

Breast cancer, endometrial cancer, and cardiovascular events in participants who used vaginal estrogen in the Women's Health Initiative Observational Study

Carolyn J. Crandall, MD, MS,¹ Kathleen M. Hovey, MS,² Christopher A. Andrews, PhD,³ Rowan T. Chlebowski, MD, PhD,⁴ Marcia L. Stefanick, PhD,⁵ Dorothy S. Lane, MD, MPH,⁶ Jan Shifren, MD,⁷ Chu Chen, PhD,⁸ Andrew M. Kaunitz, MD,⁹ Jane A. Cauley, DrPH,¹⁰ and JoAnn E. Manson, MD, DrPH¹¹

Abstract

Objective: To determine the association between use of vaginal estrogen and risk of a global index event (GIE), defined as time to first occurrence of coronary heart disease (CHD), invasive breast cancer, stroke, pulmonary embolism, hip fracture, colorectal cancer, endometrial cancer, or death from any cause.

Methods: For this prospective observational cohort study, we used data from participants of the Women's Health Initiative Observational Study, who were recruited at 40 US clinical centers, aged 50 to 79 years at baseline and did not use systemic estrogen therapy during follow-up (n = 45,663, median follow-up 7.2 years). We collected data regarding incident CHD, invasive breast cancer, stroke, pulmonary embolism, hip fracture, colorectal cancer, endometrial cancer, death, and self-reported use of vaginal estrogen (cream, tablet). We used Cox proportional-hazards regression models to adjust for covariates.

Results: Among women with an intact uterus, the risks of stroke, invasive breast cancer, colorectal cancer, endometrial cancer, and pulmonary embolism/deep vein thrombosis were not significantly different between vaginal estrogen users and nonusers, whereas the risks of CHD, fracture, all-cause mortality, and GIE were lower in users than in nonusers (GIE adjusted hazard ratio 0.68, 95% confidence interval 0.55-0.86). Among hysterectomized women, the risks of each of the individual GIE components and of the overall GIE were not significantly different in users versus nonusers of vaginal estrogen (GIE adjusted hazard ratio 0.94, 95% confidence interval 0.70-1.26).

Conclusions: The risks of cardiovascular disease and cancer were not elevated among postmenopausal women using vaginal estrogens, providing reassurance about the safety of treatment.

Key Words: Breast cancer – Cardiovascular diseases – Endometrial cancer – Genitourinary syndrome of menopause – Vaginal estradiol – Vaginal estrogen – Venous thromboembolism.

The genitourinary syndrome of menopause encompasses a constellation of symptoms, including genital symptoms of dryness, burning, and irritation; sexual symptoms of lack of lubrication, discomfort or pain, and impaired function; and urinary symptoms of urgency, dysuria,

and recurrent urinary tract infections.¹ It is highly prevalent, occurring in 20% to 45% of menopausal women.¹⁻³ Genital atrophic changes may have detrimental effects on a woman's quality of life.^{3,4} Several clinical guidelines advocate local low-dose vaginal estrogen therapy, instead of systemic

Received April 18, 2017; revised and accepted June 23, 2017.

From the ¹Department of Medicine, University of California at Los Angeles, Los Angeles, CA; ²Department of Epidemiology and Environmental Health, University at Buffalo, The State University of New York, Buffalo, New York, NY; ³Department of Ophthalmology and Visual Sciences, University of Michigan, Ann Arbor, MI; ⁴Department of Medical Oncology and Therapeutics, City of Hope National Medical Center, Duarte, CA; ⁵Departments of Medicine and Obstetrics and Gynecology, Stanford University School of Medicine, Stanford, CA; ⁶Department of Family, Population and Preventive Medicine, Stony Brook University School of Medicine, Stony Brook, New York, NY; ⁷Department of Obstetrics and Gynecology, Massachusetts General Hospital, Harvard Medical School, Boston, MA; ⁸Program in Epidemiology, Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, Washington, D.C.; ⁹Department of Obstetrics and Gynecology, University of Florida College of Medicine, Jacksonville, FL; ¹⁰Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; and ¹¹Division of Preventive

Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

Funding: The WHI program is funded by the National Heart, Lung, and Blood Institute, National Institutes of Health, US Department of Health and Human Services through contracts HHSN268201100046C, HHSN268201100001C, HHSN268201100002C, HHSN268201100003C, HHSN268201100004C, and HHSN271201100004C.

Conflicts of interest: The following authors have no conflicts to disclose: C.J.C., K.M.H., J.E.M., C.C., D.S.L., J.A.C., C.A., M.L.S. A.M.K. serves as a consultant for Allergan, Inc; AMAG, Pfizer Inc; and Shionogi. He receives research grants (funds paid to University of Florida) from TherapeuticsMD. He receives royalties from UpToDate. R.T.C. serves as consultant for Amgen, Genentech, Novartis, Astra-Zeneca, and Pfizer. He also is on speaker's panel for Genentech and Novartis. J.L.S. serves as a research consultant to the New England Research Institutes.

Address correspondence to: Carolyn J. Crandall, MD, MS, Department of Medicine, University of California at Los Angeles, Los Angeles, CA. E-mail: ccrandall@mednet.ucla.edu

estrogen therapy, for the treatment of postmenopausal women with only vaginal symptoms (ie, without hot flashes).^{1,2,5} Several formulations of low-dose vaginal estrogen therapy are available for treatment of the genitourinary syndrome of menopause, including topical creams, an intravaginal insert, and an intravaginal ring. In a recent Cochrane review, all appear to have similar efficacy and safety.⁶

The use of some formulations of low-dose vaginal estrogens acutely increases serum estrogen levels,^{7,8} but maintenance levels remain in the normal postmenopausal range.⁹ Randomized trials have shown that systemic estrogen is linked to increased risks of venous thromboembolism, stroke, and (with combination estrogen + progestogen) invasive breast cancer.¹⁰⁻¹² Despite the selection and confounding bias that apply to observational studies, these adverse health risks of systemic estrogen have been demonstrated in observational studies.¹³⁻¹⁷ Therefore, large prospective observational studies might demonstrate whether vaginal estrogen use confers similar risks. However, it is unknown whether low-dose vaginal estrogen confers similar risks. Potential systemic effects of vaginal estrogen are included as warnings on the package labeling for vaginal estrogen preparations. However, randomized controlled trials of vaginal estrogen therapy were not designed to examine major endpoints such as heart attack, stroke, pulmonary embolism (PE), deep vein thrombosis (DVT), cancer, or hip fracture. Several observational studies have examined breast cancer and fracture risk in relation to vaginal estrogen therapy. Specifically, a Finnish observational study found that the use of vaginal estradiol was not associated with increased risk of breast cancer (although oral and transdermal estradiol use were associated with increased risk),¹⁸ and a Swedish case-control study suggested that current users of low-potency vaginal estrogen had lower hip fracture risk compared with never-users of estrogen.¹⁹ In the recent American College of Obstetricians and Gynecologists Committee Opinion regarding route of estrogen administration and DVT, the conclusion was that vaginal estrogens (in contrast to oral systemic estrogens) are not associated with increased risk of venous thromboembolism,²⁰ but observational studies are lacking.⁹ Observational studies of associations of vaginal estradiol use with risk of other types of cardiovascular disease (CVD) and overall mortality are similarly lacking, though one study without a placebo comparison group reported decreased risk of coronary heart disease and stroke death in vaginal estrogen users compared with the background population.²¹ Long-term studies regarding endometrial safety of vaginal estrogen use are also needed^{6,9}; one large Danish registry study that did not exclude users of systemic hormone therapy (HT) found a two-fold increased endometrial cancer risk among users of vaginal estrogen.²² However, in our review of published studies, the overall balance of risks and benefits with the use of vaginal estrogens has not been studied in detail, even in observational studies.

