Conjugated equine oestrogen and breast cancer incidence and mortality in postmenopausal women with hysterectomy: extended follow-up of the Women's Health Initiative randomised placebo-controlled trial

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Summary

Background By contrast with many observational studies, women in the Women's Health Initiative (WHI) trial who were randomly allocated to receive oestrogen alone had a lower incidence of invasive breast cancer than did those who received placebo. We aimed to assess the influence of oestrogen use on longer term breast cancer incidence and mortality in extended follow-up of this cohort.

Methods Between 1993 and 1998, the WHI enrolled 10 739 postmenopausal women from 40 US clinical centres into a randomised, double-masked, placebo-controlled trial. Women aged 50–79 years who had undergone hysterectomy and had expected 3-year survival and mammography clearance were randomly allocated by a computerised, permuted block algorithm, stratified by age group and centre, to receive oral conjugated equine oestrogen (0·625 mg per day; n=5310) or matched placebo (n=5429). The trial intervention was terminated early on Feb 29, 2004, because of an adverse effect on stroke. Follow-up continued until planned termination (March 31, 2005). Consent was sought for extended surveillance from the 9786 living participants in active follow-up, of whom 7645 agreed. Using data from this extended follow-up (to Aug 14, 2009), we assessed long-term effects of oestrogen use on invasive breast cancer incidence, tumour characteristics, and mortality. We used Cox regression models to estimate hazard ratios (HRs) in the intention-to-treat population. This study is registered with ClinicalTrials.gov, number NCT00000611.

Findings After a median follow-up of 11·8 years (IQR 9·1–12·9), the use of oestrogen for a median of 5·9 years (2·5–7·3) was associated with lower incidence of invasive breast cancer (151 cases, 0·27% per year) compared with placebo (199 cases, 0·35% per year; HR 0·77, 95% CI 0·62–0·95; p=0·02) with no difference (p=0·76) between intervention phase (0·79, 0·61–1·02) and post-intervention phase effects (0·75, 0·51–1·09). In subgroup analyses, we noted breast cancer risk reduction with oestrogen use was concentrated in women without benign breast disease (p=0·01) or a family history of breast cancer (p=0·02). In the oestrogen group, fewer women died from breast cancer (six deaths, 0·009% per year) compared with controls (16 deaths, 0·024% per year; HR 0·37, 95% CI 0·13–0·91; p=0·03). Fewer women in the oestrogen group died from any cause after a breast cancer diagnosis (30 deaths, 0·046% per year) than did controls (50 deaths, 0·076%; HR 0·62, 95% CI 0·39–0·97; p=0·04).

Interpretation Our findings provide reassurance for women with hysterectomy seeking relief of climacteric symptoms in terms of the effects of oestrogen use for about 5 years on breast cancer incidence and mortality. However, our data do not support use of oestrogen for breast cancer risk reduction because any noted benefit probably does not apply to populations at increased risk of such cancer.

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Introduction

Elevated concentrations of endogenous oestrogen have been consistently associated with increased risk of breast cancer.[1] Exogenous oestrogen use has also been associated with higher breast cancer incidence in many[2–5] but not all[6,7] observational studies, especially in leaner women[3–5,8] and those receiving oestrogen long term.[4,5,8,9] Oestrogen use has been linked to hormone-receptor positive and early stage disease,[3,5] suggesting a better prognosis,[10] although associations with breast cancer mortality are mixed.[2,9,10–17]

During the intervention phase of the Women's Health Initiative (WHI) randomised trial[17] of 10 739 postmenopausal women who had undergone hysterectomy, a non-significant reduction in incidence of breast cancer was noted after receipt of conjugated equine oestrogens compared with placebo (hazard ratio [HR] 0·79, 95% CI 0·61–1·02). After trial intervention was stopped in February, 2004, because of an increased incidence of stroke,[18] follow-up continued until planned termination in 2005 and every year thereafter for participants who consented to extended surveillance. Results of a

prespecified analysis in 2009 suggested that most estimates of the effect of oestrogen on risk of chronic disease were attenuated. The reduced breast cancer risk in the oestrogen group persisted and reached statistical significance (HR 0·77, 95% CI 0·62–0·95).[19] We aim to provide additional details about the effects of oestrogen use on invasive breast cancer incidence during and after intervention with regard to tumour characteristics and previously identified effect modifiers. We also present results for breast cancer-related mortality.

Methods

Study design and participants

Postmenopausal women aged 50–79 years who had undergone a hysterectomy were recruited into the WHI randomised, double-masked, placebo-controlled trial at 40 clinical centres in the USA between 1993 and 1998. Previous breast cancer was an exclusion criterion and clearance by recent mammograms and clinical breast examinations was required. Women who were taking hormones underwent a 3-month washout period. Eligible women were randomly allocated in a one-to-one ratio to receive oral conjugated equine oestrogen (0·625 mg per day) or matched placebo through use of a computerised, permuted-block algorithm, stratified by age group and centre. Randomisation and drug dispensing was supported through a secure database system developed and implemented by the WHI Clinical Coordinating Center (Seattle, WA, USA). Clinical centre staff entered the eligibility data into the database and executed a database function that confirmed eligibility and did the randomisation. Double-masking was implemented through an associated database drug dispensing system. The study was approved by each centre's institutional review board. All women provided written informed consent. Details of study design, eligibility, and implementation have been published elsewhere.[18,19]

Data collection

Participants' previous hormone use was ascertained at baseline by an interviewer-administered questionnaire. Medical, reproductive, and family histories were obtained by self-reported questionnaires. Height and weight were measured by study staff. All non-study drug use was assessed by interviewer-administered questionnaire at baseline and follow-up at 1, 3, 6, and 9 years. Adherence to study drug was assessed primarily by pill counts or weighing returned bottles; self-report was used only rarely.

