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Original Contribution

Menopausal Hormone Therapy and Risk of Endometrial Carcinoma Among Postmenopausal Women in the European Prospective Investigation into Cancer and Nutrition

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Estrogen-only menopausal hormone therapy (HT) increases the risk of endometrial cancer, but less is known about the association with other types of HT. Using Cox proportional hazards regression, the authors examined the association of various types of HT with the risk of endometrial cancer among 115,474 postmenopausal women recruited into the European Prospective Investigation into Cancer and Nutrition between 1992 and 2000. After a mean follow-up period of 9 years, 601 incident cases of endometrial cancer were identified. In comparison with never users of HT, risk of endometrial cancer was increased among current users of estrogen-only HT (hazard ratio (HR) = 2.52, 95% confidence interval (CI): 1.77, 3.57), tibolone (HR = 2.96, 95% CI: 1.67, 5.26), and, to a lesser extent, estrogen-plus-progestin HT (HR = 1.41, 95% CI: 1.08, 1.83), although risks differed according to regimen and type of progestin constituent. The association of HT use with risk was stronger among women who were older, leaner, or had ever smoked cigarettes. The finding of a strong increased risk of endometrial cancer with estrogen-only HT and a weaker association with combined HT supports the hypothesis that progestins have an attenuating effect on endometrial cancer risk. The increased risk associated with tibolone use requires further investigation.

endometrial neoplasms; estrogen replacement therapy; norpregnenes; postmenopause; prospective studies

Abbreviations: CI, confidence interval; HR, hazard ratio; HT, hormone therapy.

It is well established that use of estrogen-only menopausal hormone therapy (HT) in postmenopausal women increases the risk of endometrial cancer (1, 2). This fits the ''unopposed estrogen'' hypothesis, which states that high levels of bioavailable estrogens, when not counterbalanced by progesterone, increase the mitogenic activity of endometrial cells (3), leading to endometrial hyperplasia and cancer. Because of this, many women are now routinely prescribed regimens that contain estrogens combined with progestins, which have been shown to weaken or even reverse the increase in endometrial cancer risk associated with exogenous estrogen use (2, 4–7). However, there is some uncertainty as to whether different types of regimens or progestin constituents have different effects on risk and whether the risks associated with HT differ according to other lifestyle factors.

Our aim in this study was to examine the associations of different types of HT with the risk of incident endometrial cancer in a large European cohort, where women use a wide range of HT preparations. A secondary aim was to investigate whether the associations of endometrial cancer risk with hormone use differed by body mass index, age, or other factors.

MATERIALS AND METHODS

The European Prospective Investigation into Cancer and Nutrition is a large cohort study consisting of approximately 370,000 women and 150,000 men recruited between 1992 and 2000 at 23 study centers in 10 European countries: Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom. The cohort population and data collection procedures have been described in detail elsewhere (8). Participants gave written informed consent and completed questionnaires on their diet, lifestyle, and medical history. Approval for the study was obtained from the ethical review boards of the participating institutions. Participants were almost all of white European origin.

Of the approximately 370,000 women enrolled in the study, women were not eligible for this analysis if they had prevalent cancer ($n = 19{,}707$), hysterectomy ($n = 35{,}158$), or incomplete follow-up data ($n = 2{,}296$) or if they did not return the baseline lifestyle questionnaire ($n = 509$). Women in Sweden ($n = 26{,}920$) and Greece ($n = 14{,}048$) were excluded because of a lack of detailed data on HT use. We further excluded women who were premenopausal or perimenopausal at recruitment ($n = 153{,}018$) based on an algorithm that included information on ovariectomy, menstruation status, exogenous hormone use, and age (9). Women were considered postmenopausal if they reported not having had any menses over the past 12 months, if they reported having undergone bilateral ovariectomy, or, in the absence of these data, if they were older than 55 years. Women were also excluded if they had never menstruated ($n = 22$), if they had missing data on both ever and current use of HT ($n = 825$), or if they had been diagnosed with nonepithelial endometrial cancer ($n = 16$). This left a total of 115,474 women for inclusion in these analyses.

Exposure assessment

Information on hormone use at recruitment was derived from country-specific questionnaires, all of which included questions on ever and current use of HT, ages at first and last use, total duration of use, and brand name of the current HT preparation, which was coded according to the Anatomical Therapeutic Chemical Classification System (10). Approximately 9% of current users had missing information on brand name, and thus the type of HT could not be classified further. Estrogen-only HT was further classified by type of estrogen constituent (estradiol compounds, conjugated equine estrogens, or other/missing) and route of administration (oral, cutaneous, or other/missing). Estrogen-plus-progestin HT was further classified by progestin constituent (micronized progesterone, progesterone derivative, or testosterone derivative (11)). Regimen was classified as sequential (estrogen with a progestin added for some days (usually 10–14 days) of the month) or continuous (estrogen plus a progestin daily). Tibolone, a synthetic steroid with estrogenic, progestagenic, and androgenic properties, was classified into a separate category. Other HT formulations were predominantly progestin-only or androgen-plus-estrogen preparations, and these were combined into 1 category.