The genitourinary syndrome of menopause does not improve without therapy and long duration of vaginal therapy is usually necessary.³ The high prevalence of the

genitourinary syndrome of menopause and its adverse impact on sexual function and quality of life for postmenopausal women underscore the clinical relevance of assessing the safety and efficacy of local low-dose vaginal estrogen use. The Women's Health Initiative Observational Study (WHI-OS) offers an opportunity to examine the longitudinal data regarding associations between vaginal estrogen use and cardiovascular, cancer, and other health outcomes among a large cohort of US women reporting information regarding vaginal estrogen therapy use and important covariates.

METHODS

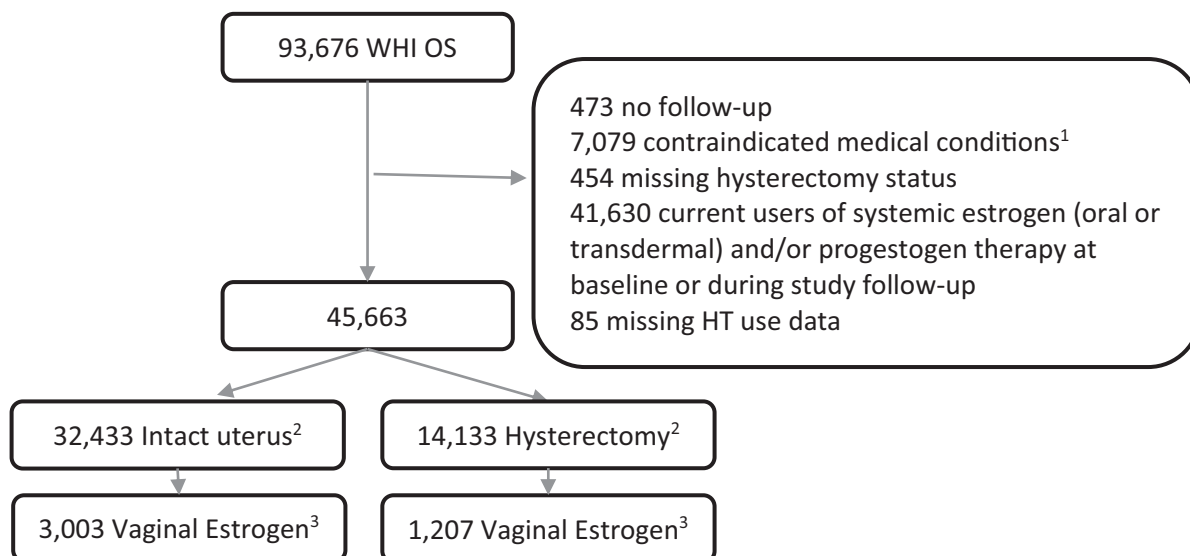
Study population and design

The WHI-OS, conducted from 1993 to 2005, enrolled 93,676 postmenopausal women aged 50 to 79 years at one of 40 clinical centers nationwide to examine risk factors and biomarkers for disease in a large prospective cohort. Details of the inclusion and exclusion criteria and the recruitment process were previously described.^{23,24} Inclusion criteria were ages between 50 and 79 years and intention to maintain residence in the same geographic area for at least 3 years. Exclusion criteria included medical conditions conferring a predicted survival of fewer than 3 years, or conditions (eg, alcohol or drug dependency, dementia, or medical illness) that would be expected to interfere with retention. Institutional review board approval was obtained at each clinical center. Each participant provided written informed consent. After the main WHI-OS ended, the WHI Extension Study (WHI Extension I, Ext 1) collected additional follow-up data from 2005 to 2010, reflecting 7.2 years of median follow-up.

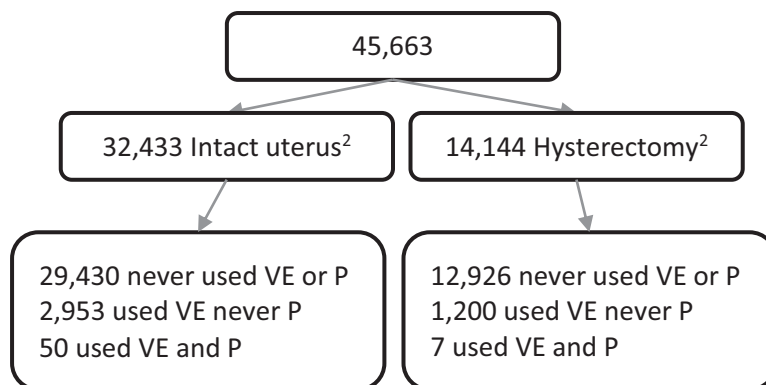
For the current analyses, of the 93,676 WHI-OS participants, we excluded data from women with prior breast, endometrial, or ovarian cancer ($n = 7,079$), women for whom information was missing regarding hysterectomy status ($n = 454$), women currently using oral or transdermal estrogen or progestogen any time during follow-up ($n = 41,630$, censoring beginning at baseline for prevalent users or at time of subsequent initiation), women for whom data regarding menopausal HT use was missing ($n = 85$), and women who did not provide follow-up data ($n = 473$), resulting in an analytic sample of 45,663 women (Fig. 1). Study participants could have initiated vaginal estrogen, or experienced hysterectomy, during the study follow-up, resulting in a sample size of 32,433 women without hysterectomy (of whom 3,003 women were users of vaginal estrogen during follow-up) and 14,133 women with hysterectomy (of whom 1,207 women were users of vaginal estrogen during follow-up).

Outcomes

Study outcomes were assessed using annual self-assessment questionnaires. The following events were adjudicated based on medical record review by WHI physicians: myocardial infarction, stroke, death from any cause, hip fracture, and any cancer. Incident venous thromboembolic events (DVT, hospitalization for PE) were ascertained based on self-report using annual questionnaires. Cause of death was determined



Participant inclusion by reported progestogen (P) use



¹ contraindicated medical conditions include past history of breast cancer, endometrial cancer or ovarian cancer.

² numbers don't add up to 45,663 due to the time varying nature of hysterectomy status: 903 change from no hysterectomy to hysterectomy and are counted in both cells

³ vaginal estrogen cream or vaginal tablet at some time during follow-up (Estradiol ring was only ascertained during the Extension Study; users are not included in this analysis)

FIG. 1. Algorithm of study participants. HT, hormone therapy; VE, vaginal estrogen.

at the Clinical Coordinating Center after medical record or death certificate review. The National Death Index, conducted serially, provided additional information on cause of death.