Until 2005, participants' vital and health status was assessed twice every year and mammography was done once per year throughout the trial. When the intervention ended on Feb 29, 2004, after a mean follow-up of 7·1 (SD 1·6) years, all participants were unmasked and asked to stop taking the study drug. Follow-up continued according to the original protocol until the planned study end (March 31, 2005). During study close-out, 7645 participants (78% of 9786 living participants in active follow-up at that time; 3778 [77·9%] of 4851 women allocated to oestrogen and 3867 [78·4%] of 4935 allocated to placebo) consented to extended follow-up.[19] From 2005, vital and health status updates have been obtained once per year. This report presents data until Aug 14, 2009, after an overall median follow-up of 11·8 years (IQR 9·1–12·9).

Breast cancers were documented by medical and pathological record review by centrally trained doctor adjudicators. Tumour characteristics were coded at the WHI Clinical Coordinating Center according to Surveillance, Epidemiology, and End Results (SEER) guidelines.[20] Deaths were documented with death certificates and medical records. We searched the national death index to identify deaths in participants lost to follow-up through Dec 31, 2008. Central doctor adjudicators reviewed medical records to establish causes of death. All adjudicators were masked to randomisation assignment.

Statistical analysis

We compared incidence of breast cancer by treatment group with failure-time methods and the intention-to-treat principle. We calculated HR and 95% CIs from Cox regression models, stratified by age group and randomisation assignment in the concurrent WHI dietary modification trial. We did analyses for three time phases: the intervention phase (from randomisation until Feb 29, 2004); the post-intervention phase (from March 1, 2004, until the data cutoff on Aug 14, 2009); and overall results. Event times during the intervention phase were censored at date of death, last follow-up, or termination of the intervention phase (Feb 29, 2004), whichever occurred first. Participants were included in post-intervention phase incidence analyses if they were alive, in follow-up, and had not developed breast cancer by March 1, 2004. Analyses of overall results began at randomisation with censoring defined as the earlier of death or date of last follow-up. Analyses of breast cancer subtypes incorporated censoring at diagnosis of any other breast cancer subtype. We assessed heterogeneity of hazard ratios across tumour subtypes with competing-risk models. Analyses of breast cancer mortality included all participants, and analysed as per the three phases defined previously. Women in active follow-up were censored at last contact date. Women in passive follow-up were censored on Dec 31, 2008 (the last date covered by the national death index linkage). To examine effects of oestrogen over time, we fitted linear, time-varying hazard ratios for randomisation assignment for the intervention and postintervention phases separately. We compared slopes for each phase with Wald tests.

We examined the influence of non-adherence to protocol-assigned treatment by censoring events 6 months after participants first became non-adherent (ie, took <80% of study drugs or started non-study hormone therapy). Additionally, we included time-varying weights, which were inversely proportional to the

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	Oestrogen group (n=3778)	Placebo group (n=3867)
Age at screening (years)		
50–59	1223 (32·4%)	1232 (31·9%)
60–69	1740 (46·1%)	1799 (46·5%)
70–79	815 (21·6%)	836 (21·6%)
Ethnic group		
White	2945 (78·0%)	3001 (77·6%)
Black	514 (13·6%)	565 (14·6%)
Hispanic	189 (5·0%)	181 (4·7%)
American Indian	31 (0·8%)	18 (0·5%)
Asian/Pacific Islander	54 (1·4%)	49 (1·3%)
Unknown	45 (1·2%)	53 (1·4%)
Education		
Up to high school/GED	1167 (31·2%)	1137 (29·6%)
Post-high school	1630 (43·6%)	1704 (44·4%)
College degree or higher	945 (25·3%)	998 (26·0%)
Family income (US$)		
<20 000	891 (24·8%)	913 (24·9%)
20 000–<35 000	1070 (29·8%)	1084 (29·6%)
35 000–<50 000	741 (20·6%)	758 (20·7%)
50 000–<75 000	550 (15·3%)	555 (15·2%)
≥75 000	340 (9·5%)	352 (9·6%)
Marital status		
Never married	123 (3·3%)	111 (2·9%)
Divorced or separated	709 (18·8%)	687 (17·9%)
Widowed	777 (20·7%)	785 (20·4%)
Married or cohabiting	2153 (57·2%)	2264 (58·9%)
Body-mass index (kg/m²)		
<25	785 (20·9%)	771 (20·1%)
25–<30	1289 (34·3%)	1391 (36·2%)
≥30	1687 (44·9%)	1683 (43·8%)
Smoking status		
Never	1988 (53·1%)	1972 (51·5%)
Past	1417 (37·9%)	1489 (38·9%)
Current	336 (9·0%)	370 (9·7%)
Age at menarche (years)		
≤11	890 (23·7%)	929 (24·1%)
12–13	2030 (54·1%)	2013 (52·3%)
≥14	833 (22·2%)	908 (23·6%)
Number of term pregnancies		
Never pregnant or no term pregnancy	350 (9·3%)	307 (8·0%)
1–2	1033 (27·5%)	1099 (28·6%)
3–4	1527 (40·7%)	1605 (41·7%)
≥5	840 (22·4%)	835 (21·7%)
Age at first birth (years)		
Never pregnant or no term pregnancy	350 (10·3%)	307 (8·8%)
<20	822 (24·2%)	872 (24·9%)
20–29	2060 (60·7%)	2128 (60·9%)
≥30	163 (4·8%)	190 (5·4%)
Number of months breastfed		
Never breastfed	1775 (47·8%)	1739 (45·8%)
Breastfed ≤1 year	1412 (38·0%)	1525 (40·1%)
Breastfed >1 year	525 (14·1%)	535 (14·1%)
Benign breast disease		
No	2758 (80·2%)	2693 (77·7%)
Yes, one biopsy	500 (14·5%)	550 (15·9%)
Yes, two or more biopsies	183 (5·3%)	222 (6·4%)
First degree female relatives with breast cancer		
None	2987 (85·5%)	3084 (86·0%)
One	459 (13·1%)	453 (12·6%)
Two or more	48 (1·4%)	49 (1·4%)
Gail model 5-year risk score		
<1·25%	1456 (38·5%)	1505 (38·9%)
1·25–<1·75%	1185 (31·4%)	1220 (31·5%)
≥1·75%	1137 (30·1%)	1142 (29·5%)
Age at hysterectomy (years)		
<40	1495 (39·8%)	1501 (39·0%)
40–49	1643 (43·7%)	1662 (43·2%)
50–54	345 (9·2%)	412 (10·7%)
≥55	275 (7·3%)	271 (7·0%)
Bilateral oophorectomy		
No	2143 (61·0%)	2094 (58·2%)
Yes	1370 (39·0%)	1507 (41·8%)
Time since menopause (years)		
<10	636 (19·9%)	623 (18·8%)
10–<20	1025 (32·1%)	1104 (33·3%)
≥20	1535 (48·0%)	1586 (47·9%)
Hormone therapy use		
Never	1929 (51·1%)	1916 (49·6%)
Past	1304 (34·5%)	1373 (35·5%)
Current	544 (14·4%)	575 (14·9%)
Unopposed oestrogen use		
Non-user	2006 (53·1%)	2007 (51·9%)
<5 years	938 (24·8%)	1008 (26·1%)
≥5 years	834 (22·1%)	852 (22·0%)
Oestrogen and progesterone use		
Non-user	3613 (95·6%)	3668 (94·9%)
<5 years	108 (2·9%)	123 (3·2%)
≥5 years	57 (1·5%)	76 (2·0%)

