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# Ovarian cancer and menopausal hormone therapy in the NIH-AARP diet and health study

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BACKGROUND: Women using unopposed estrogens during menopause are at increased risk of ovarian cancer. It is uncertain whether oestrogen plus progestin therapy exerts similar effects.

METHODS: We evaluated menopausal hormone use and incident ovarian cancer ($n = 426$) in 92 601 post-menopausal women enrolled in the National Institutes of Health-AARP (NIH-AARP) Diet and Health Study. Participants were administered questionnaires in 1996–1997 and followed through 2006. Hazard rate ratios (RR) and 95% confidence intervals (CIs) were estimated using Cox regression.

RESULTS: Increased risks were associated with long duration (10+ years) use of unopposed oestrogen (RR 2.15, 95% CI: 1.30–3.57 among women with a hysterectomy) and oestrogen plus progestin (RR 1.68, 95% CI: 1.13–2.49 among women with intact uteri) therapy. Similar risks were associated with progestins that were used sequentially (<15 days progestin per month) (RR 1.60, 95% CI: 1.10–2.33) or continuously (>25 days progestin per month) (RR 1.43, 95% CI: 1.032–2.01; *P*-value for heterogeneity = 0.63).

CONCLUSION: Our findings suggest that long duration use of both unopposed estrogens and oestrogen plus progestins are associated with increased risks of ovarian cancer, and that risk associated with oestrogen plus progestin use does not vary by regimen (sequential or continuous).

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Menopausal hormone therapy has been associated with increased ovarian cancer incidence and mortality (Rodriguez *et al,* 2001; Danforth *et al,* 2007; Greiser *et al,* 2007; Rossing *et al,* 2007). Recently, the Endocrine Society published a formal statement concluding that long-term unopposed oestrogen menopausal hormone therapy use is associated with an increased, albeit small, risk of ovarian cancer (Santen *et al,* 2010). The report cited that the association between oestrogen plus progestin menopausal hormone therapy and ovarian cancer risk is inconclusive and requires further study (Santen *et al,* 2010). A greater reduction in ovarian cancer risk with oral contraceptives containing high-dose progestins (Schildkraut *et al,* 2002) has led to the suggestion that progestins may be protective and mitigate some of the ovarian cancer risk associated with unopposed estrogens. Not all studies, however, support an antagonistic role for progestins. Some have reported increased ovarian cancer risk associated with combination oestrogen plus progestin hormone therapy compared with non-users (Lacey *et al*, 2006; Beral *et al,* 2007; Morch *et al,* 2009). Further, it remains unclear among combination users whether the regimen of progestin use is important; although some studies report lower ovarian cancer risk estimates for continuous as compared with sequential progestin use (Pearce *et al,* 2009), other studies report no substantial differences by regimen (Morch *et al,* 2009).

To address outstanding questions regarding ovarian cancer risk as related to the use of menopausal hormone therapy, specifically oestrogen plus progestin therapy, we conducted an analysis in the National Institutes of Health-AARP (NIH-AARP) Diet and Health Study, a large prospective study with comprehensive data on menopausal hormone use. An increased risk of ovarian cancer with oestrogen plus progestin use was previously reported using data from this cohort with follow-up through 2000 (Lacey *et al*, 2006). The results from the prior analysis, albeit based on small numbers, suggested that sequential oestrogen plus progestin users had a greater risk of ovarian cancer than continuous users. Given 6 additional years of follow-up and twice as many ovarian cancer cases, we were able with increased power to evaluate the risks associated with long duration oestrogen plus progestin use and regimen (sequential *vs* continuous use of progestins) of use.

## MATERIALS AND METHODS

### Study population

The NIH-AARP Diet and Health Study design and methodology has been described previously (Schatzkin *et al,* 2001). Briefly, the NIH-AARP Diet and Health Study cohort was established during

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1995 and 1996 when 3.5 million AARP members aged 50–71 years and residing in one of six states (California, Florida, Louisiana, New Jersey, North Carolina, and Pennsylvania) or two metropolitan areas (Atlanta, Georgia and Detroit, Michigan) were invited to complete a baseline questionnaire. Approximately 18% of the cohort ($n = 617\,119$) returned the self-administered questionnaire, 566 399 of which were non-duplicate and satisfactorily completed. A second questionnaire (1996–1997) collecting detailed information on menopausal hormone therapy use, family history of cancer, and physical activity was mailed to baseline questionnaire respondents who did not have self-reported colon, breast, or prostate cancer and was returned by 334 906 individuals (59%). We excluded male participants ($n = 188\,116$) and participants who used a proxy respondent for the baseline ($n = 6959$) or second questionnaire ($n = 3424$). The study population included 136 407 potentially eligible women. The Special Studies Institutional Review Board of the US National Cancer Institute approved the study and all the participants gave informed consent.