Data on smoking status, menstrual and reproductive factors, and physical activity were obtained from the recruitment questionnaire, and a validated physical activity index was calculated, as described elsewhere (12). Height and weight were measured at recruitment, except for the Oxford, United Kingdom, ''health-conscious'' cohort, the Norwegian cohort, and approximately two-thirds of the French cohort, among whom height and weight were self-reported. Body mass index was calculated as weight (kg)/height $(m)^2$.

Case ascertainment and follow-up

Incident cases of endometrial cancer were identified through linkage to population cancer registries in Denmark, Italy, the Netherlands, Norway, Spain, and the United Kingdom or through a combination of methods, including linkage to health insurance records, cancer and pathology registries, and active follow-up of study participants or their next of kin in France and Germany.

Of the 115,474 women included in these analyses, 601 developed endometrial carcinoma after recruitment and before the closure date of the study period (between December 2003 and November 2006, according to recruitment center). The cancer diagnosis was based on histology reports for 81% of cases, clinical examination for 12%, and self-report, autopsy, or death certificate for the remaining 7%. Details on tumor morphology were specified for 252 (42%) cases, of which 233 (92.5%) were endometrioid, 8 (3.2%) were serous, 3 (1.2%) were mucinous, 5 (2.0%) were clear-cell, and 3 (1.2%) were undifferentiated. Of the 601 cases, 105 were in Denmark, 187 in France, 38 in Germany, 51 in Italy, 55 in the Netherlands, 20 in Norway, 38 in Spain, and 107 in the United Kingdom.

Statistical analysis

Cox proportional hazards models were used to estimate hazard ratios for endometrial cancer and 95% confidence intervals according to various measures of HT. Age was used as the underlying time variable, with entry and exit times being defined as the subject's age at recruitment and the subject's age at endometrial cancer diagnosis or censoring (death, loss to follow-up, or the end of follow-up). Tests using Schoenfeld residuals showed no evidence that the proportional hazards assumption was violated (13). We conducted tests for linear trend by including categorical variables as continuous terms in the models. Tests for heterogeneity were performed using likelihood ratio chi-square tests. Data were stratified by recruitment center and age, and adjustments were made for variables known to be associated with risk: body mass index (<25, 25–29, or ≥ 30), parity (0,

1, 2, or ≥3 births), age at menopause (<46, 46–49, 50–52, or >52 years), and ever use of oral contraceptives (yes, no). There was a low proportion of missing values for each of these covariates (<5%), with the exception of age at menopause (missing for 28%); most of those women were current HT users. All missing values were assigned to a separate stratum for each variable. Sensitivity analyses that excluded women with missing data on any covariates produced risk estimates similar to those of the full data set and are not presented. Further adjustments for physical activity, waist circumference, alcohol intake, age at menarche, smoking, education, breastfeeding, diabetes, and time since last birth produced no material difference in the risk estimates and were not included in the final model.

Tests for interaction between current HT use and a priori variables of interest, including age at recruitment (dichotomized at the median: <57 years vs. ≥57 years), smoking status (ever vs. never), body mass index (<25, 25–29, or ≥30), and oral contraceptive use (ever vs. never), were carried out by inclusion in the model of the relevant exposure variable (current HT use), indicator variables for the potentially modifying factors, and interaction terms for the product of the 2 variables. The statistical significance of the interaction terms was evaluated by means of the Wald test. To test for heterogeneity of associations according to time between recruitment and diagnosis and by country, we used a meta-analytic approach and calculated the Q statistic. We performed a sensitivity analysis to evaluate the assumption that HT use reported at recruitment remained constant throughout follow-up, by censoring participants at 2, 4, 6, 8, and 10 years of follow-up. All statistical tests presented are 2-tailed, and P values below 0.05 were considered statistically significant. Analyses were performed using Stata, version 10 (Stata Corporation, College Station, Texas).

RESULTS

In total, 115,474 postmenopausal women were followed for a mean of 9.0 years, during which time 601 incident cases of endometrial carcinoma were identified. The average age at recruitment was 57 years, and the mean age at diagnosis was 65 years. Overall, 50,894 women (44%) had ever used HT and 33,853 (29%) were current users at recruitment. Both ever and current use of HT were lowest in Spain (16% and 8%, respectively) and highest in Norway (70% and 59%, respectively; Table 1). The mean total duration of use ranged from 1.8 years in Spain to 5.4 years in Denmark. Among current users, 13% used estrogen-only HT, 74% used combined estrogen-plus-progestin HT, 2.9% used tibolone, and 1.9% used other preparations. Among women who had information on the type of progestin regimen (61% of combined users), most reported using sequential rather than continuous regimens (45% and 16%, respectively), and most preparations contained synthetic derivatives of testosterone or progesterone (55% and 36%, respectively) rather than micronized progesterone (9%; Table 1). Testosterone derivatives were most common in Northern Europe, and progesterone derivatives were most common in Southern Europe; micronized progesterone was commonly used only in France (Table 1).