Data are n (%). GED=general educational development. Some categories do not add up to total number of women who consented to extended follow-up due to missing data.

Table 1: **Baseline characteristics of participants in the Women's Health Initiative trial of conjugated equine oestrogen who consented to extended follow-up**

estimated probability of continued adherence, in the proportional-hazards models to adjust for changes in the distribution of sample characteristics during follow-up.[21]

In secondary analyses, we tested interactions between randomisation assignment and 16 baseline characteristics within the primary Cox model, expanded to include the designated baseline factor, randomisation assignment, and interaction term(s). We omitted participants with missing

	Intervention phase (events on or before Feb 29, 2004)			Post-intervention phase* (events on March 1, 2004–Aug 14, 2009)				Overall (events to Aug 14, 2009)			
	Oestrogen (n=5310)	Placebo (n=5429)	HR†‡ (95% CI)	Oestrogen (n=4794)	Placebo (n=4877)	HR†§ (95% CI)	$p_{interaction}$¶	Oestrogen (n=5310)	Placebo (n=5429)	HR†‡ (95% CI)	$p_{heterogeneity}$‖
Follow-up, months	85·8 (77·1–96·8)	86·3 (76·7–97·6)	..	56·7 (42·0–61·0)	57·0 (42·2–61·0)	..	..	141·8 (108·5–154·2)	141·8 (109·1–154·4)	..	..
Invasive breast cancer	104 (0·28%)	135 (0·35%)	0·79 (0·61–1·02)	47 (0·26%)	64 (0·34%)	0·75 (0·51–1·09)	0·76	151 (0·27%)	199 (0·35%)	0·77 (0·62–0·95)	
Histology**											0·33
Ductal	60 (0·16%)	88 (0·23%)	0·70 (0·51–0·98)	26 (0·14%)	43 (0·23%)	0·62 (0·38–1·01)	0·63	86 (0·15%)	131 (0·23%)	0·67 (0·51–0·88)	
Lobular	18 (0·048%)	12 (0·031%)	1·56 (0·75–3·24)	2 (0·011%)	7 (0·038%)	0·28 (0·06–1·34)	0·06	20 (0·036%)	19 (0·033%)	1·09 (0·58–2·04)	
Ductal and lobular	12 (0·032%)	13 (0·034%)	0·93 (0·42–2·03)	7 (0·038%)	5 (0·027%)	1·38 (0·44–4·34)	0·55	19 (0·034%)	18 (0·032%)	1·06 (0·55–2·01)	
Other	14 (0·037%)	21 (0·055%)	0·68 (0·34–1·33)	12 (0·065%)	9 (0·048%)	1·37 (0·58–3·25)	0·22	26 (0·047%)	30 (0·053%)	0·88 (0·52–1·49)	
Oestrogen receptor status**											0·78
Positive	72 (0·19%)	96 (0·25%)	0·77 (0·57–1·05)	38 (0·21%)	53 (0·29%)	0·72 (0·48–1·10)	0·79	110 (0·20%)	149 (0·26%)	0·75 (0·59–0·96)	
Negative	19 (0·051%)	22 (0·058%)	0·88 (0·48–1·63)	6 (0·033%)	9 (0·048%)	0·70 (0·25–1·96)	0·68	25 (0·045%)	31 (0·055%)	0·81 (0·48–1·38)	
Progesterone receptor status**											0·34
Positive	57 (0·15%)	71 (0·19%)	0·83 (0·58–1·17)	35 (0·19%)	41 (0·22%)	0·86 (0·55–1·36)	0·91	92 (0·17%)	112 (0·20%)	0·84 (0·63–1·10)	
Negative	32 (0·086%)	43 (0·11%)	0·76 (0·48–1·20)	9 (0·049%)	20 (0·11%)	0·46 (0·21–1·02)	0·27	41 (0·074%)	63 (0·11%)	0·66 (0·45–0·98)	
HER2 overexpression**											0·045
Yes	18 (0·048%)	12 (0·031%)	1·58 (0·76–3·27)	5 (0·027%)	4 (0·022%)	1·32 (0·35–4·94)	0·84	23 (0·041%)	16 (0·028%)	1·50 (0·79–2·83)	
No	53 (0·14%)	67 (0·18%)	0·81 (0·56–1·16)	36 (0·20%)	54 (0·29%)	0·67 (0·44–1·03)	0·50	89 (0·16%)	121 (0·21%)	0·74 (0·56–0·97)	
Triple-negative tumour											0·26
Yes	12 (0·032%)	8 (0·021%)	1·54 (0·63–3·79)	4 (0·022%)	6 (0·032%)	0·69 (0·19–2·44)	0·30	16 (0·029%)	14 (0·025%)	1·14 (0·56–2·34)	
No	92 (0·25%)	127 (0·33%)	0·74 (0·57–0·97)	43 (0·23%)	58 (0·31%)	0·75 (0·51–1·12)	>0·99	135 (0·24%)	185 (0·33%)	0·74 (0·60–0·93)	
Stage**											0·19
Local	67 (0·18%)	100 (0·26%)	0·69 (0·51–0·94)	34 (0·18%)	44 (0·24%)	0·79 (0·50–1·24)	0·67	101 (0·18%)	144 (0·25%)	0·72 (0·56–0·92)	
Regional or distant	35 (0·094%)	31 (0·081%)	1·15 (0·71–1·86)	13 (0·071%)	18 (0·097%)	0·72 (0·36–1·48)	0·30	48 (0·086%)	49 (0·086%)	0·98 (0·66–1·46)	
Grade**											0·56
Well differentiated	19 (0·051%)	26 (0·068%)	0·74 (0·41–1·33)	11 (0·060%)	16 (0·086%)	0·70 (0·32–1·51)	0·91	30 (0·054%)	42 (0·074%)	0·72 (0·45–1·16)	
Moderately differentiated	31 (0·083%)	52 (0·14%)	0·61 (0·39–0·96)	20 (0·11%)	31 (0·17%)	0·65 (0·37–1·14)	0·91	51 (0·092%)	83 (0·15%)	0·62 (0·44–0·88)	
Poorly differentiated	29 (0·078%)	40 (0·10%)	0·75 (0·46–1·21)	14 (0·076%)	13 (0·070%)	1·11 (0·52–2·37)	0·40	43 (0·077%)	53 (0·093%)	0·83 (0·55–1·24)	
Tumour size (cm)**											0·06
≤1	31 (0·083%)	51 (0·13%)	0·63 (0·40–0·98)	11 (0·060%)	21 (0·11%)	0·53 (0·26–1·11)	0·70	42 (0·075%)	72 (0·13%)	0·60 (0·41–0·87)	
1–<2	35 (0·094%)	52 (0·14%)	0·69 (0·45–1·06)	16 (0·087%)	21 (0·11%)	0·78 (0·41–1·50)	0·77	51 (0·092%)	73 (0·13%)	0·71 (0·50–1·02)	
≥2	25 (0·067%)	23 (0·060%)	1·10 (0·63–1·95)	16 (0·087%)	16 (0·086%)	0·99 (0·49–1·97)	0·79	41 (0·074%)	39 (0·069%)	1·05 (0·68–1·63)	
Positive lymph nodes**											0·14
No	61 (0·16%)	93 (0·24%)	0·68 (0·49–0·94)	27 (0·15%)	41 (0·22%)	0·67 (0·41–1·09)	0·94	88 (0·16%)	134 (0·24%)	0·67 (0·51–0·88)	
Yes	32 (0·086%)	28 (0·073%)	1·16 (0·70–1·92)	11 (0·060%)	16 (0·086%)	0·69 (0·32–1·49)	0·27	43 (0·077%)	44 (0·077%)	0·98 (0·64–1·49)	
All-cause mortality after breast cancer	7 (0·018%)	12 (0·031%)	0·60 (0·22–1·48)	23 (0·087%)	38 (0·14%)	0·62 (0·36–1·03)	0·92	30 (0·046%)	50 (0·076%)	0·62 (0·39–0·97)	
Breast cancer deaths	4 (0·011%)	9 (0·023%)	0·45 (0·12–1·37)	2 (0·008%)	7 (0·026%)	0·29 (0·04–1·21)	0·66	6 (0·009%)	16 (0·024%)	0·37 (0·13–0·91)	