As for the WHI HT trials, the global index event (GIE) was defined as the time to first occurrence of CHD (nonfatal myocardial infarction or CHD death), invasive breast cancer, stroke, PE, hip fracture, colorectal cancer, endometrial cancer, or death from any cause through the end of WHI Ext I.²⁵

Assessment of vaginal estrogen use

Use of vaginal estrogen was ascertained using self-assessment questionnaires at baseline, annually from years 3 through 8, and at Ext 1 year 4 (Table 1). Information

regarding specific type of vaginal estrogen (cream, ring, or tablet) or dose was not collected (use of the vaginal estradiol ring was only assessed at the end of the follow-up period in the Extension Study, so users are not included in this analysis).

We imputed missing information regarding vaginal estrogen use from the previous year's questionnaire if the same preparation was reported in the year before, compared with the year after, the missing questionnaire.

Other measures

We used baseline questionnaires to collect information regarding age, race/ethnicity, education level, household income, medical history (previous cancer, cardiovascular disease, DVT,

TABLE 1. Questionnaire assessment of vaginal estrogen use

Annual follow-up visit #	Form #	Questionnaire item	Response choices
Baseline	43	Did you ever take any type of estrogen, such as Premarin, progesterone, such as Provera, testosterone, or any other hormone medications. . .were these hormones in the form of a vaginal cream or suppository? Did you take them for 3 straight months or more? What is the name of the vaginal cream or suppository you used?	Yes, no Yes, no Name, # times per day/week/month/year
3	143	In the past 2 years, did you use a vaginal cream or suppository containing estrogen which was prescribed by a doctor? In the past 2 years, how many months did you use the vaginal cream or suppository	Yes, no, don't know <1 month, 1-6 months, 7-10 months, 11-12 months, 13-18 months, 19-24 months
4, 5, 6, 7, 8	144, 145, 146, 147, 148	In the past year, did you use a vaginal cream or suppository containing estrogen which was prescribed by a doctor? In the past year, how many months did you use the vaginal cream or suppository	Yes, no, don't know <1 month, 1-6 months, 7-10 months, 11-12 months
Extension 1	153	Medication and Supplement Inventory. For each of the prescription medications you are currently taking, please answer the questions below using the label on the prescription bottle.	Name of medication Strength of medication Medication type

PE, fracture, hypertension, or diabetes mellitus), reproductive history (oophorectomy, hysterectomy, age at menopause, age at first birth), medication use (past HT use, oral contraceptive use, aspirin use, or statin use), breast cancer risk factors, cigarette smoking, and alcohol intake (servings/week). Gail breast cancer risk score was calculated for each participant.²⁶ Physical activity (total metabolic equivalent-h/wk) was assessed using the WHI validated physical activity questionnaire.^{27,28}

At baseline, using a standardized protocol, blood pressure, height, and weight were directly measured. Body mass index (BMI) was calculated as body weight in kilograms (kg) divided by the square of height in meters.

Role of the funding source

The WHI-OS was funded by National Institutes of Health. Representatives from the National Institutes of Health participated in the design, monitoring, and conduct of the WHI-OS.

Statistical analysis

We used Cox proportional-hazards models to examine associations between vaginal estrogen use and individual endpoints and GIE risk. The main predictor was time-varying exposure to vaginal estrogen. The time to outcome was computed from the date of enrollment to the date of the first event (among the events included in the GIE index), or last available follow-up visit, or end of study follow-up period, whichever came first. The end of the study follow-up period was defined as 2.5 years after the last ascertainment of vaginal estrogen use. Because of the different risk profiles of estrogen alone (in women with hysterectomy) and estrogen plus progesterone (in women without hysterectomy), we made the a priori decision to present the results separately for women with an intact uterus and women who have had a hysterectomy. Hysterectomy status was time-varying. Covariates were chosen a priori based on previously published studies and included age, education, past estrogen use, history of cancer other than breast, endometrial or ovarian cancer before study

baseline, history of cardiovascular disease before study baseline, history of DVT or PE before study baseline, race/ethnicity, baseline BMI, baseline diagnosis of diabetes, baseline physical activity (total metabolic equivalent-h/wk), hypertension, Gail breast cancer risk score, fracture after age 55 before study enrollment, smoking, income, and alcohol use (servings/wk) All analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC).

RESULTS

Mean follow-up duration was 6.4 years; median follow-up was 7.2 years. Among women who initiated vaginal estrogen during the follow-up period, median duration of use was 2 years. Among women already using vaginal estrogen at baseline, median duration of use was 3 years before enrollment in WHI. Among vaginal estrogen users, on average, use encompassed 40% of the time during follow-up. At baseline, mean (standard deviation) age of participants was 64.8 (7.4) years in women who did not use vaginal estrogen at any time during follow-up and 65.5 (7.0) years in women who used vaginal estrogen during follow-up. In the overall analytic sample (women with hysterectomy and women without hysterectomy combined), compared with nonusers of vaginal estrogen, vaginal estrogen users were less likely to be black or African American (4.9% vs 11.2%), Hispanic (4.4% vs 2.3%), current smokers (3.6% vs 7.4%), diabetic (3.0% vs 5.5%), and to have BMI ≥ 30 kg/m² (19.1% vs 29.9%); vaginal estrogen users were more likely to be white (89.2% vs 79.8%), college graduates (46.3% vs 37.8%), have household income \geq \$100,000, and to have BMI < 25 kg/m² (45.5% vs 35.9%) (Table 2). The same pattern was true when participants were stratified by hysterectomy status. At baseline, the frequencies of other cardiovascular risk factors, cardiovascular disease events before study enrollment, and previous cancer diagnosis were similar among users and nonusers of vaginal estrogen. Bilateral oophorectomy, BMI ≥ 30 kg/m², treated hypertension, history of cardiovascular

TABLE 2. Baseline characteristics by vaginal estrogen use^a in those with or without hysterectomy (N = 45,663)