Compared with never users, current HT users were more likely to be younger, to be leaner, to smoke cigarettes, to be more highly educated, to have ever used oral contraceptives, and to be more physically active than never users (Table 2). Similar differences were found for estrogen-plus-progestin HT users compared with estrogen-only HT users, with the exception of smoking. Users of tibolone were older, less likely to smoke, less highly educated, and less likely to have used oral contraceptives and were more likely to be nulliparous than users of all other types of HT.

Figure 1 shows the association of HT use with risk of endometrial carcinoma after stratification for age and center and adjustment for body mass index, parity, age at menopause, and oral contraceptive use. Compared with women who had never used HT, the multivariate hazard ratio was 1.44 (95% confidence interval (CI): 1.20, 1.74) among ever users, 1.72 (95% CI: 1.39, 2.14) among current users, and 1.16 (95% CI: 0.90, 1.48) among former users. Among current users, increasing duration of use was associated with a progressively increased risk (P for trend < 0.001).

Compared with never use, current use of estrogen-only HT was associated with over a 2-fold increased risk of endometrial carcinoma (hazard ratio (HR) = 2.52, 95% CI: 1.77, 3.57; Figure 1), which increased with duration of use (for <2 years of use, HR = 1.84, 95% CI: 0.88, 3.83; for ≥2 years of use, HR = 2.59, 95% CI: 1.32, 5.07; P for trend ≤ 0.01). There was no evidence of heterogeneity in risk estimates according to estrogen constituent (estradiol compounds vs. conjugated equine estrogens) or route of administration (oral vs. cutaneous; data not shown). Use of tibolone was also associated with a significantly increased risk of endometrial carcinoma (HR = 2.96, 95% CI: 1.67, 5.26; Figure 1); there were too few cases to examine this association according to duration of use.

Table 3 shows the associations of estrogen-plus-progestin HT with risk according to duration of use, regimen, and type of progestin constituent, where these data were available. Overall, current use of estrogen-plus-progestin HT was associated with increased risk (HR = 1.41, 95% CI: 1.08, 1.83), which increased with duration of use (P for trend = 0.01). Use of sequential combined HT was positively associated with risk (HR = 1.52, 95% CI: 1.00, 2.29), while use of continuous combined HT was inversely associated with risk (HR = 0.24, 95% CI: 0.08, 0.77; P for heterogeneity = 0.003), although this finding was based on only 3 cases. The association also varied by type of progestin constituent (P for heterogeneity = 0.02); preparations that contained micronized progesterone were associated with a significantly increased risk, while those that contained progesterone or testosterone derivatives were not associated with risk (Table 3).

Table 4 shows the association of estrogen-plus-progestin HT with risk of endometrial carcinoma according to age, smoking, body mass index, and use of oral contraceptives; there were too few data to allow such subgroup analyses for other types of HT. The association of estrogen-plus-progestin HT with risk of endometrial carcinoma varied by age: There was a positive association among women aged 57 years or older and no association among younger women (P for interaction = 0.001). The association also varied by

Table 1. Use of Menopausal Hormone Therapy at Recruitment Among Postmenopausal Women, by Country, European Prospective Investigation into Cancer and Nutrition, 1992–2000

	All Countries (n = 115,474)	Denmark (n = 18,709)	France (n = 29,304)	Germany (n = 9,052)	Italy (n = 12,193)	The Netherlands (n = 10,304)	Norway (n = 10,426)	Spain (n = 7,863)	United Kingdom (n = 17,623)
Median year of recruitment	1996	1996	1993	1996	1995	1995	1998	1994	1996
No. of cases	601	105	187	38	51	55	20	38	107
HT use, %									
Never use	55.9	52.3	43.8	42.2	75.7	75.3	30.5	83.9	64.2
Ever use	44.1	47.7	56.2	57.8	24.3	24.7	69.5	16.1	35.8
Former use	13.6	16.6	17.7	13.3	12.8	11.6	9.7	7.7	10.4
Current use	29.3	30.9	35.7	43.9	11.0	12.4	59.3	8.4	23.7
Mean age at first HT use, years (SD)[a]	49.9 (5.0)	48.9 (4.7)	51.9 (4.5)	50.1 (3.9)	48.5 (5.3)	49.1 (5.5)	47.1 (4.0)	49.1 (4.4)	50.5 (5.8)
Mean total duration of HT use, years (SD)[a]	3.8 (3.3)	5.4 (4.3)	3.4 (2.8)	4.1 (2.8)	2.3 (2.1)	3.5 (3.3)	3.7 (2.8)	1.8 (1.5)	3.6 (3.0)
Type of HT among current users, %									
Estrogen-only	12.8	12.3	10.0	17.6	22.9	23.1	14.8	13.6	6.0
Estrogen plus progestin	73.9	63.1	89.3	68.3	38.5	27.8	80.2	63.1	73.0
Regimen (% among combined users)									
Sequential	45.2	71.6	7.2	69.7	18.4	66.0	61.4	3.1	89.3
Continuous	15.8	24.4	2.3	24.2	0.6	7.9	38.0	0	8.8
Missing data	39.0	4.0	90.5	6.1	81.0	26.1	0.6	96.9	1.9
Progestin component									
Micronized progesterone	9.0	0	24.0	0.1	2.3	0.8	0	1.0	0
Progesterone derivatives	35.8	19.5	70.7	18.8	84.9	29.8	0.7	93.1	5.5
Testosterone derivatives	54.9	80.5	4.9	81.0	12.8	69.4	99.3	3.4	94.5
Missing data	0.3	0	0.4	0.1	0	0	0	2.5	0
Tibolone	2.9	5.4	0	0	6.5	8.9	0	0	11.4
Other	1.9	1.3	0	1.0	11.6	18.2	0.2	11.2	1.5
Missing data on HT type	8.5	17.9	0.7	13.1	20.5	22.0	4.8	12.1	8.1
Mean current duration of HT use, years (SD)	2.6 (2.2)	NA[b]	2.2 (1.8)	NA	NA	4.7 (3.7)	3.0 (2.3)	NA	NA