Data are median (IQR) or n (% per year). HR=hazard ratio. *Post-intervention phase includes data obtained during the extended follow-up (after March 31, 2005); 3778 (77·9%) of 4851 eligible participants randomly allocated to oestrogen and 3867 (78·4%) of 4935 eligible participants randomly allocated to placebo consented to extended follow-up. †From a proportional hazards model, stratified by age group (50–54, 55–59, 60–69, and 70–79 years) and dietary modification randomisation group. ‡Time to event measured from date of randomisation. §Time to event measured from March 1, 2004. ¶$p_{interaction}$ based on tests for equality of the intervention and post-intervention phase hazard ratios in a proportional hazards model, stratified by age group (50–54, 55–59, 60–69, and 70–79 years), dietary modification randomisation arm, and trial phase (time dependent), with time to event measured from date of randomisation. ‖$p_{heterogeneity}$ from a competing risks analysis that tested whether hazard ratios differed between tumour types. **Hazard ratios for a specific tumour characteristic are from proportional hazards model in which incidence of tumour with alternate characteristics were censored; tumour characteristics were missing for the following numbers of cases for histology (none in the active group *vs* one in the placebo group), oestrogen receptor status (16 *vs* 19), progesterone receptor status (18 *vs* 24), HER2 overexpression (39 *vs* 62), stage (two *vs* six), grade (27 *vs* 21), tumour size (ten *vs* 11), and positive lymph nodes (20 *vs* 21). 11 tumours of indeterminate size were reported (seven in the active group *vs* four in the placebo group).

***Table 2*: Associations between conjugated equine oestrogen and breast cancer incidence and mortality during and after the intervention in the Women's Health Initiative randomised trial**

values from the corresponding analysis. No adjustment for multiple testing was made; at most, one interaction was expected to be significant by chance alone.

All analyses were done with SAS version 9.1.3 and figures were drawn with R 2.11. All p values are two-sided and p values of 0·05 or less were regarded as significant at the 0·05 level. The WHI study is registered with ClinicalTrials.gov, number NCT00000611.