The cohort follow-up included periodic matching of the cohort database with the National Change of Address database maintained by the US Postal Service and through processing of undeliverable mail, other address update services, and direct responses from participants. Vital status was ascertained by linkage to the US Social Security Administration Death Master File with verification in the National Death Index Plus. The loss to follow-up of cohort participants was $<5\%$.

### Analytic population

We excluded women who reported a previous diagnosis of cancer other than non-melanoma skin cancer or a prior diagnosis of cancer other than non-melanoma skin cancer on their death certificate ($n = 9471$). We further excluded women who were premenopausal at baseline ($n = 4421$) and women who reported a bilateral oophorectomy ($n = 27\,512$), unknown oophorectomy status ($n = 2107$), or menstrual periods that stopped due to radiation or chemotherapy ($n = 66$). Women who developed non-epithelial ovarian cancer during follow-up ($n = 35$), borderline histology ($n = 1$) or non-primary ovarian cancer ($n = 113$) during follow-up were also excluded. Finally, women with missing values for menopausal hormone use variables ($n = 80$) were excluded. After these additional exclusions the analytic cohort consisted of 92 601 women with at least one intact ovary.

### Exposure ascertainment

Information on menopausal hormone use was based on data collected in the second questionnaire only, this questionnaire included detailed questions on 'replacement hormone use', as described previously (Lacey *et al*, 2006). Briefly, this questionnaire collected information about oestrogen and progestin use separately and did not include questions about the combined oestrogen plus progestin pill, which was first marketed in 1995. Participants reported the dates of first and last use, recency of use, total duration of use, usual dose, and name of the pill that they took for the longest time for each pill type. For duration of use, women were asked to report the total duration of use in 1-year increments up to 10 years, with a single category for use $>10$ years.

### Incident ovarian cancer ascertainment

Incident ovarian cancers were identified through probabilistic linkage with cancer registries in the original recruitment areas and three common relocation states (Arizona, Nevada and Texas). The state cancer registries have been certified by the North American Association of Central Cancer Registries and capture at least 95% of the cancer incidences within 24 months of cancer occurrence. Further, a validation study that compared registry findings with self-reports and medical records estimated that registry linkage validly identified $\sim 90\%$ of all incident cancers (Michaud *et al*, 2005). Of the 92 601 post-menopausal women available for analysis, 426 were diagnosed with incident epithelial ovarian cancer after completing the second questionnaire and on or before December 31, 2006. We further classified the incident epithelial ovarian cancers as serous ($n = 228$), endometrioid ($n = 31$), mucinous ($n = 20$), clear cell ($n = 14$), and other ($n = 133$) based on International Classification of Diseases for Oncology morphology codes as described previously (Yang *et al*, 2012).

### Statistical analysis

Cox proportional hazards regression was used to estimate rate ratios (RR) and 95% confidence intervals (CIs) with age as the time metric and ties handled by complete enumeration. Follow-up time began at the scan date of the second questionnaire and continued until diagnosis of first primary cancer, date of death, date moved out of registry ascertainment area, or December 31, 2006, whichever came first.

Oestrogen plus progestin only was defined if the reported date of progestin therapy use was within 90 days of unopposed oestrogen use. In the oestrogen plus progestin only analyses, we modelled unopposed oestrogen followed by oestrogen plus progestin as a separate variable to avoid any residual influence on risk of former unopposed oestrogen use. We defined sequential regimen usage as oestrogen with progestin use for $<15$ days per month, and continuous regimen usage as oestrogen with 'daily' use of progestin ($>25$ days per month). The usage pattern that included progestin use 15–25 days per month was modelled as a separate variable in statistical models; however, the number of cases was small and the effect estimates were consistent with sequential and continuous regimens and are not reported.

All models were adjusted for age at cohort entry, race, body mass index (BMI), age at menopause, parity, and duration of oral contraceptive use at baseline. Models evaluating unopposed oestrogen therapy were restricted to women who had reported a hysterectomy at baseline, and models evaluating combination oestrogen plus progestin therapy were restricted to women with intact uteri at baseline. Tests for linear trends across hormone use categories were calculated using a grouped linear variable. Likelihood ratio tests for interaction across levels of oral contraceptive use, parity, smoking history, BMI, and family history of breast cancer were computed based on cross-product terms with hormone use. The assumption of proportional hazards for each adjustment variable and main effect was tested using a likelihood ratio test of interaction with the time-scale (continuous) based on cross-product terms. The *P*-values for all comparisons were two-sided and an alpha $<0.05$ indicated statistical significance. SAS statistical software, version 9.2 (SAS Institute Inc., Cary, NC, USA) was used for all analyses.

## RESULTS

### Characteristics of the cohort

A total of 92 601 women with at least one intact ovary at baseline contributed 822 409 person-years, with mean follow-up of 4.7 years for ovarian cancer cases and 8.9 years for non-cases. The mean age at baseline of all participants was 62.3 years and the study population was predominantly white (91.2%). Ovarian cancer was inversely associated with duration of oral contraceptive use and parity (results not shown).