Abbreviations: HT, hormone therapy; NA, not applicable; SD, standard deviation.

[a] Among ever users.

[b] Data not available.

Table 2. Baseline Characteristics of Participants According to Use of Menopausal Hormone Therapy, European Prospective Investigation into Cancer and Nutrition, 1992–2000

	All Women[a]						Current Use of Hormone Therapy[b]							
	Never Use (n = 64,506)		Former Use (n = 15,716)		Current Use (n = 33,853)		Estrogen-Only (n = 4,318)		Estrogen Plus Progestin (n = 25,000)		Tibolone (n = 990)		Other (n = 656)	
	%	Mean (SD)	%	Mean (SD)	%	Mean (SD)	%	Mean (SD)	%	Mean (SD)	%	Mean (SD)	%	Mean (SD)
Age, years		58.7 (6.2)		57.7 (5.1)		54.6 (4.9)		55.9 (5.2)		54.3 (4.7)		57.9 (5.0)		52.3 (5.0)
Body mass index[c]		26.0 (4.6)		25.1 (4.2)		24.2 (3.7)		24.6 (3.8)		24.0 (3.6)		25.1 (3.6)		25.4 (4.3)
Physical activity (moderately active/active)	30.6		34.2		33.6		31.2		32.4		42.3		42.2	
Current smoker	16.9		18.6		20.0		19.7		19.5		17.4		24.7	
University degree	14.4		18.7		21.7		17.7		23.6		16.0		18.0	
Self-reported diabetes	3.6		2.4		1.6		1.4		1.5		1.0		3.2	
Alcohol consumption, g/day[d]		9.2 (12.6)		10.6 (13.7)		10.4 (13.2)		10.1 (13.4)		10.4 (13.1)		11.1 (13.4)		11.0 (13.1)
Age at menarche, years[e]														
<12	13.8		14.3		12.9		12.1		12.9		16.8		15.6	
12–15	75.8		76.9		79.0		79.0		79.6		70.9		77.7	
>15	8.2		7.2		6.4		6.7		6.0		8.7		5.5	
Nulliparous	12.6		11.5		10.7		11.5		10.4		13.5		11.1	
No. of livebirths[f]		2.5 (1.2)		2.4 (1.0)		2.3 (0.9)		2.2 (0.9)		2.3 (0.9)		2.3 (1.0)		2.3 (1.0)
Ever breastfed	68.3		69.8		69.5		69.8		69.0		68.2		73.8	
Ever used oral contraceptives	37.1		50.5		62.7		58.1		63.6		57.4		61.4	
Age at menopause, years		49.5 (4.3)		49.5 (4.7)		49.3 (4.7)		49.3 (4.5)		49.4 (4.6)		50.1 (5.0)		47.6 (5.5)

Abbreviation: SD, standard deviation.

[a] Excludes 1,399 women for whom current or former use was not known.

[b] Excludes 20,004 women for whom type of current hormone therapy was not known.

[c] Weight (kg)/height $(m)^2$.

[d] Among alcohol drinkers only.

[e] Women with missing data on age at menarche were excluded.

[f] Among parous women only.

