maryland Cancer Registry, Maryland Department of Health and Mental Hygiene; Michigan Cancer Surveillance Program within the Division of Vital Records and Health Statistics, Michigan Department of Community Health; Pennsylvania Department of Health; Tennessee Cancer Registry; Texas Department of Health; and the states of Arizona, Georgia, Hawaii, Idaho, Iowa, New Jersey, New York, North Carolina, Ohio, Oregon, and Rhode Island for providing data from their cancer registries for use in these analyses. The views expressed in this manuscript are solely those of the authors, and do not necessarily reflect the opinions of any state agency listed above.

Reference List

- 1 Whittemore AS, Harris R, Itnyre J. Characteristics relating to ovarian cancer risk: collaborative analysis of 12 US case-control studies. II. Invasive epithelial ovarian cancers in white women. Collaborative Ovarian Cancer Group. *Am J Epidemiol* 1992; 136(10):1184-1203.
- 2 Negri E, Tzonou A, Beral V, Laggiou P, Trichopoulos D, Parazzini F et al. Hormonal therapy for menopause and ovarian cancer in a collaborative re- analysis of European studies. *Int J Cancer* 1999; 80(6):848-851.
- 3 Beral V, Banks E, Reeves G, Appleby P. Use of HRT and the subsequent risk of cancer. *J Epidemiol Biostat* 1999; 4(3):191-210.
- 4 Garg PP, Kerlikowske K, Subak L, Grady D. Hormone replacement therapy and the risk of epithelial ovarian carcinoma: a meta-analysis [see comments]. *Obstet Gynecol* 1998; 92(3):472-479.
- 5 Coughlin SS, Giustozzi A, Smith SJ, Lee NC. A meta-analysis of estrogen replacement therapy and risk of epithelial ovarian cancer. *J Clin Epidemiol* 2000; 53(4):367-375.
- 6 Weiss NS, Rossing MA. Oestrogen-replacement therapy and risk of ovarian cancer. *Lancet* 2001; 358(9280):438.
- 7 Hoover R, Gray LA, Sr., Fraumeni JF, Jr. Stilboestrol (diethylstilbestrol) and the risk of ovarian cancer. *Lancet* 1977; 2(8037):533-534.
- 8 Persson I, Yuen J, Bergkvist L, Schairer C. Cancer incidence and mortality in women receiving estrogen and estrogen- progestin replacement therapy--long-term follow-up of a Swedish cohort. *Int J Cancer* 1996; 67(3):327-332.
- 9 Rodriguez C, Patel AV, Calle EE, Jacob EJ, Thun MJ. Estrogen replacement therapy and ovarian cancer mortality in a large prospective study of US women. *JAMA* 2001; 285(11):1460-1465.
- 10 Brett KM, Madans JH. Use of postmenopausal hormone replacement therapy: estimates from a nationally representative cohort study. *Am J Epidemiol* 1997; 145(6):536-545.
- 11 Risch HA, Marrett LD, Jain M, Howe GR. Differences in risk factors for epithelial ovarian cancer by histologic type. Results of a case-control study. *Am J Epidemiol* 1996; 144(4):363-372.
- 12 Schairer C, Byrne C, Keyl PM, Brinton LA, Sturgeon SR, Hoover RN. Menopausal estrogen and estrogen-progestin replacement therapy and risk of breast cancer (United States). *Cancer Causes Control* 1994; 5(6):491-500.