Role of the funding source

The WHI Project Office at the US National Heart, Lung, and Blood Institute (NHLBI) reviewed and approved the final manuscript but had no other role in the preparation of this report. Decisions about study design, data collection and analysis, result interpretation, manuscript preparation, and the decision to submit the manuscript for publication resided with committees comprised of WHI investigators that included NHLBI representatives. AKA had access to the raw data. The corresponding author had full access to all of the data and the final responsibility to submit for publication.

Results

Baseline breast cancer risk factors in women who consented to extended follow-up were much the same as those of the original cohort[18] and much the same between randomised groups, apart from a small imbalance in bilateral oophorectomy and benign breast disease (table 1). During the intervention phase, 80–90% of participants had mammograms every year with equivalent frequencies in the two treatment groups.[17,22] 3894 (81·2%) of 4794 of women in the oestrogen group and 3965 (81·3%) of 4877 controls had at least one mammogram after the trial intervention was stopped.

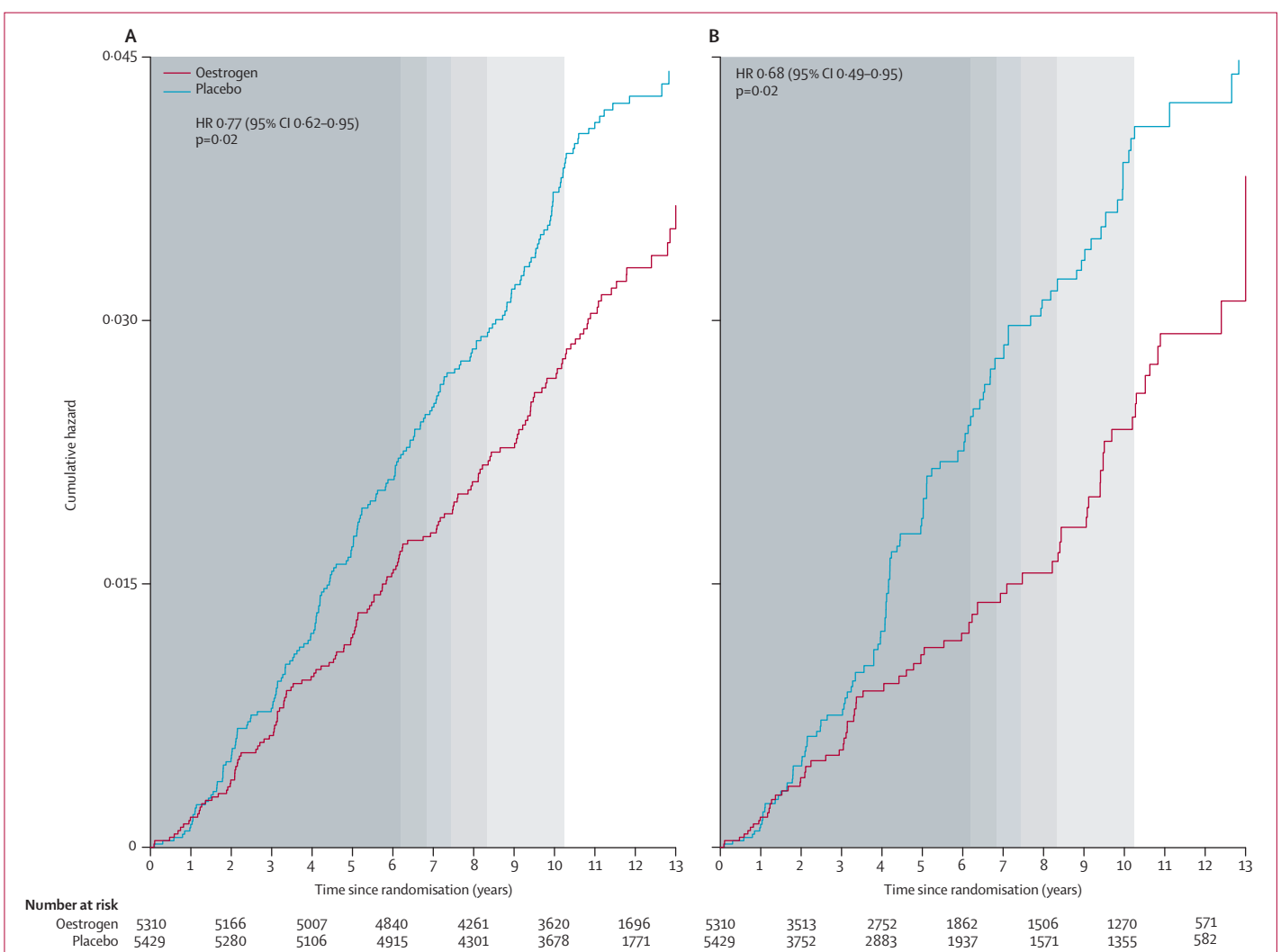

Figure 1: **Kaplan-Meier estimates of cumulative hazards of invasive breast cancer in the WHI randomised trial of conjugated equine oestrogen with the intention-to-treat principle (A) and with adjustments for adherence (B)**

Background shading shows the distribution of the duration of intervention (in quintiles). HR=hazard ratio.