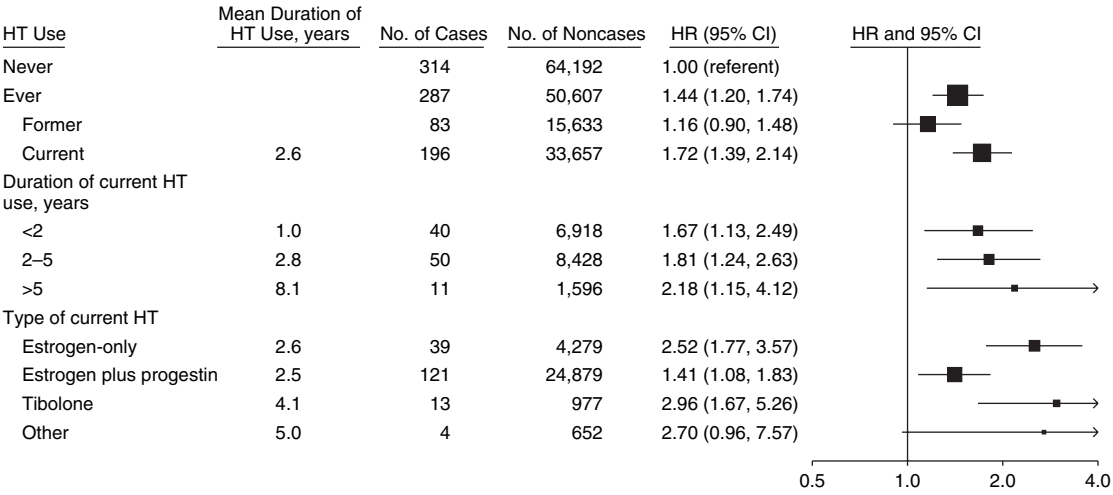

Figure 1. Hazard ratios (HRs) for risk of endometrial carcinoma (black squares) according to use of menopausal hormone therapy (HT), by recruitment center and age, European Prospective Investigation into Cancer and Nutrition, 1992–2006. The size of each square is inversely proportional to the variance of the logarithm of the relative risk. HRs were adjusted for body mass index, parity, age at menopause, and oral contraceptive use. Bars, 95% confidence interval (CI).

smoking status, with a stronger association among ever smokers compared with never smokers (P for interaction = 0.01). Combined HT use was also more strongly associated with risk among normal-weight women, with weaker associations being found among overweight and obese women, although these differences were of marginal statistical significance (P for interaction = 0.07). There was no difference in the association of combined HT use with risk according to ever use of oral contraceptives.

There was no significant heterogeneity in the association of current HT use with risk of endometrial carcinoma according to time between recruitment and diagnosis (<2, 2–4, or ≥5 years) or by country (data not shown). Analyses in which participants were censored after 2, 4, 6, 8, and 10

Table 3. Hazard Ratio[a] for Endometrial Carcinoma Among Current Users (vs. Never Users) of Estrogen-Plus-Progestin Menopausal Hormone Therapy, According to Regimen and Progestin Constituent, European Prospective Investigation into Cancer and Nutrition, 1992–2006

Characteristic	No. of Cases	No. of Noncases	Hazard Ratio	95% Confidence Interval	*P* for Heterogeneity
HT use					
Never user	314	64,192	1.00		
Current user	121	24,879	1.41	1.08, 1.83	
Duration of HT use, years					
<2	30	5,763	1.46	0.94, 2.28	
≥2	47	8,483	1.64	1.11, 2.42	0.01[b]
HT regimen[c]					
Sequential	50	11,240	1.52	1.00, 2.29	
Continuous	3	3,940	0.24	0.08, 0.77	0.003
Progestin constituent[d]					
Micronized progesterone	26	2,231	2.42	1.53, 3.83	
Progesterone derivative	46	8,909	1.23	0.84, 1.79	
Testosterone derivative	46	13,685	1.09	0.74, 1.61	0.02

Abbreviation: HT, hormone therapy.

[a] Hazard ratio estimates were stratified by recruitment center and age and were adjusted for body mass index, parity, age at menopause, and oral contraceptive use.

[b] *P* for trend.

[c] Data on type of regimen were available for 61.0% of estrogen-plus-progestin users.

[d] Data on progestin constituent were available for 99.7% of estrogen-plus-progestin users.

Table 4. Hazard Ratio[a] for Endometrial Carcinoma Among Current Users (vs. Never Users) of Estrogen-Plus-Progestin Menopausal Hormone Therapy, According to Age, Smoking, Body Mass Index, and Use of Oral Contraceptives, European Prospective Investigation into Cancer and Nutrition, 1992–2006

Characteristic	No. of Cases	No. of Noncases	Hazard Ratio	95% Confidence Interval
Age at recruitment, years				
<57	54	17,547	0.92	0.63, 1.34
≥57	67	7,332	1.62	1.20, 2.19
P for interaction			0.001	
Smoking status[b]				
Never smoker	66	13,350	1.15	0.82, 1.62
Ever smoker	51	10,867	2.10	1.35, 3.27
P for interaction			0.01	
Body mass index[c]				
<25	79	16,970	1.49	1.05, 2.13
25–29	28	6,272	1.24	0.74, 2.07
≥30	14	1,637	1.29	0.65, 2.55
P for interaction			0.07	
Use of oral contraceptives[d]				
Never user	64	8,912	1.60	1.13, 2.25
Ever user	55	15,856	1.13	0.75, 1.72
P for interaction			0.13	

[a] Hazard ratio estimates were stratified by recruitment center and age and were adjusted for body mass index, parity, age at menopause, and oral contraceptive use, where appropriate. For all analyses, the reference category was never use of hormone therapy within each stratum.