- 13 Schairer C, Lubin J, Troisi R, Sturgeon S, Brinton L, Hoover R. Menopausal estrogen and estrogen-progestin replacement therapy and breast cancer risk. *JAMA* 2000; 283(4):485-491.
- 14 Breslow NE, Day NE. *Statistical methods in cancer research*. Lyon: International Agency for Research on Cancer, 1987.
- 15 Preston DL, Lubin J, Pierce DA, McConney ME. EPICURE [software]. Release 2.0 ed. Seattle, WA: HiroSoft International Corp, 1996.
- 16 La Vecchia C, Liberati A, Franceschi S. Noncontraceptive estrogen use and the occurrence of ovarian cancer. *J Natl Cancer Inst* 1982; 69(6):1207.
- 17 Booth M, Beral V, Smith P. Risk factors for ovarian cancer: a case-control study. *Br J Cancer* 1989; 60(4):592-598.
- 18 Parazzini F, La Vecchia C, Negri E, Villa A. Estrogen replacement therapy and ovarian cancer risk. *Int J Cancer* 1994; 57(1):135-136.
- 19 Polychronopoulou A, Tzonou A, Hsieh CC, Kaprinis G, Rebelakos A, Toupadaki N et al. Reproductive variables, tobacco, ethanol, coffee and somatometry as risk factors for ovarian cancer. *Int J Cancer* 1993; 55(3):402-407.
- 20 Purdie DM, Bain CJ, Siskind V, Russell P, Hacker NF, Ward BG et al. Hormone replacement therapy and risk of epithelial ovarian cancer. *Br J Cancer* 1999; 81(3):559-563.
- 21 Risch HA. Estrogen replacement therapy and risk of epithelial ovarian cancer. *Gynecol Oncol* 1996; 63(2):254-257.
- 22 Tzonou A, Day NE, Trichopoulos D, Walker A, Saliaraki M, Papapostolou M et al. The epidemiology of ovarian cancer in Greece: a case-control study. *Eur J Cancer Clin Oncol* 1984; 20(8):1045-1052.
- 23 Weiss NS, Lyon JL, Krishnamurthy S, Dietert SE, Liff JM, Daling JR. Noncontraceptive estrogen use and the occurrence of ovarian cancer. *J Natl Cancer Inst* 1982; 68(1):95-98.
- 24 Cramer DW, Hutchison GB, Welch WR, Scully RE, Ryan KJ. Determinants of ovarian cancer risk. I. Reproductive experiences and family history. *J Natl Cancer Inst* 1983; 71(4):711-716.
- 25 Lee NC, Wingo PA, Peterson HB, Rubin GL, Sattin RW. Estrogen therapy and the risk of breast, ovarian, and endometrial cancer. In: Mastroianni LJr, Paulsen C.A., editors. *Aging, reproduction, and the climacteric*. New York: Plenum Press, 1986: 287-303.
- 26 Annegers JF, Strom H, Decker DG, Dockerty MB, O'Fallon WM. Ovarian cancer: incidence and case-control study. *Cancer* 1979; 43(2):723-729.

- 27 Hartge P, Hoover R, McGowan L, Leshner L, Norris HJ. Menopause and ovarian cancer. *Am J Epidemiol* 1988; 127(5):990-998.
- 28 John EM, Whittemore AS, Harris R, Itnyre J. Characteristics relating to ovarian cancer risk: collaborative analysis of seven U.S. case-control studies. Epithelial ovarian cancer in black women. Collaborative Ovarian Cancer Group. *J Natl Cancer Inst* 1993; 85(2):142-147.
- 29 Hempling RE, Wong C, Piver MS, Natarajan N, Mettlin CJ. Hormone replacement therapy as a risk factor for epithelial ovarian cancer: results of a case-control study [see comments]. *Obstet Gynecol* 1997; 89(6):1012-1016.
- 30 Kaufman DW, Kelly JP, Welch WR, Rosenberg L, Stolley PD, Warshauer ME et al. Noncontraceptive estrogen use and epithelial ovarian cancer. *Am J Epidemiol* 1989; 130(6):1142-1151.
- 31 Hildreth NG, Kelsey JL, LiVolsi VA, Fischer DB, Holford TR, Mostow ED et al. An epidemiologic study of epithelial carcinoma of the ovary. *Am J Epidemiol* 1981; 114(3):398-405.
- 32 Purdie D, Green A, Bain C, Siskind V, Ward B, Hacker N et al. Reproductive and other factors and risk of epithelial ovarian cancer: an Australian case-control study. Survey of Women's Health Study Group. *Int J Cancer* 1995; 62(6):678-684.
- 33 Mink PJ, Folsom AR, Sellers TA, Kushi LH. Physical activity, waist-to-hip ratio, and other risk factors for ovarian cancer: a follow-up study of older women. *Epidemiology* 1996; 7(1):38-45.
- 34 Garg PP, Kerlikowske K, Subak L, Grady D. Hormone replacement therapy and the risk of epithelial ovarian carcinoma: a meta-analysis. *Obstet Gynecol* 1998; 92(3):472-479.
- 35 Adami HO, Persson I, Hoover R, Schairer C, Bergkvist L. Risk of cancer in women receiving hormone replacement therapy. *Int J Cancer* 1989; 44(5):833-839.
- 36 Rodriguez C, Calle EE, Coates RJ, Miracle-McMahill HL, Thun MJ, Heath CW, Jr. Estrogen replacement therapy and fatal ovarian cancer. *Am J Epidemiol* 1995; 141(9):828-835.
- 37 Ziel HK, Finkle WD, Greenland S. Decline in incidence of endometrial cancer following increase in prescriptions for opposed conjugated estrogens in a prepaid health plan. *Gynecol Oncol* 1998; 68(3):253-255.
- 38 Risch HA. Hormonal etiology of epithelial ovarian cancer, with a hypothesis concerning the role of androgens and progesterone [see comments]. *J Natl Cancer Inst* 1998; 90(23):1774-1786.