### Hormone usage patterns

Forty-six percent ($n = 42\,204$) of the post-menopausal women with at least one intact ovary reported never using menopausal

hormones on the second questionnaire, whereas 19.4% ($n=17\,922$) reported using oestrogen-only hormone therapy, 31.0% ($n=28\,707$) reported using any oestrogen plus progestin, and 4.1% ($n=3768$) reported other or unknown type of hormone therapy. Of the women who reported using any oestrogen plus progestin, 68.7% ($n=19\,726$) reported using oestrogen plus progestin only, 13.3% ($n=3822$) reported using oestrogen alone followed by some combination of oestrogen plus progestin, and the remaining 18.0% ($n=5159$) used other combinations of hormones.

Baseline characteristics of the women included in our analyses are presented in Table 1 according to menopausal hormone use (never/ever) and type (oestrogen-only/oestrogen plus progestin-only). Compared with non-hormone users, women who used unopposed estrogens were more often oral contraceptive users, multiparous, and thin, while women who used oestrogen plus progestin were more frequently young, white, older when they experienced natural menopause, oral contraceptive users and thin. As expected, unopposed oestrogen users (72.3%) were more likely to have had a hysterectomy as compared with oestrogen plus progestin only users (2.6%) or non-hormone users (14.9%).

## Risk of ovarian cancer among women using unopposed oestrogen only

Among women with a hysterectomy, exclusive use of unopposed estrogens was associated with an increased risk of ovarian cancer compared with women who reported never using menopausal hormones (RR 1.69, 95% CI: 1.05, 2.71) (Table 2). The risk was substantially elevated among women who reported long duration ($\geqslant$10 years) unopposed oestrogen only use (RR 2.151, 95% CI: 1.30, 3.57); whereas shorter duration ($<$10 years) unopposed oestrogen only use was not associated with increased risk (RR 1.25, 95% CI: 0.71, 2.20).

## Risk of ovarian cancer among women using only oestrogen plus progestin

Exclusive use of oestrogen plus progestin therapy was associated with an increased risk of ovarian cancer compared with women who reported never using menopausal hormones (RR 1.43; 95% CI: 1.09, 1.86) (Table 3). Long duration ($\geqslant$10 years) oestrogen plus progestin use was also associated with increased risk (RR 1.68; 95% CI: 1.13, 2.49). The risk estimate associated with short-duration use was also elevated, although the estimate did not achieve statistical significance. Higher-dose progestins (5 mg and 10 mg) were associated with increased, albeit not statistically significant, risk (5 mg RR 1.60; 95% CI: 0.95, 2.68; 10 mg RR 1.58; 95% CI: 0.90, 2.79). The number of days per month on progestin did not influence ovarian cancer risk ($P$-trend $=0.70$). Use of either sequential ($<$15 days progestin per month) or continuous ($>$25 days progestin per month) progestins were associated with similar increases in ovarian cancer risk (sequential RR 1.60; 95% CI: 1.10, 2.33; continuous RR 1.43; 95% CI: 1.03, 2.01; $P$-value for heterogeneity $=0.63$). We attempted to evaluate how duration of use affected the risk associated with sequential or continuous regimen use, but small numbers of long-term users precluded any definitive conclusions.

Based on the likelihood ratio test of interaction, the association between menopausal hormone use and ovarian cancer was not significantly modified by prior oral contraceptive use (all $P$-values for interaction in Table 4 $>0.05$). Although the interaction models in Table 4 suggest that the menopausal hormone-ovarian cancer association is apparent only among women who reported never or short-duration ($<$1 years) oral contraceptive use, the risk estimates among women who used oral contraceptives for longer durations ($\geqslant$1 years) should be interpreted with caution as they are based on a small numbers in some categories. Other factors associated with ovarian cancer risk including parity, smoking history, BMI, or family history of breast cancer did not significantly modify the associations between menopausal hormone use and ovarian cancer risk (results not shown).

We evaluated associations for serous and other histological types, combined as a single group as there were too few

**Table 1** Select baseline characteristics by the use of menopausal hormone therapy and hormone type among 92 601 women in NIH-AARP Diet and Health Study, 1995–2006