[b] Data on smoking were available for 97% of current hormone therapy users.

[c] Weight (kg)/height (m)^2.

[d] Data on use of oral contraceptives were available for 99.4% of current hormone therapy users.

years since recruitment also did not appreciably change any of the risk estimates (data not shown).

DISCUSSION

This large European cohort study with a wide variety of HT preparations confirmed the well-established association of estrogen-only HT with risk of endometrial carcinoma. It also showed a strong positive association with tibolone and a smaller increased risk for estrogen-plus-progestin HT.

Progestins counteract the proliferative effect of estrogens on the endometrium (3), and there is a wealth of data from randomized controlled trials showing that use of estrogen-plus-progestin HT reduces endometrial hyperplasia in comparison with use of estrogen-only preparations (14). It has therefore been suggested that the addition of progestin to HT may also weaken the excess risk of endometrial cancer associated with exogenous estrogen use. Our finding that sequential combined HT was associated with a smaller increased risk than that found for estrogen-only HT is consistent with this hypothesis. Evidence from other studies also suggests that use of short-term sequential progestin (where progestin is provided for fewer than 10 days per month) is clearly associated with increased risk (2, 7, 15–20), while use of longer-term sequential regimens is not strongly associated with risk (5, 7, 15, 16, 18–23).

Our finding of a significantly reduced risk with use of continuous progestin regimens, while based on very small numbers, is consistent with findings from most other studies (2, 6, 16, 17, 23, 24). Indeed, in the largest prospective study to date, which had repeated HT information on over 700,000 women, Beral et al. (5) reported a 30% reduction in risk associated with continuous regimens (based on 73 cases, relative risk = 0.71, 95% CI: 0.56, 0.93). The limited trial evidence also suggests that continuous regimens may be associated with a reduced risk, although the numbers of cases were low and the associations were not statistically significant (4, 25). However, other observational studies have shown either no association (7, 21) or increased risks with use of continuous regimens (18–20, 26, 27), with some also showing evidence of a dose-response relation with increasing duration of use (19, 27) or increasing progestin dose (20). The inconsistencies between studies might be explained by the low numbers of users in individual studies and/or the different lengths, doses, or types of progestin used. In particular, the present study found that use of micronized progesterone was associated with an increased risk, whereas testosterone or progesterone-derived derivatives were not. Different progestins have been shown to induce different responses in target tissue such as the breast (28) and endometrium (29). Results from the E3N cohort study in France showed that progesterone-containing HT was more weakly associated with breast cancer risk than was HT containing other progestins (30), and it is conceivable that this weaker prostagenic effect could also apply to the endometrium, whereby micronized progesterone is not as effective in preventing estrogen-induced endometrial cancer in HT users as other progestins. These findings, while based on small numbers, warrant confirmation in other populations in which micronized progesterone is commonly used.

Our finding that tibolone was associated with a significantly increased risk of endometrial cancer is consistent with findings from the United Kingdom Million Women Study, in which Beral et al. (5) reported a relative risk of 1.79 (95% CI: 1.43, 2.25) that remained when analyses were restricted to women who were likely to have used tibolone exclusively. In a record-linkage study carried out in the United Kingdom, de Vries et al. (31) reported similar findings, although many cases had used other types of HT before or after using tibolone and information on other potential confounders was not taken into account. In the current study, tibolone users were, on average, older, less likely to smoke, and more likely to be nulliparous than users of other types of HT. However, these variables were either included in the final model or did not influence the risk estimates, making it unlikely that these factors could explain the association. However, we did not have information on whether tibolone users were more likely to have had previous dysfunctional uterine bleeding or to be past users of estrogen-only HT, as has been suggested (32). Nonetheless, the consistency of our

findings with those from other observational studies suggests that tibolone treatment could be more estrogenic and/or less progestagenic with regard to the endometrium than previously anticipated, and future research on the long-term effects of tibolone on the endometrium is clearly warranted.

It is well established that obesity is associated with an increased risk of endometrial cancer, most likely through higher circulating levels of bioavailable estrogens (3). Our finding that the association of estrogen-plus-progestin HT with risk becomes somewhat weaker with increasing body mass index is consistent with the well-regarded hypothesis that overweight and obese women may be progressively less responsive to the harmful effects of exogenous estrogens because of their already-high circulating levels (5, 33). We had limited statistical power with which to examine these differences by other types of HT, although other studies have also shown greater risks associated with use of estrogen-only HT and tibolone among normal-weight women, with little or no increase in risk among overweight and obese women (5, 18, 20, 33).

In the current study, HT use was associated with an increased risk of endometrial cancer among older women and ever smokers, which is consistent with some (33, 34) but not all (5, 20, 35, 36) studies. While it is plausible that HT may exert a stronger estrogenic effect in older women and in smokers, both of whom have lower circulating estrogen levels, these women also had a higher duration of total HT use, which may account for some, if not all, of this difference in risk. However, a more detailed examination of the association between duration of exposure and risk is hampered by the lack of information on HT use during the follow-up period.