- 39 Baldwin WS, Curtis SW, Cauthen CA, Risinger JI, Korach KS, Barrett JC. BG-1 ovarian cell line: an alternative model for examining estrogen- dependent growth in vitro. *In Vitro Cell Dev Biol Anim* 1998; 34(8):649-654.
- 40 Auersperg N, Edelson MI, Mok SC, Johnson SW, Hamilton TC. The biology of ovarian cancer. *Semin Oncol* 1998; 25(3):281-304.
- 41 Bai W, Oliveros-Saunders B, Wang Q, Acevedo-Duncan ME, Nicosia SV. Estrogen stimulation of ovarian surface epithelial cell proliferation. *In Vitro Cell Dev Biol Anim* 2000; 36(10):657-666.
- 42 Brandenberger AW, Tee MK, Jaffe RB. Estrogen receptor alpha (ER-alpha) and beta (ER-beta) mRNAs in normal ovary, ovarian serous cystadenocarcinoma and ovarian cancer cell lines: down-regulation of ER-beta in neoplastic tissues. *J Clin Endocrinol Metab* 1998; 83(3):1025-1028.
- 43 Lau KM, Mok SC, Ho SM. Expression of human estrogen receptor-alpha and -beta, progesterone receptor, and androgen receptor mRNA in normal and malignant ovarian epithelial cells. *Proc Natl Acad Sci U S A* 1999; 96(10):5722-5727.
- 44 Brinton LA, Hoover RN, Szklo M, Fraumeni JF, Jr. Menopausal estrogen use and risk of breast cancer. *Cancer* 1981; 47(10):2517-2522.
- 45 Rockhill B, Colditz GA, Rosner B. Bias in breast cancer analyses due to error in age at menopause. *Am J Epidemiol* 2000; 151(4):404-408.
- 46 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* 1998; 147(8):718-721.
- 47 Greendale GA, James MK, Espeland MA, Barrett-Connor E. Can we measure prior postmenopausal estrogen/progestin use? The Postmenopausal Estrogen/Progestin Interventions Trial. The PEPI Investigators. *Am J Epidemiol* 1997; 146(9):763-770.
- 48 Pike MC, Ross RK. Progestins and menopause: epidemiological studies of risks of endometrial and breast cancer. *Steroids* 2000; 65(10 -11):659-664.

Table 1. Prevalence of HRT* use by selected factors.

| | None | ERT only | EPRT following ERT | EPRT only | ERT only, unknown PRT | Unknown HRT | Total Person-Years‡ |
|-----------------------------|---------------------------------|----------|--------------------|-----------|-----------------------|-------------|---------------------|
| | Percentage (%) of Person-Years† | | | | | | |
| <u>Attained age (years)</u> | | | | | | | |
| <55 | 65 | 24 | 1 | 2 | 1 | 6 | 22,437 |
| 55-59.9 | 56 | 25 | 3 | 7 | 3 | 6 | 67,667 |
| 60-64.9 | 48 | 27 | 6 | 11 | 4 | 5 | 123,359 |
| 65-69.9 | 43 | 31 | 7 | 9 | 5 | 5 | 128,771 |
| 70-74.9 | 41 | 34 | 7 | 7 | 7 | 5 | 104,005 |
| 75-79.9 | 40 | 35 | 7 | 5 | 8 | 5 | 70,405 |
| ≥80 | 46 | 32 | 4 | 2 | 10 | 6 | 72,566 |
| <u>Menopausal status</u> | | | | | | | |
| Natural menopause | 52 | 19 | 6 | 9 | 8 | 5 | 408,625 |
| Surgical menopause§ | 30 | 57 | 6 | 3 | 0 | 4 | 170,464 |
| Unknown | 55 | 30 | 6 | 5 | 0 | 4 | 10,123 |
| <u>Age at menopause</u> | | | | | | | |
| <45 | 35 | 49 | 5 | 4 | 2 | 5 | 168,809 |
| 45-49.9 | 49 | 28 | 6 | 7 | 6 | 5 | 172,312 |
| 50-53.9 | 52 | 20 | 6 | 9 | 7 | 6 | 181,859 |
| ≥54 years | 43 | 19 | 10 | 15 | 7 | 5 | 10,433 |
| Unknown age | 52 | 19 | 6 | 11 | 7 | 5 | 55,799 |