| | Menopausal hormone use | | | | Menopausal hormone type | | | |
|---|---|---|---|---|---|---|---|---|
| | Never use | | Ever use | | Oestrogen only | | Oestrogen plus progestin-only | |
| | (*N* = 42 204) | | (*N* = 50 397) | | (*N* = 17 922) | | (*N* = 19 726) | |
| | *N*[a] | % | *N*[a] | % | *N*[a] | % | *N*[a] | % |
| *Age at study entry (years)* | | | | | | | | |
| <55 | 3789 | 9.0 | 6474 | 12.9 | 2069 | 11.6 | 3154 | 16.0 |
| 55–59 | 7832 | 18.6 | 13 483 | 26.8 | 4036 | 22.5 | 6423 | 32.6 |
| 60–64 | 12 374 | 29.3 | 15 038 | 29.8 | 5186 | 28.9 | 5870 | 29.8 |
| 65–69 | 16 330 | 38.7 | 13 874 | 27.5 | 5898 | 32.9 | 3921 | 19.9 |
| 70+ | 1879 | 4.4 | 1528 | 3.0 | 733 | 4.1 | 358 | 1.8 |
| *Race/ethnicity* | | | | | | | | |
| White | 37 790 | 89.5 | 46 711 | 92.7 | 16 373 | 91.4 | 18 594 | 94.3 |
| Other | 4414 | 10.5 | 3686 | 7.3 | 1549 | 8.6 | 1132 | 5.7 |
| *Smoking status* | | | | | | | | |
| Never | 19 163 | 45.4 | 22 096 | 43.8 | 8012 | 44.7 | 8524 | 43.2 |
| Former | 15 353 | 36.4 | 21 045 | 41.8 | 7084 | 39.5 | 8578 | 43.5 |
| Current | 6575 | 15.6 | 5989 | 11.9 | 2388 | 13.3 | 2166 | 11.0 |
| *Age at menarche* | | | | | | | | |
| <13 | 20 323 | 48.1 | 24 243 | 48.1 | 8888 | 49.6 | 9260 | 46.9 |
| 13–14 | 17 780 | 42.1 | 21 496 | 42.7 | 7418 | 41.4 | 8712 | 44.2 |
| 15+ | 3951 | 9.4 | 4543 | 9.0 | 1579 | 8.8 | 1720 | 8.7 |
| *Age at natural menopause (years)* | | | | | | | | |
| <45 | 4477 | 10.6 | 3321 | 6.6 | 862 | 4.8 | 1438 | 7.3 |
| 45–49 | 9992 | 23.7 | 8501 | 16.9 | 1645 | 9.2 | 4548 | 23.1 |
| 50–54 | 17 538 | 41.6 | 15 835 | 31.4 | 2388 | 13.3 | 9393 | 47.6 |
| 55+ | 3524 | 8.4 | 4026 | 8.0 | 466 | 2.6 | 2426 | 12.3 |
| Surgical | 6138 | 14.5 | 16 416 | 32.5 | 12 370 | 69.0 | 524 | 2.7 |
| Unknown | 535 | 1.3 | 2298 | 4.6 | 191 | 1.1 | 1397 | 7.1 |
| *Oral contraceptive use* | | | | | | | | |
| Never/<1 year | 29 254 | 69.3 | 26 638 | 52.9 | 10 301 | 57.5 | 9435 | 47.8 |
| 1–9 years | 9865 | 23.4 | 17 186 | 34.1 | 5781 | 32.3 | 7271 | 36.9 |
| 10+ years | 2816 | 6.7 | 6252 | 12.4 | 1734 | 9.7 | 2900 | 14.7 |
| *Parity* | | | | | | | | |
| Nulliparous | 7117 | 16.9 | 6980 | 13.9 | 1821 | 10.2 | 3342 | 16.9 |
| 1 | 4186 | 9.9 | 4888 | 9.7 | 1575 | 8.8 | 2120 | 10.8 |
| 2 | 9871 | 23.4 | 13 704 | 27.2 | 4575 | 25.5 | 5664 | 28.7 |
| 3+ | 20 876 | 49.5 | 24 663 | 48.9 | 9881 | 55.1 | 8549 | 43.3 |
| *Body mass index in kg m$^{-2}$* | | | | | | | | |
| <25 | 16 733 | 39.7 | 25 071 | 49.8 | 8035 | 44.8 | 10 623 | 53.9 |
| 25–29.9 | 13 395 | 31.7 | 15 462 | 30.7 | 5825 | 32.5 | 5754 | 29.2 |
| 30+ | 10 705 | 25.4 | 8711 | 17.3 | 3630 | 20.3 | 2949 | 15.0 |
| *Hysterectomy at baseline* | | | | | | | | |
| No | 35 669 | 84.5 | 32 927 | 65.3 | 4902 | 27.4 | 19 140 | 97.0 |
| Yes | 6303 | 14.9 | 17 281 | 34.3 | 12 959 | 72.3 | 520 | 2.6 |

Abbreviation: NIH-AARP = National Institutes of Health-AARP. [a]Numbers may not add to total because of missing values.