	Intact Uterus ^b		Hysterectomy		Overall	
	No VE 29,430 n (%)	Yes VE 3,003 n (%)	No VE 12,926 n (%)	Yes VE 1,207 n (%)	No VE 41,563 n (%)	Yes VE 4,100 n (%)
Age at baseline, y						
Mean (SD)	64.7 (7.3)	65.3 (7.0)	65.1 (7.4)	66.0 (7.1)	64.8 (7.4)	65.5 (7.0)
<60	7,914 (26.9)	667 (22.2)	3,134 (24.2)	238 (19.7)	10,873 (26.2)	881 (21.5)
60-69	12,983 (44.1)	1,419 (47.3)	5,747 (44.5)	556 (46.1)	18,323 (44.1)	1,912 (46.6)
≥70	8,533 (29.0)	917 (30.5)	4,045 (31.3)	413 (34.2)	12,367 (29.8)	1,307 (31.9)
Ethnicity						
Asian or Pacific Islander	885 (3.0)	64 (2.1)	237 (1.8)	28 (2.3)	1,107 (2.7)	91 (2.2)
Black or African-American	2,526 (8.6)	122 (4.1)	2,176 (16.9)	80 (6.7)	4,653 (11.2)	201 (4.9)
Hispanic/Latino	1,223 (4.2)	66 (2.2)	639 (5.0)	32 (2.7)	1,829 (4.4)	95 (2.3)
White (not of Hispanic origin)	24,198 (82.5)	2,706 (90.5)	9,569 (74.2)	1,041 (86.5)	33,085 (79.8)	3,645 (89.2)
Other	510 (1.7)	33 (1.1)	270 (2.1)	22 (1.8)	769 (1.9)	53 (1.3)
Education						
High school or less	6,897 (23.6)	531 (17.8)	3,846 (30.1)	262 (22.0)	10,521 (25.5)	773 (19.0)
Some college or vocational training	10,320 (35.4)	999 (33.5)	5,057 (39.5)	447 (37.5)	15,110 (36.7)	1,409 (34.7)
College graduate or more	11,959 (41.0)	1,450 (48.7)	3,892 (30.4)	484 (40.6)	15,556 (37.8)	1,882 (46.3)
Family income						
Less than \$50,000	17,477 (64.9)	1,532 (55.4)	8,535 (72.2)	712 (63.6)	25,521 (67.2)	2,183 (57.7)
\$50,000 to \$99,999	7,131 (26.5)	898 (32.5)	2,626 (22.2)	280 (25.0)	9,573 (25.2)	1,146 (30.3)
\$100,000 or more	2,303 (8.6)	337 (12.2)	659 (5.6)	127 (11.3)	2,910 (7.7)	453 (12.0)
Any insurance						
No	1,258 (4.3)	75 (2.5)	642 (5.0)	26 (2.2)	1,867 (4.6)	100 (2.5)
Yes	27,805 (95.7)	2,905 (97.5)	12,110 (95.0)	1,162 (97.8)	39,162 (95.4)	3,961 (97.5)
Mammogram (past 2 y)						
Mammogram within 2 y	22,479 (79.2)	2,650 (90.3)	9,715 (78.6)	1,022 (87.7)	31,561 (79.0)	3,575 (89.6)
No mammogram within 2 y	5,887 (20.8)	285 (9.7)	2,650 (21.4)	143 (12.3)	8,397 (21.0)	417 (10.4)
Bilateral oophorectomy						
No	29,046 (99.4)	2,972 (99.5)	7,341 (60.7)	755 (64.9)	35,606 (87.9)	3,617 (89.5)
Yes	165 (0.6)	15 (0.5)	4,761 (39.3)	409 (35.1)	4,922 (12.1)	424 (10.5)
Alcohol servings/wk						
Mean (SD)	2.5 (5.2)	2.7 (4.9)	1.9 (4.6)	2.1 (4.5)	2.3 (5.0)	2.5 (4.8)
None	12,693 (43.2)	1,111 (37.1)	6,822 (52.9)	580 (48.2)	19,158 (46.2)	1,644 (40.2)
<2 servings/wk	8,223 (28.0)	881 (29.4)	3,281 (25.5)	306 (25.4)	11,281 (27.2)	1,157 (28.3)
2 or more servings/wk	8,433 (28.7)	1,004 (33.5)	2,787 (21.6)	318 (26.4)	11,009 (26.6)	1,290 (31.5)
Smoking status						
Never smoked	15,039 (51.8)	1,545 (52.1)	6,665 (52.5)	654 (55.0)	21,305 (52.1)	2,139 (52.9)
Past smoker	11,915 (41.1)	1,318 (44.4)	5,004 (39.4)	493 (41.5)	16,586 (40.5)	1,763 (43.6)
Current smoker	2,058 (7.1)	103 (3.5)	1,018 (8.0)	42 (3.5)	3,021 (7.4)	145 (3.6)
Physical activity (total MET-h/wk)						
Mean (SD)	13.4 (14.4)	15.5 (14.7)	11.7 (13.7)	13.2 (12.9)	12.9 (14.2)	14.9 (14.3)
Body mass index, kg/m ²						
Mean (SD)	27.6 (6.1)	26.2 (5.1)	28.9 (6.5)	27.2 (5.4)	28.0 (6.3)	26.5 (5.2)
<25	11,137 (38.4)	1,412 (47.7)	3,850 (30.2)	469 (39.2)	14,722 (35.9)	1,843 (45.5)
25-<30	9,891 (34.1)	1,036 (35.0)	4,422 (34.7)	444 (37.2)	14,017 (34.2)	1,433 (35.4)
≥30	8,005 (27.6)	513 (17.3)	4,486 (35.2)	282 (23.6)	12,267 (29.9)	774 (19.1)
Diabetes treated (pills or shots)						
No	28,050 (95.4)	2,931 (97.6)	11,932 (92.5)	1,154 (95.7)	39,213 (94.5)	3,977 (97.0)
Yes	1,343 (4.6)	72 (2.4)	972 (7.5)	52 (4.3)	2,292 (5.5)	122 (3.0)
Systolic blood pressure						
Mean (SD)	128 (18.3)	126 (17.6)	130 (18.6)	128 (18.3)	128 (18.4)	127 (17.9)
Diastolic blood pressure						
Mean (SD)	74.8 (9.5)	73.8 (9.1)	75.5 (9.8)	74.9 (9.5)	75.0 (9.6)	74.1 (9.2)
Hypertension						
Never hypertensive	19,496 (67.6)	2,105 (71.6)	7,383 (58.5)	717 (60.7)	26,357 (64.8)	2,746 (68.4)
Untreated hypertensive	2,334 (8.1)	243 (8.3)	1,209 (9.6)	123 (10.4)	3,470 (8.5)	356 (8.9)
Treated hypertensive	7,004 (24.3)	590 (20.1)	4,039 (32.0)	342 (28.9)	10,866 (26.7)	910 (22.7)
Age at menopause						
Mean (SD)	50.2 (4.8)	50.6 (4.7)	45.3 (7.4)	46.0 (7.1)	48.8 (6.1)	49.3 (5.8)
Fracture after age 55+						
No	19,612 (72.5)	1,987 (72.3)	8,560 (72.3)	811 (73.4)	27,626 (72.4)	2,722 (72.6)
Yes	4,396 (16.2)	518 (18.9)	1,921 (16.2)	209 (18.9)	6,193 (16.2)	706 (18.8)
Age <55	3,052 (11.3)	243 (8.8)	1,360 (11.5)	85 (7.7)	4,351 (11.4)	321 (8.6)
Menopausal hormone therapy usage at baseline						
Never used	23,535 (80.0)	2,226 (74.1)	8,106 (62.7)	742 (61.5)	30,980 (74.5)	2,884 (70.3)
Past user	5,895 (20.0)	777 (25.9)	4,820 (37.3)	465 (38.5)	10,583 (25.5)	1,216 (29.7)

(Continued on next page)