Oral contraceptive use

| | | | | | | | |
|-------------------------------|----|----|---|----|---|---|---------|
| None¶ | 48 | 31 | 5 | 5 | 6 | 4 | 429,785 |
| ≤2 years | 39 | 30 | 8 | 11 | 5 | 6 | 74,234 |
| >2 years | 40 | 26 | 9 | 12 | 5 | 8 | 85,193 |
| <u>BMI (kg/m²)</u> | | | | | | | |
| ≤21.4 | 43 | 30 | 8 | 10 | 6 | 5 | 157,696 |
| 21.5-23.4 | 43 | 32 | 7 | 8 | 6 | 5 | 150,056 |
| 23.5-26.6 | 46 | 31 | 5 | 6 | 6 | 5 | 147,898 |
| >26.6 | 53 | 29 | 3 | 4 | 5 | 6 | 133,563 |

*HRT, hormone replacement therapy; ERT, estrogen replacement therapy; EPRT, estrogen-progestin replacement therapy, BMI, body mass index.

†Percentages may not sum to 100 because of rounding.

‡Excludes 3,884 PY among women who used progestin-only and 402 PY among women who used “progestin, estrogen unknown”.

§Hysterectomy with or without unilateral oophorectomy at menopause; see Methods.

¶Includes 1,851 PY among women with unknown oral contraceptive use.

Table 2. Rate ratios (RRs) and 95 per cent confidence intervals (CIs) for ever-use of HRT and ovarian cancer.

| HRT | PY | Cases | Age-Adjusted* | | Multivariate-Adjusted† | |
|------------------------|---------|-------|---------------|----------|------------------------|----------|
| | | | RR | 95% CI | RR | 95% CI |
| No use | 270,520 | 120 | 1.0 | Referent | 1.0 | Referent |
| ERT-only | 179,065 | 116 | 1.4 | 1.1-1.8 | 1.6 | 1.2-2.0 |
| ERT, unknown PRT | 32,565 | 40 | 2.4 | 1.7-3.5 | 2.6 | 1.8-3.7 |
| Any EPRT | 77,019 | 39 | 1.2 | 0.80-1.7 | 1.2 | 0.86-1.8 |
| <i>EPRT after ERT‡</i> | 34,619 | 21 | 0.85 | 0.35-2.1 | 0.96 | 0.39-2.4 |
| <i>EPRT-only</i> | 42,400 | 18 | 1.0 | 0.61-1.6 | 1.1 | 0.64-1.7 |
| Unknown HRT | 30,043 | 14 | 1.0 | 0.59-1.8 | 1.1 | 0.63-1.9 |

*Adjusted for attained age.

†Adjusted for attained age, menopause type (natural menopause, surgical menopause, unknown type), and duration of oral contraceptive use (none, ≤ 2 years, > 2.0 years).

‡Also adjusted for duration of prior ERT use (< 10 years vs. ≥ 10 years).

Table 3. Rate ratios (RRs) and 95 per cent confidence intervals (CIs) for duration of HRT use and ovarian cancer.

| Duration of use (years) | PY | Cases | Age-adjusted* | | Multivariate-adjusted† | |
|---|-----------|--------------|---------------|---------------|------------------------|---------------|
| | | | RR | 95% CI | RR | 95% CI |
| No use | 270,520 | 120 | 1.0 | Referent | 1.0 | Referent |
| ERT-only | | | | | | |
| <4 | 93,804 | 51 | 1.2 | 0.87-1.7 | 1.3 | 0.96-1.9 |
| 4-9.9 | 40,451 | 25 | 1.4 | 0.89-2.1 | 1.6 | 1.0-2.6 |
| 10-19.9 | 30,058 | 21 | 1.5 | 0.93-2.4 | 1.8 | 1.1-3.0 |
| ≥ 20 | 11,567 | 16 | 2.5 | 1.5-4.3 | 3.2 | 1.7-5.7 |
| P-value for trend | | | | | 0.0002 | |
| Increase in RR per year of use (95% CI) | | | | | 0.07 (0.02 to 0.13) | |
| | <u>PY</u> | <u>Cases</u> | <u>RR‡</u> | <u>95% CI</u> | <u>RR‡</u> | <u>95% CI</u> |
| EPRT | | | | | | |
| <2 | 22,730 | 10 | 0.89 | 0.45-1.8 | 0.98 | 0.50-1.9 |
| 2-4 | 12,783 | 8 | 1.2 | 0.58-2.6 | 1.3 | 0.62-2.8 |
| ≥ 4 | 18,792 | 11 | 0.98 | 0.47-2.0 | 1.1 | 0.51-2.3 |
| P-value for trend | | | | | 0.99 | |

*Adjusted for attained age.

†Adjusted for attained age, menopause type (natural menopause, surgical menopause, unknown type), and duration of oral contraceptive use (none, ≤2 years, >2.0 years).

‡ Also adjusted for duration of prior ERT use (<10 years vs. ≥10 years).