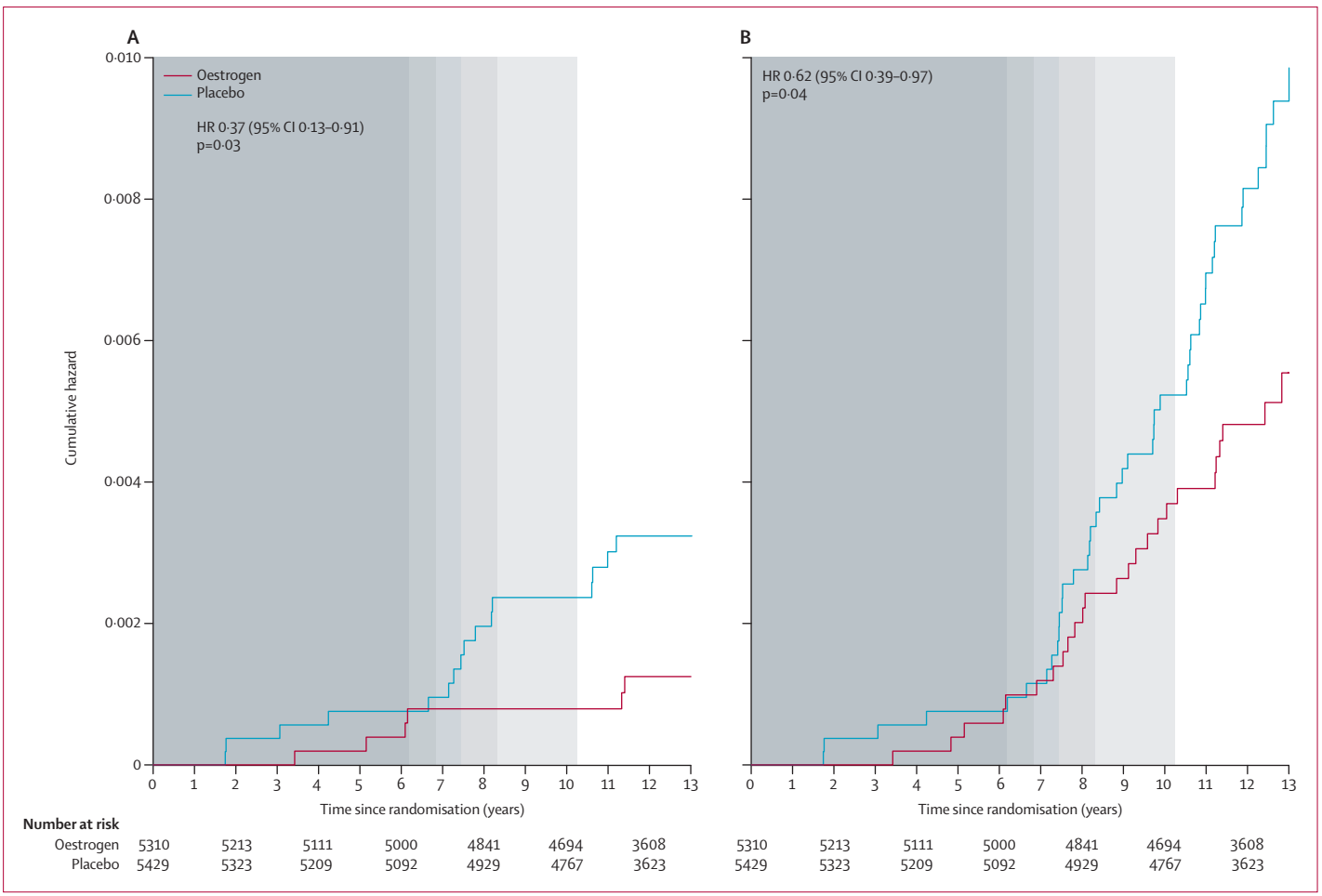

Figure 2: **Kaplan-Meier estimates of cumulative hazards for breast cancer deaths (A) and all-cause mortality after breast cancer (B) in the WHI randomised trial of conjugated equine oestrogen**
Background shading shows the distribution of the duration of intervention (in quintiles). HR=hazard ratio.

We previously reported[18] that 53·8% of 10739 participants had stopped study drugs by trial termination with very similar frequencies noted between randomisation groups. By that time, 5·7% of 5310 women in the oestrogen group and 9·1% of 5429 controls had started hormones provided by their health-care providers.[18] Of 7472 participants in extended follow-up with available data, 604 (8·1%) reported use of hormones after intervention, with slightly more in the oestrogen group than the placebo group (334 [9·0%] of 3699 in the oestrogen group *vs* 270 [7·2%] of 3773 controls; p=0·003).

In the intention-to-treat analyses, data were available for a median follow-up of 7·2 years (IQR 6·4–8·1) from randomisation until early termination of the trial intervention, a median follow-up of 4·7 years (3·5–5·1) after intervention, and a median of 11·8 years (9·1–12·9) follow-up overall. In these analyses, use of oestrogen was associated with lower overall invasive breast cancer incidence (151 events, 0·27% per year) compared with placebo (199 events, 0·35% per year; HR 0·77, 95% CI 0·62–0·95; p=0·02;[19] table 2, figure 1), with no difference between intervention and post-intervention hazard ratios ($p_{interaction}$=0·76). Adjustment for the small imbalances noted in the characteristics of extended follow-up participants had no appreciable effect on the post-intervention results. We noted non-significant increasing trends in oestrogen HRs by time since randomisation (p_{slope}=0·19) and by time since trial cessation (p_{slope}=0·32).

Median duration of study drug use was 5·9 years (IQR 2·5–7·3) and median time to non-adherence (ie, taking <80% of study pills or starting other hormone therapy) was 3·5 years (1·5–6·5). Sensitivity analyses adjusting for non-adherence yielded a stronger association between oestrogen use and lower breast cancer risk overall (HR 0·68, 95% CI 0·49–0·95; figure 1), which seemed even higher when restricted to the intervention period (0·58, 0·39–0·84).

In analyses of tumour characteristics (table 2), oestrogen use was associated with a reduced risk of infiltrating ductal carcinoma (HR 0·67, 95% CI 0·51–0·88) compared with placebo but not infiltrating lobular cancers; however, the test for heterogeneity was not significant (p=0·33). Hazard