TABLE 2. Continued

	Intact Uterus ^b		Hysterectomy		Overall	
	No VE 29,430 n (%)	Yes VE 3,003 n (%)	No VE 12,926 n (%)	Yes VE 1,207 n (%)	No VE 41,563 n (%)	Yes VE 4,100 n (%)
Age at first birth, y						
Never had term pregnancy	4,096 (15.7)	373 (13.8)	1,422 (12.6)	125 (11.7)	5,445 (14.9)	490 (13.3)
<20	2,665 (10.2)	191 (7.1)	2,030 (17.9)	123 (11.5)	4,618 (12.6)	307 (8.4)
20-29	16,573 (63.6)	1,851 (68.7)	7,123 (63.0)	736 (68.7)	23,231 (63.4)	2,518 (68.6)
30+	2,721 (10.4)	281 (10.4)	737 (6.5)	88 (8.2)	3,372 (9.2)	358 (9.7)
Gail breast cancer risk score						
Mean (SD)	1.8 (1.0)	2.0 (1.0)	1.8 (1.1)	2.0 (1.1)	1.8 (1.1)	2.0 (1.1)
Female relative had breast cancer						
No	22,426 (80.8)	2,272 (79.5)	9,693 (79.8)	912 (79.0)	31,521 (80.5)	3,092 (79.2)
Yes	5,344 (19.2)	586 (20.5)	2,452 (20.2)	243 (21.0)	7,636 (19.5)	813 (20.8)
Oral contraceptive use ever						
No	19,445 (66.1)	1,877 (62.5)	8,893 (68.8)	810 (67.1)	27,805 (66.9)	2,620 (63.9)
Yes	9,985 (33.9)	1,126 (37.5)	4,033 (31.2)	397 (32.9)	13,758 (33.1)	1,480 (36.1)
Aspirin use med inventory						
No	23,428 (79.6)	2,288 (76.2)	10,290 (79.6)	932 (77.2)	33,105 (79.7)	3,141 (76.6)
Yes	6,002 (20.4)	715 (23.8)	2,636 (20.4)	275 (22.8)	8,458 (20.3)	959 (23.4)
Statin use						
No	26,975 (91.7)	2,766 (92.1)	11,630 (90.0)	1,070 (88.6)	37,885 (91.2)	3,738 (91.2)
Yes	2,455 (8.3)	237 (7.9)	1,296 (10.0)	137 (11.4)	3,678 (8.8)	362 (8.8)
Stroke ever						
No	28,952 (98.4)	2,961 (98.6)	12,584 (97.4)	1,184 (98.3)	40,755 (98.1)	4,035 (98.5)
Yes	469 (1.6)	41 (1.4)	335 (2.6)	21 (1.7)	793 (1.9)	62 (1.5)
Myocardial infarction ever						
No	28,713 (97.6)	2,958 (98.5)	12,376 (95.9)	1,170 (96.9)	40,314 (97.1)	4,020 (98.0)
Yes	695 (2.4)	45 (1.5)	535 (4.1)	37 (3.1)	1,212 (2.9)	80 (2.0)
Coronary artery bypass graft or percutaneous transluminal coronary artery angioplasty ever						
No	28,386 (98.1)	2,919 (98.6)	12,293 (97.2)	1,158 (97.6)	39,917 (97.8)	3,974 (98.4)
Yes	537 (1.9)	42 (1.4)	360 (2.8)	28 (2.4)	881 (2.2)	65 (1.6)
History of cardiovascular disease ^c						
No	28,053 (95.3)	2,898 (96.5)	11,952 (92.5)	1,140 (94.4)	39,248 (94.4)	3,933 (95.9)
Yes	1,377 (4.7)	105 (3.5)	974 (7.5)	67 (5.6)	2,315 (5.6)	167 (4.1)
Deep vein thrombosis or pulmonary embolism before study enrollment						
No	28,232 (95.9)	2,861 (95.3)	11,969 (92.6)	1,108 (91.8)	39,433 (94.9)	3,864 (94.2)
Yes	1,198 (4.1)	142 (4.7)	957 (7.4)	99 (8.2)	2,130 (5.1)	236 (5.8)
History of cancer ^d						
No	27,687 (94.1)	2,834 (94.4)	11,650 (90.1)	1,115 (92.4)	38,585 (92.8)	3,841 (93.7)
Yes	1,743 (5.9)	169 (5.6)	1,276 (9.9)	92 (7.6)	2,978 (7.2)	259 (6.3)

MET, metabolic equivalent task; VE, vaginal estrogen.

^aVaginal estrogen (cream or suppository) use is time-varying.

^bHysterectomy status is time-varying.

^cHistory of cardiovascular disease includes a past history of myocardial infarction, stroke, or revascularization prior to study enrollment.

^dNot including breast, endometrial, or ovarian cancers (which were exclusion criteria).

disease, and past use of HT were more frequent in women who had undergone hysterectomy than among women without hysterectomy. The frequencies of specific types of cardiovascular events and cardiovascular risk factors at baseline were similar in women with versus without hysterectomy.

In the study cohort overall, after adjustment for age, education, past HT use, history of cancer, history of CVD, history of venous thromboembolism, race/ethnicity, BMI, diabetes, physical activity level, hypertension, Gail breast cancer risk score, previous fracture, smoking, household income, and alcohol intake level, the risk of invasive breast cancer, death, stroke, colorectal cancer, and venous thromboembolism were similar among users versus nonusers of vaginal estrogen (Table 3). Compared with nonusers of vaginal estrogen, users of vaginal estrogen had 48% lower risk of CHD (adjusted hazard ratio [aHR] 0.52, 95% confidence interval [CI] 0.31-0.85), and 60% lower risk of hip fracture (aHR 0.40, 95% CI 0.19-0.85). The risk of GIE was lower in users than in nonusers of vaginal estrogen (aHR 0.76, 95% CI 0.64-0.91).

When we analyzed results based on hysterectomy status, the risk of invasive breast cancer, stroke, colorectal cancer, endometrial cancer, and venous thromboembolism was not significantly different in vaginal estrogen users than in nonusers of vaginal estrogen. In women with prior hysterectomy, the aHR estimates for GIE and for each of the individual components of the global index were not significantly different in users of vaginal estrogen than in nonusers of vaginal estrogen. In contrast, in women without hysterectomy, the risk of several outcomes was significantly lower in vaginal estrogen users than in nonusers of vaginal estrogen: GIE aHR 0.68 (95% CI 0.55-0.86), death aHR 0.62 (95% CI 0.41-0.93), CHD aHR 0.39 (95% CI 0.19-0.78), and hip fracture aHR 0.40 (95% CI 0.16-0.96). The aHRs are illustrated graphically in Figure 2.

DISCUSSION

In this large prospective cohort study, postmenopausal women who used vaginal estrogen had similar risks of invasive breast cancer, stroke, colorectal cancer, endometrial

TABLE 3. Hazard ratio (HR) and 95% confidence interval (CI) for global index events (GIEs) and components by vaginal estrogen (VE) use