**Table 2** Associations between unopposed oestrogen-only menopausal hormone therapy and ovarian cancer among 23 584 women with a hysterectomy at baseline, NIH-AARP Diet and Health Study Cohort, 1995–2006

| | Number of cancers | Person-years | RR[a] (95% CI) | *P*-value[b] |
|---|---|---|---|---|
| No MHT use | 23 | 55 868 | 1.00 (reference) | |
| ET only | 76 | 116 139 | 1.69 (1.05, 2.71) | |
| *Duration of use (years)* | | | | |
| < 10 | 27 | 58 393 | 1.25 (0.71, 2.20) | 0.01 |
| ⩾ 10 | 49 | 55 878 | 2.15 (1.30, 3.57) | |

Abbreviations: CI = confidence interval; ET = unopposed oestrogen therapy; MHT = menopausal hormone therapy; NIH-AARP = National Institutes of Health-AARP; RR = hazard rate ratio. [a]RR adjusted for continuous age (years), race (white, other/unknown), parity (nulliparous, 1, 2, 3+, unknown), duration of oral contraceptive use (none, <10 years, ⩾10 years, or unknown), and body mass index (<25, 25–29, ⩾30 kg m$^{-2}$, or unknown); models included terms for ever use of other MHT formulations (oestrogen plus progestin, other formulations, or unknown). [b]*P*-value (two-sided) for trend was calculated using ordinal duration variable based on the categories and referent group shown.

**Table 3** Associations between oestrogen plus progestin therapy only and ovarian cancer among 68 596 women with an intact uteri at baseline, NIH-AARP Diet and Health Study Cohort, 1995–2006

| | Numbers of cancers | Person-years | RR[a] (95% CI) | *P*-value[b] |
|---|---|---|---|---|
| No MHT use | 150 | 316 239 | 1.00 (reference) | |
| EPT only | 98 | 170 556 | 1.43 (1.09, 1.86) | |
| *Duration of EPT only use (years)* | | | | |
| < 10 | 67 | 126 465 | 1.33 (0.98, 1.79) | 0.26 |
| ⩾ 10 | 31 | 43 752 | 1.68 (1.13, 2.49) | |
| *Progestin dose*[c] | | | | |
| < 1 mg | 2 | 5854 | 0.83 (0.21, 3.36) | 0.15 |
| 2.5 mg | 49 | 85 828 | 1.35 (0.98, 1.86) | |
| 5 mg | 16 | 23 968 | 1.60 (0.95, 2.68) | |
| 10 mg | 13 | 19 792 | 1.58 (0.90, 2.79) | |
| *Days on progestin per month*[c] | | | | |
| < 10 | 10 | 17 482 | 1.41 (0.74, 2.69) | 0.70 |
| 10–14 | 26 | 39 150 | 1.69 (1.10, 2.58) | |
| 15–19 | 2 | 3300 | 1.48 (0.37, 6.00) | |
| 20–25 | 11 | 17 499 | 1.51 (0.81, 2.79) | |
| Daily | 48 | 83 396 | 1.43 (1.03, 2.01) | |
| *Regimen of EPT only use*[d] | | | | |
| Sequential | 36 | 56 632 | 1.60 (1.10, 2.33) | |
| Continuous | 48 | 83 396 | 1.43 (1.03, 2.01) | |
| *Regimen and duration of EPT only use* | | | | |
| Sequential | | | | |
| < 10 years | 26 | 37 417 | 1.81 (1.18, 2.78) | 0.10 |
| ⩾ 10 years | 9 | 18 840 | 1.13 (0.57, 2.23) | |
| Continuous | | | | |
| < 10 years | 36 | 66 345 | 1.37 (0.94, 1.99) | 0.03 |
| ⩾ 10 years | 12 | 16 737 | 1.72 (0.95, 3.11) | |

Abbreviations: CI = confidence interval; EPT = oestrogen plus progestin therapy; ET = unopposed oestrogen therapy; MHT = menopausal hormone therapy; NIH-AARP = National Institutes of Health-AARP; RR = hazard rate ratio. [a]RR adjusted for continuous age (years), race (white, other/unknown), parity (nulliparous, 1, 2, 3+, unknown), duration of oral contraceptive use (none, <10 years, ⩾10 years, or unknown), and body mass index (<25, 25–29, ⩾30 kg m$^{-2}$, or unknown); models included terms for use of other MHT formulations (ET only, other formulations, or unknown). [b]*P*-values (two-sided) for trend were calculated using ordinal (duration, progestin dose, days on progestin per month, regimen and duration) variables based on the categories and referent group shown. [c]Cases do not add to total because of missing values ($n = 18$ missing progestin dose and $n = 1$ missing days on progestin per month). [d]Sequential regimen usage was defined as oestrogen with <15 days progestin per month and the continuous regimen usage as oestrogen with 'daily' use of progestin (>25 days progestin per month). The usage pattern that included 15–25 days per month was modelled as a separate parameter in the statistical model (results not shown).

endometrioid, clear cell, or mucinous cancers to reliably evaluate their separate relationships (Table 5). We found that the increased risks associated with unopposed oestrogen (RR 2.82; 95% CI: 1.31, 6.04) and oestrogen plus progestin (RR 1.83; 95% CI: 1.28, 2.61) menopausal hormones were limited to women with serous histology. Long duration unopposed oestrogen (RR 3.32; 95% CI: 1.49, 7.44) and long duration oestrogen plus progestin use (RR 1.97; 95% CI: 1.17, 3.34) were associated with serous ovarian cancer risk, as well as sequential (RR 1.87; 95% CI: 1.14, 3.08) and continuous regimen (RR 2.02; 95% CI: 1.32, 3.08) use. The combined category of 'other' histologic subtypes was not associated with unopposed oestrogen or oestrogen plus progestin menopausal hormone use.