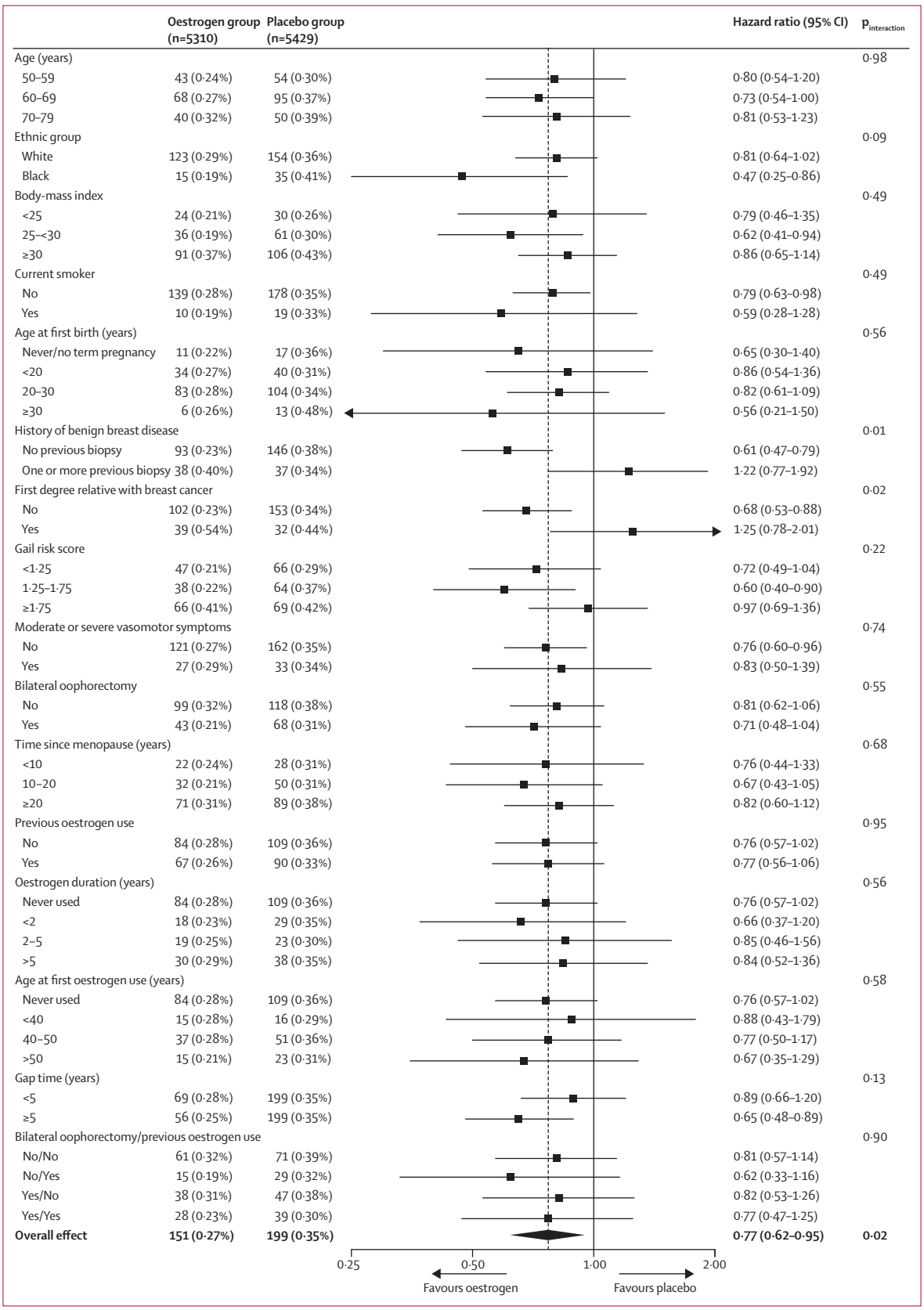

Figure 3: **Hazard ratios for invasive breast cancer incidence by baseline characteristics in the WHI trial of conjugated equine oestrogen**
Data are n (% per year) unless otherwise stated.

ratios for hormone receptor-positive tumours and hormone receptor-negative tumours were much the same. We noted a reduced risk for HER2-negative tumours (0·74, 0·56–0·97) but not HER2-positive tumours (1·50, 0·79–2·83) in women who received oestrogen compared with placebo ($p_{heterogeneity}$=0·045), although missing HER2 data were common. Compared with placebo, we noted fewer small and node-negative tumours but no reduction in the number of large tumours (≥2 cm) or node-positive tumours in the oestrogen group, but comparisons between subtypes did not reach significance.

In analyses starting at randomisation (table 2 and figure 2), fewer women diagnosed with breast cancer died in the oestrogen group (30 deaths, 0·046% per year) compared with the placebo group (50 deaths, 0·076% per year; HR 0·62, 95% CI 0·39–0·97; p=0·04). Of these deaths, six were directly attributed to breast cancer in the oestrogen group (0·009% per year) compared with 16 in the placebo group (0·024% per year; 0·37, 0·13–0·91; p=0·03).

Subgroup analyses provided a mostly consistent pattern of lower breast cancer incidence with oestrogen use (figure 3). Of 16 interactions tested, two were nominally statistically significant: history of benign breast disease (p=0·01) and first degree family history of breast cancer (p=0·02). In both of these analyses, evidence for lower breast cancer incidence was restricted to women without these risk factors. No interactions were reported with age (p=0·98), body-mass index (p=0·49), Gail model risk score (p=0·22), oophorectomy status (p=0·55), years since menopause onset (p=0·68), previous oestrogen use (p=0·95), or vasomotor symptoms (p=0·74).

Of women who first used hormone therapy 5 years or more after menopause (ie, gap time ≥5 years), the estimated effect of oestrogen (HR 0·65, 95% CI 0·48–0·89) seemed lower than that for women who started hormone therapy sooner after menopause (0·89, 0·66–1·20) but the interaction was not significant (p=0·13).

Discussion

Use of oestrogen for a median of 5·9 years in postmenopausal women with hysterectomy in the WHI randomised trial was associated with a significant reduction in incidence of invasive breast cancer, a reduction that continued for the median 4·7 years of follow-up after discontinuation of intervention. Adjustment for adherence suggested somewhat stronger protective effects of oestrogen therapy compared with placebo. Potential effect modification with benign breast disease (p=0·01) and family history of breast cancer (p=0·02) suggests that these reductions might not apply to women at increased risk. We noted a significant reduction in breast cancer-related mortality and all-cause mortality after breast cancer diagnosis with oestrogen use, but the number of such deaths was small.