	Intact uterus		Hysterectomy		Overall		
	N (rate/1,000 person-y) ^a	Adjusted model 1 ^b HR (95% CI)	Adjusted model 1 HR (95% CI)	Adjusted model 2 HR (95% CI)	N (rate/1,000 person-y)	Adjusted model 1 HR (95% CI)	Adjusted model 2 HR (95% CI)
GIE							
No VE	4,110 (19.9)	1 (ref)	1 (ref)	1 (ref)	5,906 (21.0)	1 (ref)	1 (ref)
VE	96 (12.0)	0.63 (0.51-0.77)	0.68 (0.55-0.86)	0.83 (0.63-1.08)	153 (14.0)	0.69 (0.59-0.81)	0.76 (0.64-0.91)
Invasive breast cancer							
No VE	858 (4.0)	1 (ref)	1 (ref)	1 (ref)	1,185 (4.1)	1 (ref)	1 (ref)
VE	26 (3.2)	0.78 (0.53-1.15)	0.79 (0.51-1.22)	1.06 (0.61-1.85)	40 (3.6)	0.86 (0.62-1.18)	0.91 (0.64-1.29)
Death							
No VE	1,683 (7.7)	1 (ref)	1 (ref)	1 (ref)	2,592 (8.7)	1 (ref)	1 (ref)
VE	32 (3.9)	0.58 (0.41-0.82)	0.62 (0.41-0.93)	0.85 (0.58-1.25)	59 (5.3)	0.67 (0.52-0.87)	0.78 (0.58-1.04)
CHD							
No VE	865 (4.0)	1 (ref)	1 (ref)	1 (ref)	1,311 (4.5)	1 (ref)	1 (ref)
VE	10 (1.2)	0.35 (0.19-0.65)	0.39 (0.19-0.78)	0.61 (0.33-1.15)	20 (1.8)	0.44 (0.29-0.69)	0.52 (0.31-0.85)
Stroke							
No VE	619 (2.9)	1 (ref)	1 (ref)	1 (ref)	902 (3.1)	1 (ref)	1 (ref)
VE	9 (1.1)	0.41 (0.21-0.80)	0.54 (0.28-1.04)	1.08 (0.59-1.99)	20 (1.8)	0.63 (0.40-0.98)	0.78 (0.49-1.24)
Colorectal cancer							
No VE	342 (1.6)	1 (ref)	1 (ref)	1 (ref)	486 (1.6)	1 (ref)	1 (ref)
VE	9 (1.1)	0.70 (0.36-1.36)	0.63 (0.28-1.41)	0.76 (0.28-2.07)	13 (1.2)	0.72 (0.41-1.25)	0.67 (0.35-1.31)
Hip fracture							
No VE	409 (1.9)	1 (ref)	1 (ref)	1 (ref)	582 (2.0)	1 (ref)	1 (ref)
VE	6 (0.7)	0.42 (0.19-0.94)	0.40 (0.16-0.96)	0.67 (0.25-1.81)	10 (0.9)	0.49 (0.26-0.92)	0.40 (0.19-0.85)
Endometrial cancer							
No VE	222 (1.0)	1 (ref)	1 (ref)	NA ^d	NA	NA	NA
VE	11 (1.3)	1.27 (0.69-2.33)	1.47 (0.75-2.90)	NA	NA	NA	NA
PE/DVT							
No VE	385 (1.8)	1 (ref)	1 (ref)	1 (ref)	600 (2.0)	1 (ref)	1 (ref)
VE	9 (1.1)	0.68 (0.35-1.31)	0.72 (0.34-1.53)	0.43 (0.14-1.33)	12 (1.1)	0.59 (0.33-1.05)	0.68 (0.36-1.28)

CHD, coronary heart disease; DVT, deep vein thrombosis; GIE, global index event (defined as time to first coronary heart disease, breast cancer, stroke, pulmonary embolism, hip fractures, colorectal cancer, endometrial cancer, or death); MET, metabolic equivalent task; NA, not applicable; PE, pulmonary embolism.

^aRates are crude rates per 1,000 person-years (N = 45,663).

^bModel 1 is adjusted for age, education, past estrogen use, history of cancer before study baseline, history of cardiovascular disease before study baseline, history of deep vein thrombosis or pulmonary embolism before study baseline. Model 1 in the overall sample also includes hysterectomy status.

^cModel 2 is adjusted for variables in model 1 and race/ethnicity, baseline body mass index, baseline physical activity (total MET-h/wk), hypertension, Gail breast cancer risk score, fracture after age 55 before study enrollment, smoking, income, and alcohol use (servings/wk).

^dEndometrial cancer analysis was not conducted in participants with hysterectomy or in the overall sample.