The main strengths of this study lie in its prospective, population-based design, the ability to investigate specific types of HT used at recruitment, and the ability to take potentially confounding factors into account. However, this study had several limitations. Women were classified according to self-reported use of HT at recruitment, and information on adherence to HT during the follow-up period was not available. Following termination of the Women's Health Initiative in 2002 (37) and publication of the Million Women Study results on breast cancer in 2003 (38), many women are likely to have ceased HT use (39, 40), which may have attenuated the associations found between HT and endometrial cancer risk. However, sensitivity analyses that censored participants at various cutoff years during the follow-up period produced results similar to those of the main analysis, suggesting that our findings were robust to changes in exposure status. Furthermore, the risk estimates remained very similar when analyses were stratified by year of recruitment, suggesting that the impact of any secular trends in HT use during the recruitment period is likely to have been small. Another limitation is that some women would have had a hysterectomy during the follow-up period, which may have led to underestimation of the effect of HT on endometrial cancer risk, as this procedure is more common among HT users than among never users. However, the rate of hysterectomy during follow-up is likely to have been low in this study population (subjects' average age at entry was 57 years), so hysterectomy is unlikely to have unduly influenced risk estimates. Finally, our findings for the effect of estrogen-only HT on risk, while important, may not be so relevant today, since women with an intact uterus are now routinely being prescribed estrogen-plus-progestin HT.

In conclusion, our findings of a substantially increased risk of endometrial cancer with use of estrogen-only HT and a smaller positive association with use of combined HT support the hypothesis that progestins have an attenuating effect on endometrial cancer risk, although the risk differs according to regimen and type of progestin constituent. The increased risk associated with use of tibolone requires further investigation.

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REFERENCES

1. Grady D, Gebretsadik T, Kerlikowske K, et al. Hormone replacement therapy and endometrial cancer risk: a meta-analysis. *Obstet Gynecol*. 1995;85(2):304–313.
2. Weiderpass E, Adami HO, Baron JA, et al. Risk of endometrial cancer following estrogen replacement with and without progestins. *J Natl Cancer Inst*. 1999;91(13):1131–1137.
3. Key TJ, Pike MC. The dose-effect relationship between 'unopposed' oestrogens and endometrial mitotic rate: its central role in explaining and predicting endometrial cancer risk. *Br J Cancer.* 1988;57(2):205–212.
4. Anderson GL, Judd HL, Kaunitz AM, et al. Effects of estrogen plus progestin on gynecologic cancers and associated diagnostic procedures: the Women's Health Initiative randomized trial. *JAMA*. 2003;290(13):1739–1748.
5. Beral V, Bull D, Reeves G. Endometrial cancer and hormone-replacement therapy in the Million Women Study. *Lancet*. 2005;365(9470):1543–1551.
6. Hill DA, Weiss NS, Beresford SA, et al. Continuous combined hormone replacement therapy and risk of endometrial cancer. *Am J Obstet Gynecol*. 2000;183(6):1456–1461.
7. Pike MC, Peters RK, Cozen W, et al. Estrogen-progestin replacement therapy and endometrial cancer. *J Natl Cancer Inst*. 1997;89(15):1110–1116.
8. Riboli E, Hunt KJ, Slimani N, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public Health Nutr.* 2002;5(6B):1113–1124.
9. Lahmann PH, Hoffmann K, Allen N, et al. Body size and breast cancer risk: findings from the European Prospective Investigation into Cancer and Nutrition (EPIC). *Int J Cancer.* 2004;111(5):762–771.
10. WHO Collaborating Centre for Drug Statistics Methodology. *ATC/DDD Index 2010*. Oslo, Norway: Norwegian Institute of Public Health; 2010. (http://www.whocc.no/atc_ddd_index/). (Accesssed March 2, 2010).
11. Stanczyk FZ. All progestins are not created equal. *Steroids*. 2003;68(10–13):879–890.
12. Wareham NJ, Jakes RW, Rennie KL, et al. Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *Public Health Nutr.* 2003;6(4):407–413.
13. Schoenfeld D. Partial residuals for the proportional hazards regression model. *Biometrika*. 1982;69(1):239–241.
14. Lethaby A, Suckling J, Barlow D, et al. Hormone replacement therapy in postmenopausal women: endometrial hyperplasia and irregular bleeding. *Cochrane Database Syst Rev.* 2004(3):CD000402. (doi: 10.1002/14651858.CD000402.pub2).
15. Beresford SA, Weiss NS, Voigt LF, et al. Risk of endometrial cancer in relation to use of oestrogen combined with cyclic progestagen therapy in postmenopausal women. *Lancet*. 1997;349(9050):458–461.
16. Doherty JA, Cushing-Haugen KL, Saltzman BS, et al. Long-term use of postmenopausal estrogen and progestin hormone therapies and the risk of endometrial cancer. *Am J Obstet Gynecol*. 2007;197(2):139.e1–139.e7.
17. Reed SD, Voigt LF, Beresford SA, et al. Dose of progestin in postmenopausal-combined hormone therapy and risk of endometrial cancer. *Am J Obstet Gynecol*. 2004;191(4):1146–1151.
18. Karageorgi S, Hankinson SE, Kraft P, et al. Reproductive factors and postmenopausal hormone use in relation to endometrial cancer risk in the Nurses' Health Study cohort 1976–2004. *Int J Cancer.* 2010;126(1):208–216.
19. Razavi P, Pike MC, Horn-Ross PL, et al. Long-term postmenopausal hormone therapy and endometrial cancer. *Cancer Epidemiol Biomarkers Prev.* 2010;19(2):475–483.
20. Newcomb PA, Trentham-Dietz A. Patterns of postmenopausal progestin use with estrogen in relation to endometrial