Associations were not significantly different when evaluated by tumour stage or grade (results not shown). The results were essentially unchanged after further adjustment for calendar time and several additional ovarian cancer risk factors, including age at menarche, age at natural menopause, first-degree family history of breast cancer, marital status, or educational attainment (results not shown). We performed a sensitivity analysis of the association between menopausal hormone therapy and ovarian cancer whereby we truncated the follow-up at June 30th, 2002, to account for potential changes in menopausal hormone therapy use or prescribing patterns following the WHI trial report, suggesting that menopausal hormones should not be recommended for chronic disease prevention in post-menopausal women (Rossouw *et al,* 2002; Anderson *et al,* 2003). The truncated results were not substantially different (results not shown).

## DISCUSSION

In this large prospective analysis in the NIH-AARP Diet and Health Study cohort, long duration use of either unopposed oestrogen or oestrogen plus progestin menopausal hormone therapy was associated with increased ovarian carcinoma risk. Further, the increased risk related to oestrogen plus progestin use was not reduced by increasing days per month of progestin nor was there a difference in risk comparing sequential and continuous regimen usage, suggesting that the relationship between estrogens and ovarian cancer risk is not attenuated by the addition of progestins, even when prescribed continuously.

The increased risks we observed for long duration unopposed oestrogen and oestrogen plus progestin therapy are consistent with results from a previous investigation within the NIH-AARP Diet and Health study (Lacey *et al*, 2002). In contrast to the prior study, the current analysis reports similar magnitude increased risk of ovarian cancer for both sequential and continuous regimen oestrogen plus progestin. In the prior analysis, ovarian cancer risk was greater for sequential than continuous oestrogen plus progestin users, but given the shorter follow-up period the risk estimate for continuous regimen oestrogen plus progestin did not attain statistical significance.

Cohort studies have consistently reported an increased risk of ovarian cancer with unopposed oestrogen use (Lacey *et al*, 2002; Danforth *et al,* 2007; Beral *et al,* 2007; Morch *et al,* 2009; Hildebrand *et al,* 2010; Tsilidis *et al,* 2011). An association with oestrogen plus progestin use has been less consistent; however, a meta-analysis of population-based case–control, cohort and clinical trial data published through 2007 reported an increased

**Table 4** Association between menopausal hormone use categories and ovarian cancer risk by oral contraceptive use status, NIH-AARP Diet and Health Study Cohort, 1995–2006

| | Oral contraceptive use | | | | | | |
|---|---|---|---|---|---|---|---|
| | Never or <1 years | | | ⩾1 years | | | |
| | Number of cancers | Person-years | HR[a] (95% CI) | Number of cancers | Person-years | HR[a] (95% CI) | *P*-intx[b] |
| *ET only among women with a hysterectomy at baseline (n = 23 584)* | | | | | | | |
| No MHT use | 17 | 39 722 | 1.00 (reference) | 6 | 17 600 | 1.03 (0.54, 1.98) | |
| ET only | 54 | 65 118 | 1.97 (1.17, 3.30) | 22 | 50 841 | 1.12 (0.60, 2.10) | 0.15 |
| *Duration of ET only use (years)* | | | | | | | |
| <10 | 19 | 31 107 | 1.54 (0.81, 2.92) | 8 | 27 145 | 0.80 (0.34, 1.89) | 0.38 |
| ⩾10 | 35 | 32 886 | 2.52 (1.43, 4.43) | 14 | 22 961 | 1.50 (0.74, 3.03) | |
| *EPT only among women with an intact uteri at baseline (n = 68 596)* | | | | | | | |
| No MHT use | 117 | 220 524 | 1.00 (reference) | 32 | 95 645 | 0.83 (0.60, 1.14) | |
| EPT only | 55 | 81 534 | 1.46 (1.06, 2.01) | 41 | 88 566 | 1.11 (0.76, 1.63) | 0.75 |
| *Duration of EPT only use (years)* | | | | | | | |
| <10 | 37 | 60 414 | 1.30 (0.90, 1.90) | 30 | 65 755 | 1.11 (0.73, 1.71) | 0.65 |
| ⩾10 | 18 | 20 903 | 1.88 (1.17, 3.03) | 11 | 22 670 | 1.09 (0.59, 2.04) | |
| *Regimen of EPT only use* | | | | | | | |
| Sequential | 19 | 26 799 | 1.60 (0.99, 2.58) | 16 | 29 744 | 1.29 (0.76, 2.22) | 0.80 |
| Continuous | 29 | 38 970 | 1.55 (1.03, 2.34) | 19 | 44 147 | 1.04 (0.63, 1.72) | |

Abbreviations: CI = confidence interval; EPT = oestrogen plus progestin therapy; ET = unopposed oestrogen therapy; HR, hazard ratio; MHT = menopausal hormone therapy; NIH-AARP = National Institutes of Health-AARP; RR = hazard rate ratio. [a]HR adjusted for continuous age (years), race (white, other/unknown), parity (nulliparous, 1, 2, 3+, unknown), and body mass index (<25, 25–29.9, ⩾30 kg m$^{-2}$, or unknown); models also contained terms for other formulations of menopausal hormone use and unknown use. [b]*P*-value (two-sided) from likelihood ratio test for the interaction of BMI and MHT formulations based on the categories and referent group shown.