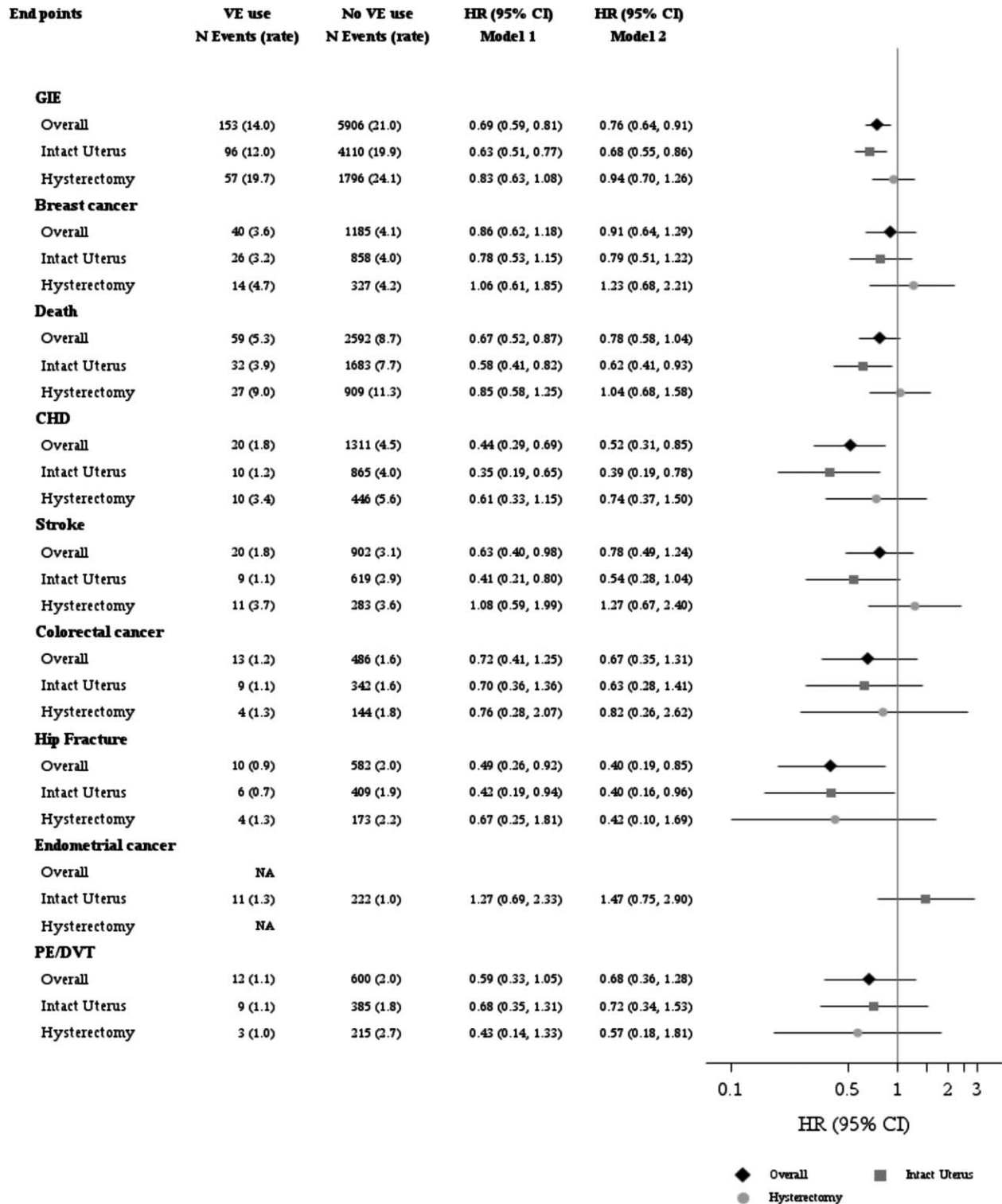


FIG. 2. Hazard Ratio (HR) and 95% Confidence Interval (CI) for Global Index Events (GIE) and Components by Vaginal Estrogen (VE) Use Overall and by Hysterectomy Status. VE includes vaginal cream or vaginal tablet. VE and hysterectomy status were included in the model as timevarying covariates. Global Index Event (GIE) is defined as time to first coronary heart disease, breast cancer, stroke, pulmonary embolism, hip fractures, colorectal cancer, endometrial cancer, or death. Analysis for endometrial cancer was not conducted in participants with hysterectomy or in the overall analytic sample. Rate are crude rates per 1000 person-years (N= 45,663). Model 1 is adjusted for age, education, past estrogen use, history of cancer before study baseline, history of cardiovascular disease before study baseline, history of deep vein thrombosis or pulmonary embolism before study baseline. Model for overall analytic sample also includes hysterectomy status. (N = 45,251). Model 2 was adjusted for variables in Model 1 and race/ethnicity, baseline body mass index, baseline diagnosis of diabetes, baseline physical activity (total MET-hours/week), hypertension, Gail breast cancer risk score, fracture after age 55 prior to study enrollment, smoking, income, and alcohol use (servings/week) (N = 36,629).

cancer, and PE/DVT than nonusers of vaginal estrogen. We did not find evidence for elevated risk of CHD or death in vaginal estrogen users compared with nonusers.

Previously published studies of vaginal estrogen preparations have not focused on summary indices of risks versus benefits of vaginal estrogen preparations, nor have they focused on colorectal cancer, PE, or hip fracture as specific individual outcomes. However, our results are consistent with a few prospective observational studies that evaluated CHD, stroke, breast cancer, and endometrial cancer risk among vaginal estrogen users.

First, a nationwide cohort study in Finland from 1994 to 2009 found a lower risk for CHD death and for stroke death in vaginal estrogen users, compared with the background population²¹ (of note, the background population of the Finnish study included women using both vaginal estrogen and systemic HT, and the study lacked a placebo group).

A second Finnish observational study with 648,022 women-years of follow-up found that the use of vaginal estradiol was not associated with increased risk of breast cancer.¹⁸

Finally, with regard to endometrial cancer, two randomized controlled trials documented no increase in endometrial hyperplasia or cancer after 12 months of treatment with 10 µg estradiol vaginal tablets.^{29,30} However, in a study of all Danish women aged 50 to 79 years followed from 1995 to 2009 (representing 9 million person-years of follow-up), the relative risk of endometrial cancer was increased with vaginal estrogen (1.96 [1.77-2.17]).²² The differences in results between the Danish study and the current study might be related to differing doses or formulations of vaginal estrogen preparations in the two studies, or due to the fact that we censored data from vaginal estrogen users if they initiated oral or transdermal progestogen.

Our findings help to fill important knowledge gaps regarding the safety of vaginal estradiol. Randomized trials have shown that systemic estrogen is linked to increased risks of venous thromboembolism, stroke, and (with combination estrogen + progestogen) invasive breast cancer.^{10-12,31,32} These risks of systemic estrogen and estrogen + progestogen were adopted into estrogen class labeling that is applied to all estrogen preparations, despite the lack of clinical trial evidence demonstrating that vaginal estrogens confer these same risks. (Clinical trials of vaginal estrogens were not designed to evaluate most key clinical outcomes such as CVD and fracture.) Low-dose vaginal estrogen preparations approved by the US Food and Drug Administration carry the same boxed warning about health risks as systemic formulations of estrogen alone and combination estrogen + progestogen. The boxed warning, which reflects estrogen class labeling, states: "WARNING: endometrial cancer, cardiovascular disorders, breast cancer, and probable dementia." This warning is based on extrapolations of data from clinical trials of systemic HT, which involved substantially higher levels of systemic exposure. The boxed warning is not based on evidence from clinical trials of vaginal estrogen and may discourage the use of a highly effective local treatment for a common

condition with adverse effects on quality of life.³³ Currently, the US FDA is considering a proposal to modify package labeling so that it better reflects the safety profile of vaginal estrogen.

In the short term, serum levels of estrogens may rise with use of vaginal estrogen. A study using modern mass spectrometry assays of estrogen levels showed that the use of conjugated equine estrogens vaginal cream (Premarin 0.625 mg) caused a five-fold increase in serum estradiol after 1 week of daily treatment in postmenopausal women.⁷ However, estrogen levels do not increase above the postmenopausal range with initial daily dosing during use of the estradiol vaginal insert (Vagifem 10 mcg),³⁴ and during the maintenance phase of low-dose vaginal estrogen therapy, blood levels remain in the normal postmenopausal range.⁹

Our study has limitations. Although we adjusted for multiple potential confounders, there could have been residual confounding in this observational study. There have been reports of alterations in serum lipoproteins and increased bone density associated with low-dose vaginal estradiol rings.^{35,36} Whereas the lower CHD risk that we observed in vaginal users was consistent with other observational studies of oral HT, clinical trials have not addressed the effects of vaginal estrogen on clinical cardiovascular events. In addition, we could not compare health outcomes among the individual types of vaginal estrogen preparations (eg, estradiol ring, vaginal estradiol tablet, estrogen cream). Study strengths include the prospective data collection, the large sample size, the availability of detailed information regarding relevant confounders, and the adjudication of all GIE events except PE/DVT. To our knowledge, this is the first large prospective study to evaluate the overall health risks and benefits of vaginal estrogen therapy.

CONCLUSIONS

In conclusion, we did not observe an increased risk of cardiovascular disease or cancer among women using vaginal estrogen compared with nonusers. These findings should provide reassurance to women and their health providers regarding the safety of vaginal estrogen and will help to inform menopausal HT clinical decision-making.

Acknowledgments: The authors thank the WHI investigators, staff, and the study participants for their outstanding dedication and commitment.

Program Office: National Heart, Lung, and Blood Institute, Bethesda, MD. Jacques Rossouw, Shari Ludlam, Joan McGowan, Leslie Ford, and Nancy Geller—Clinical Coordinating Center: Fred Hutchinson Cancer Research Center, Seattle, WA; Garnet Anderson, Ross Prentice, Andrea LaCroix, and Charles Kooperberg—Investigators and Academic Centers: Brigham and Women's Hospital, Harvard Medical School, Boston, MA; JoAnn E. Manson: MedStar Health Research Institute/Howard University, Washington, D.C.; Barbara V. Howard: Stanford Prevention Research Center, Stanford, CA; Marcia L. Stefanick: The Ohio State University, Columbus, OH; Rebecca Jackson: University of Arizona, Tucson/Phoenix, AZ; Cynthia A. Thomson: University at Buffalo, Buffalo, NY; Jean Wactawski-Wende: University of Florida, Gainesville/Jacksonville, FL; Marian Limacher: University of Iowa, Iowa City/Davenport, IA;

Jennifer Robinson: University of Pittsburgh, Pittsburgh, PA; Lewis Kuller: Wake Forest University School of Medicine, Winston-Salem, NC; Sally Shumaker: University of Nevada, Reno, NV; Robert Brunner: University of Minnesota, Minneapolis, MN; Karen L. Margolis.