cancer (United States). *Cancer Causes Control*. 2003;14(2):195–201.
21. Lacey JV Jr, Leitzmann MF, Chang SC, et al. Endometrial cancer and menopausal hormone therapy in the National Institutes of Health-AARP Diet and Health Study cohort. *Cancer*. 2007;109(7):1303–1311.
22. Persson I, Adami HO, Bergkvist L, et al. Risk of endometrial cancer after treatment with oestrogens alone or in conjunction with progestogens: results of a prospective study. *BMJ*. 1989;298(6667):147–151.
23. Strom BL, Schinnar R, Weber AL, et al. Case-control study of postmenopausal hormone replacement therapy and endometrial cancer. *Am J Epidemiol*. 2006;164(8):775–786.
24. Epstein E, Lindqvist PG, Olsson H. A population-based cohort study on the use of hormone treatment and endometrial cancer in southern Sweden. *Int J Cancer*. 2009;125(2):421–425.
25. Hulley S, Furberg C, Barrett-Connor E, et al. Noncardiovascular disease outcomes during 6.8 years of hormone therapy: Heart and Estrogen/Progestin Replacement Study follow-up (HERS II). *JAMA*. 2002;288(1):58–66.
26. Jain MG, Rohan TE, Howe GR. Hormone replacement therapy and endometrial cancer in Ontario, Canada. *J Clin Epidemiol*. 2000;53(4):385–391.
27. Lacey JV Jr, Brinton LA, Lubin JH, et al. Endometrial carcinoma risks among menopausal estrogen plus progestin and unopposed estrogen users in a cohort of postmenopausal women. *Cancer Epidemiol Biomarkers Prev*. 2005;14(7):1724–1731.
28. Wood CE, Sitruk-Ware RL, Tsong YY, et al. Effects of estradiol with oral or intravaginal progesterone on risk markers for breast cancer in a postmenopausal monkey model. *Menopause*. 2007;14(4):639–647.
29. Jondet M, Maroni M, Yaneva H, et al. Comparative endometrial histology in postmenopausal women with sequential hormone replacement therapy of estradiol and, either chlormadinone acetate or micronized progesterone. *Maturitas*. 2002;41(2):115–121.
30. 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*. 2008;107(1):103–111.
31. de Vries CS, Bromley SE, Thomas H, et al. Tibolone and endometrial cancer: a cohort and nested case-control study in the UK. *Drug Saf*. 2005;28(3):241–249.
32. Wierik EJ, Hendricks PT, Boerstoel-Streefland M. Clinical background of women prescribed tibolone or combined estrogen + progestogen therapies: a UK MediPlus study. *Climacteric*. 2004;7(2):197–209.
33. Brinton LA, Hoover RN. Estrogen replacement therapy and endometrial cancer risk: unresolved issues. The Endometrial Cancer Collaborative Group. *Obstet Gynecol*. 1993;81(2):265–271.
34. Rubin GL, Peterson HB, Lee NC, et al. Estrogen replacement therapy and the risk of endometrial cancer: remaining controversies. *Am J Obstet Gynecol*. 1990;162(1):148–154.
35. Shields TS, Weiss NS, Voigt LF, et al. The additional risk of endometrial cancer associated with unopposed estrogen use in women with other risk factors. *Epidemiology*. 1999;10(6):733–738.
36. Newcomer LM, Newcomb PA, Trentham-Dietz A, et al. Hormonal risk factors for endometrial cancer: modification by cigarette smoking (United States). *Cancer Causes Control*. 2001;12(9):829–835.
37. 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*. 2002;288(3):321–333.
38. Beral V. Breast cancer and hormone-replacement therapy in the Million Women Study. *Lancet*. 2003;362(9382):419–427.
39. Faber A, Bouvy ML, Loskamp L, et al. Dramatic change in prescribing of hormone replacement therapy in the Netherlands after publication of the Million Women Study: a follow-up study. *Br J Clin Pharmacol*. 2005;60(6):641–647.
40. Watson J, Wise L, Green J. Prescribing of hormone therapy for menopause, tibolone, and bisphosphonates in women in the UK between 1991 and 2005. *Eur J Clin Pharmacol*. 2007;63(9):843–849.