**Table 5** Associations between unopposed oestrogen therapy and oestrogen plus progestin therapy and ovarian cancer by histology, NIH-AARP Diet and Health Study Cohort, 1995–2006

| | Serous cases (*n* = 228) | | | Other cases (*n* = 198) | | |
|---|---|---|---|---|---|---|
| | Number of cancers | Person-years | HR[a] (95% CI) | No. of cancers | Person-years | HR[a] (95% CI) |
| *Among women with a hysterectomy at baseline (n = 23 584)* | | | | | | |
| No MHT use | 8 | 55 796 | 1.00 (reference) | 15 | 55 839 | 1.00 (reference) |
| ET only | 43 | 116 004 | 2.82 (1.31, 6.04) | 33 | 115 930 | 1.02 (0.56, 1.84) |
| *Duration of use (years)* | | | | | | |
| <10 | 17 | 58 341 | 2.20 (0.94, 5.15) | 10 | 58 310 | 0.72 (0.32, 1.62) |
| ⩾10 | 26 | 55 796 | 3.32 (1.49, 7.44) | 23 | 55 753 | 1.52 (0.78, 2.96) |
| *Among women with an intact uteri at baseline (n = 68 596)* | | | | | | |
| No MHT use | 73 | 315 892 | 1.00 (reference) | 78 | 315 883 | 1.00 (reference) |
| EPT only | 61 | 170 392 | 1.83 (1.28, 2.61) | 37 | 170 265 | 1.05 (0.70, 1.58) |
| *Duration of EPT only use (years)* | | | | | | |
| <10 | 43 | 126 355 | 1.73 (1.16, 2.56) | 24 | 126 235 | 0.92 (0.57, 1.49) |
| ⩾10 | 18 | 43 697 | 1.97 (1.17, 3.34) | 13 | 43 691 | 1.37 (0.76, 2.50) |
| *Regimen of EPT only use* | | | | | | |
| Sequential | 21 | 56 571 | 1.87 (1.14, 3.08) | 15 | 56 545 | 1.31 (0.74, 2.31) |
| Continuous | 33 | 83 334 | 2.02 (1.32, 3.08) | 15 | 83 223 | 0.87 (0.49, 1.53) |

Abbreviations: CI = confidence interval; EPT = oestrogen plus progestin therapy; ET = unopposed oestrogen therapy; HR, hazard ratio; MHT = menopausal hormone therapy; NIH-AARP = National Institutes of Health-AARP; RR = hazard rate ratio. [a]HR adjusted for continuous age (years), race (white, other/unknown), parity (nulliparous, 1, 2, 3+, unknown), duration of oral contraceptive use (none, <10 years, ⩾10 years, or unknown), and body mass index (<25, 25–29.9, ⩾30 kg m$^{-2}$, or unknown); models also contained terms for other formulations of menopausal hormone use and unknown use.

risk of ovarian cancer per 5 years of use for both unopposed oestrogen and oestrogen plus progestin therapies (Pearce *et al,* 2009). Further, the authors reported higher risk estimate for unopposed oestrogen use than oestrogen plus progestin use (Pearce *et al,* 2009). Three additional cohort studies have been published since the meta-analysis (Morch *et al,* 2009; Hildebrand *et al,* 2010; Tsilidis *et al,* 2011); the largest of which, the Danish Sex Hormone Register Study, included 2681 ovarian cancers and

reported an increased risk of ovarian cancer with current use of oestrogen plus progestin (Morch *et al,* 2009). Our finding of an increased risk of ovarian cancer with oestrogen plus progestin use is consistent with the results from the Danish Sex Hormone Register Study (Morch *et al,* 2009) and the meta-analysis (Pearce *et al,* 2009) and provides evidence that progestins do not mitigate the ovarian cancer risk associated with post-menopausal exposure to unopposed estrogens. Further supporting the notion that ovarian cancer risk does not depend on whether progestins were prescribed sequentially or continuously, the current analysis and the Danish Sex Hormone Register Study also reported increased risk for continuous oestrogen plus progestin use, which was not significantly different than the risk observed for sequential oestrogen plus progestin use (Morch *et al,* 2009).