REFERENCES

- Portman DJ, Gass ML. Vulvovaginal Atrophy Terminology Consensus Conference P. Genitourinary syndrome of menopause: new terminology for vulvovaginal atrophy from the International Society for the Study of Women's Sexual Health and The North American Menopause Society. *J Sex Med* 2014;11:2865-2872.
- ACOG Practice Bulletin No 141: management of menopausal symptoms. *Obstet Gynecol* 2014;123:202-216.
- The North American Menopause Society. Management of symptomatic vulvovaginal atrophy: 2013 position statement of The North American Menopause Society. *Menopause* 2013;20:888-902; quiz 903-904.
- American College of Obstetricians and Gynecologists Committee on Practice Bulletins-Gynecology. ACOG Practice Bulletin No. 119: female sexual dysfunction. *Obstet Gynecol* 2011;117:996-1007.
- National Institute for Health and Care Excellence. Menopause: diagnosis and management. Available at: <https://www.nice.org.uk/guidance/ng23>. Accessed June 29, 2017.
- Lethaby A, Ayeleke RO, Roberts H. Local oestrogen for vaginal atrophy in postmenopausal women. *Cochrane Database Syst Rev* (8):2016;CD001500.
- Labrie F, Cusan L, Gomez JL, et al. Effect of one-week treatment with vaginal estrogen preparations on serum estrogen levels in postmenopausal women. *Menopause* 2009;16:30-36.
- Kunovac Kallak T, Baumgart J, Stavreus Evers A, et al. Higher than expected estradiol levels in aromatase inhibitor-treated, postmenopausal breast cancer patients. *Climacteric* 2012;15:473-480.
- Santen RJ. Vaginal administration of estradiol: effects of dose, preparation and timing on plasma estradiol levels. *Climacteric* 2015;18:121-134.
- Manson JE, Chlebowski RT, Stefanick ML, et al. Menopausal hormone therapy and health outcomes during the intervention and extended post-stopping phases of the Women's Health Initiative randomized trials. *JAMA* 2013;310:1353-1368.
- Boardman HM, Hartley L, Eisinga A, et al. Hormone therapy for preventing cardiovascular disease in post-menopausal women. *Cochrane Database Syst Rev* (3):2015;CD002229.
- Nelson HD, Walker M, Zakher B, Mitchell J. Menopausal hormone therapy for the primary prevention of chronic conditions: a systematic review to update the U.S. Preventive Services Task Force recommendations. *Ann Intern Med* 2012;157:104-113.
- Beral V. Breast cancer and hormone-replacement therapy in the Million Women Study. *Lancet* 2003;362:419-427.
- Grodstein F, Manson JE, Stampfer MJ, Rexrode K. Postmenopausal hormone therapy and stroke: role of time since menopause and age at initiation of hormone therapy. *Arch Intern Med* 2008;168:861-866.
- Bergendal A, Kieler H, Sundstrom A, Hirschberg AL, Kocoska-Maras L. Risk of venous thromboembolism associated with local and systemic use of hormone therapy in peri- and postmenopausal women and in relation to type and route of administration. *Menopause* 2016;23:593-599.
- Bhupathiraju SN, Grodstein F, Stampfer MJ, Willett WC, Hu FB, Manson JE. Exogenous hormone use: oral contraceptives, postmenopausal hormone therapy, and health outcomes in the Nurses' Health Study. *Am J Public Health* 2016;106:1631-1637.
- Prentice RL, Manson JE, Langer RD, et al. Benefits and risks of postmenopausal hormone therapy when it is initiated soon after menopause. *Am J Epidemiol* 2009;170:12-23.
- Lyytinen H, Pukkala E, Ylikorkala O. Breast cancer risk in postmenopausal women using estrogen-only therapy. *Obstet Gynecol* 2006;108:1354-1360.
- Michaelsson K, Baron JA, Farahmand BY, Ljunghall S. Use of low potency estrogens does not reduce the risk of hip fracture. *Bone* 2002;30:613-618.
- American College of Obstetricians and Gynecologists. ACOG committee opinion no. 556: postmenopausal estrogen therapy: route of administration and risk of venous thromboembolism. *Obstet Gynecol* 2013;121:887-890.
- Mikkola TS, Tuomikoski P, Lyytinen H, et al. Vaginal estradiol use and the risk for cardiovascular mortality. *Hum Reprod* 2016;31:804-809.
- Morch LS, Kjaer SK, Keiding N, Lokkegaard E, Lidegaard O. The influence of hormone therapies on type I and II endometrial cancer: a nationwide cohort study. *Int J Cancer* 2016;138:1506-1515.
- Design of the Women's Health Initiative clinical trial and observational study. The Women's Health Initiative Study Group. *Control Clin Trials* 1998;19:61-109.
- Hays J, Hunt JR, Hubbell FA, et al. The Women's Health Initiative recruitment methods and results. *Ann Epidemiol* 2003;13(9 Suppl):S18-77.
- 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;288:321-333.
- Gail MH, Brinton LA, Byar DP, et al. Projecting individualized probabilities of developing breast cancer for white females who are being examined annually. *J Natl Cancer Inst* 1989;81:1879-1886.
- Meyer AM, Evenson KR, Morimoto L, Siscovick D, White E. Test-retest reliability of the Women's Health Initiative physical activity questionnaire. *Med Sci Sports Exerc* 2009;41:530-538.
- Pettee Gabriel K, McClain JJ, Lee CD, et al. Evaluation of physical activity measures used in middle-aged women. *Med Sci Sports Exerc* 2009;41:1403-1412.
- Ulrich LS, Naessen T, Elia D, Goldstein JA, Eugster-Hausmann M; investigators VAGt. Endometrial safety of ultra-low-dose Vagifem 10 microg in postmenopausal women with vaginal atrophy. *Climacteric* 2010;13:228-237.
- Simon J, Nachtigall L, Ulrich LG, Eugster-Hausmann M, Gut R. Endometrial safety of ultra-low-dose estradiol vaginal tablets. *Obstet Gynecol* 2010;116:876-883.
- 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;288:321-333.
- Anderson GL, Limacher M, Assaf AR, et al. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial. *JAMA* 2004;291:1701-1712.
- Manson JE, Goldstein SR, Kagan R, et al. Why the product labeling for low-dose vaginal estrogen should be changed. *Menopause* 2014;21:911-916.
- Eugster-Hausmann M, Waitzinger J, Lehnick D. Minimized estradiol absorption with ultra-low-dose 10 microg 17beta-estradiol vaginal tablets. *Climacteric* 2010;13:219-227.
- Naessen T, Berglund L, Ulmsten U. Bone loss in elderly women prevented by ultralow doses of parenteral 17 beta-estradiol. *Am J Obstet Gynecol* 1997;177:115-119.
- Naessen T, Rodriguez-Macias K, Lithell H. Serum lipid profile improved by ultra-low doses of 17 beta-estradiol in elderly women. *J Clin Endocrinol Metab* 2001;86:2757-2762.