A number of case–control (Purdie *et al,* 1995; Risch *et al,* 1996; Pike and Spicer, 2000; Riman *et al,* 2002; Moorman *et al,* 2005; Rossing *et al,* 2007) and cohort (Lacey *et al*, 2002; Danforth *et al,* 2007; Hildebrand *et al,* 2010; Tsilidis *et al,* 2011) studies have reported null associations for oestrogen plus progestin use and ovarian cancer risk. The inconsistency in reports may partly be explained by the small number of menopausal hormone users in some studies, differences in the categorisation of days per month on progestin, type of progestin prescribed in the United States *vs* Europe, evaluation of current *vs* ever users of oestrogen plus progestin, or lack of adjustment for or stratification by hysterectomy status.

Consistent with previous investigations (Lacey *et al*, 2002; Beral *et al,* 2007), we found that the menopausal hormone therapy-ovarian cancer associations did not vary significantly according to the presence or absence of ovarian cancer risk factors. The analyses evaluating the association between menopausal hormone use and ovarian cancer across categories of prior oral contraception use were based on small numbers and should be interpreted with caution.

Few cohort studies have reported analyses by histologic type (Beral *et al,* 2007; Danforth *et al,* 2007; Tsilidis *et al,* 2011; Morch *et al,* 2012). The current use of menopausal hormones was associated with serous tumours in the Million Women Study (Beral *et al,* 2007); however, current use of any menopausal hormone was not associated with any histologic type in the European Prospective Investigation into Cancer and Nutrition (Tsilidis *et al,* 2011). Unopposed oestrogen use was associated with serous and endometrioid tumours in the Nurse's Health Study and the Danish Sex Hormone Register Study (Danforth *et al,* 2007; Morch *et al,* 2012). Oestrogen plus progestins use was not associated with serous or endometrioid tumours in the Nurse's Health Study, but it was associated with increased serous and endometrioid tumours in the Danish Sex Hormone Register Study (Danforth *et al,* 2007; Morch *et al,* 2012). Other prospective studies have not reported risk estimates by subtype (Lacey *et al*, 2002; Hildebrand *et al,* 2010). We report increased risk of serous tumours associated with both unopposed oestrogen and oestrogen plus progestin menopausal hormone therapy, regardless of whether progestins were prescribed sequentially or continuously—a finding that is consistent with results from the Danish Sex Hormone Register Study (Morch *et al,* 2012).

The major strengths of the current study include the use of a large prospective cohort of primarily post-menopausal women with extensive data on menopausal hormone therapy use and long duration of follow-up. The detailed questionnaire provided information on potential confounders and effect modifiers, which allowed for a thorough assessment of the independence of menopausal hormone use from other related factors and the evaluation of potential joint effects between menopausal hormones and known ovarian cancer risk factors.

As participants of the NIH-AARP study represent mainly white, post-menopausal women who consented to participate, the results of the current study may not apply to all women. Another limitation of this study is that menopausal hormone usage patterns were only collected as part of second questionnaire administered in 1996–1997. We therefore could not evaluate whether cessation of or changes in menopausal hormone usage patterns after exposure assessment differed by ovarian cancer status. Hormone usage patterns among our cohort participants likely changed during follow-up given increases in hormone use in the United States leading up to the early termination of the WHI trial in 2002 and subsequent changes in prescribing patterns. However, a sensitivity analysis truncating follow-up at June 30th 2002 did not materially attenuate our results. Further, the reported duration of use at baseline would have systematically underestimated the true total duration of menopausal hormone therapy use in the population during the study period. We did not have information on gynaecologic surgery after baseline; therefore the inability to censor women who had an oophorectomy during follow-up was also a study limitation. Hysterectomy prevalence during the study period increased, with bilateral oophorectomy accompanying approximately half of those hysterectomy procedures. However, hysterectomy rates decline markedly after menopause (Merrill, 2001); therefore the expected number of women in the NIH-AARP Diet and Health Study who received an oophorectomy after baseline is small.

Menopausal hormone therapy is the most effective treatment available for vasomotor symptoms associated with oestrogen deficiency during the menopausal transition. Current guidelines recommend use of the lowest effective dose of menopausal hormone therapy for the shortest possible duration. Chronic disease associations with short duration, low dose use have not been thoroughly evaluated. When risks are resolved they will need to be weighed in relation to a number of benefits (Santen *et al,* 2010).

In conclusion, our results suggest that long duration use of both unopposed estrogens and oestrogen plus progestins are associated with increased risks of ovarian cancer, and that risk associated with oestrogen plus progestin use does not vary by regimen (sequential or continuous). Although there has been previous uncertainty regarding whether oestrogen plus progestin hormone therapy has a substantial impact on ovarian cancer risk, there appears to be growing evidence that long-term oestrogen plus progestin confers increased risk. Larger studies are still needed to evaluate ovarian cancer risk with short-duration oestrogen plus progestin use according to different regimens, as well as evaluate effect modification with known risk factors of ovarian cancer (e.g., oral contraception use, smoking, BMI, parity, etc.).

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