Although many observational studies have reported an increased risk of breast cancer with oestrogen use,[2–5] some have reported lower risks.[6,23] In previous studies reporting an adverse effect of such therapy on breast cancer, most[2–5,8] but not all,[5,9] showed an increased risk of breast cancer only after prolonged (>5 years) oestrogen use. In this trial, with substantial variation in exposure length (median duration of the intervention 5·9 years [range <1–10 years]; median adherent time 3·5 years; and 2291 [21%] of 10739 participants reporting previous oestrogen use for >5 years at baseline), we noted no time-trends in terms of duration of use or time since cessation and no interactions with previous oestrogen use, although the power for interaction tests was low (panel). The continued, postintervention effect of oestrogen on breast cancer incidence is akin to that reported for other hormone-targeted drugs shown to reduce breast cancer incidence.[33,34]

Panel: Research in context

Systematic review

Conjugated equine oestrogen was approved for management of climacteric symptoms in several countries in the early 1940s. By the 1990s, about 40% of postmenopausal women in the USA and UK were receiving hormone therapy (oestrogen alone or oestrogen plus progesterone).[24–26] However, the risks and benefits of this commonly used therapy had never been established in a clinical trial setting. Against this background, scientists at the US National Institutes of Health, working in conjunction with experts in a number of disciplines, developed the Women's Health Initiative (WHI) hormone therapy programme to meet this unmet need with potential implications for a large number of postmenopausal women in the USA and around the world.[27]

The WHI hormone therapy programme consisted of two full-scale randomised, placebo-controlled clinical trials to separately assess the effect of oestrogen alone and oestrogen plus progesterone on chronic disease in postmenopausal women with and without previous hysterectomy, respectively, at 40 clinical sites in the USA. When the WHI trials were developed, observational study evidence suggested that oestrogen, alone or with progesterone, would modestly increase breast cancer risks[2–5] but the cancers would have a favourable prognosis.[3–5]

The results from the WHI randomised, placebo-controlled trial of oestrogen alone contradicts most previous observational studies in that oestrogen use was associated with reduced breast cancer incidence and reduced breast cancer-associated mortality. Previous efforts to reconcile these results have pointed to the issue of timing of first hormone therapy.[28,29] Additionally, some of the differences between previous observational studies and the present randomised clinical trial results might reflect variation in mammography frequency in observational study populations. In that setting, oestrogen users more frequently have mammography, leading to more common breast cancer diagnosis at an early stage. The WHI oestrogen-alone findings also differ from those seen in the WHI randomised trial assessing oestrogen plus progesterone in women without previous hysterectomy in which combined hormone therapy significantly increased breast cancer incidence and breast cancer mortality.[30–32]

Interpretation

Our findings provide reassuring evidence about breast safety of oestrogen use for climacteric symptom management in postmenopausal women with hysterectomy for durations consistent with those reported in this trial. Although a reduced risk of breast cancer incidence and mortality was noted in recipients of oestrogen, these findings do not support its use for breast cancer risk reduction in light of the lack of benefit for populations at higher risk, the adverse effects on stroke and venous thromboembolism,[18] and the increased risk reduction available with other drugs.

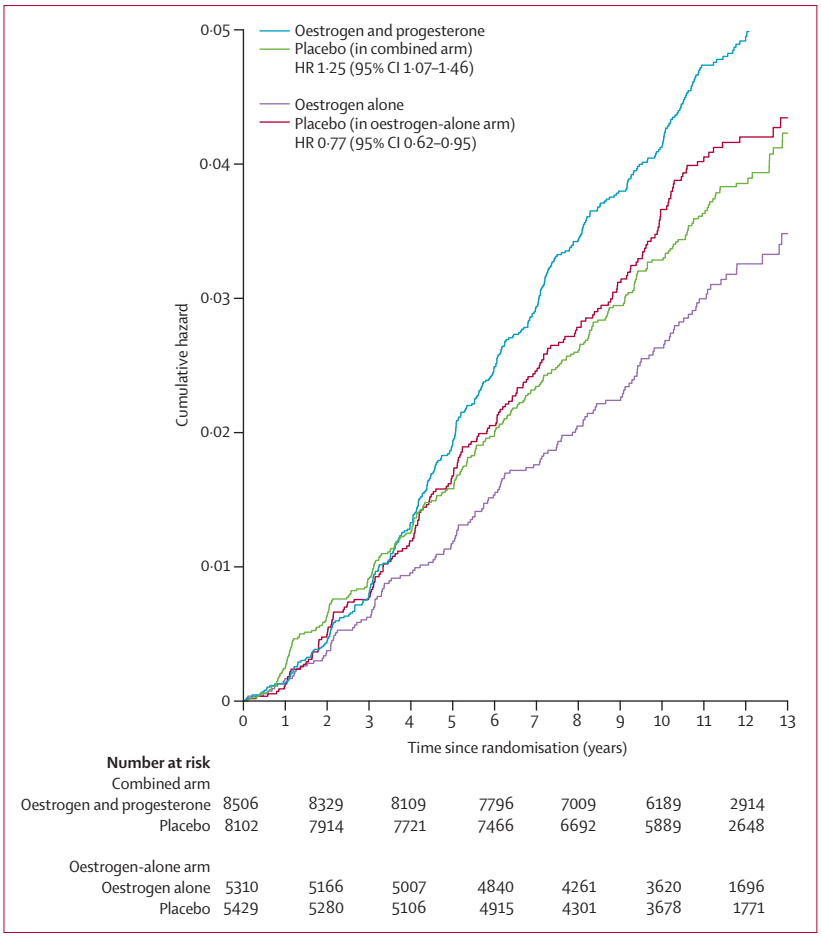

Figure 4: **Cumulative hazards, adjusted for age and ethnic group, of invasive breast cancer by random allocation in the WHI trials of conjugated equine oestrogen alone and conjugated equine oestrogen plus medroxyprogesterone acetate trials derived from Chlebowski and colleagues[32]**
HR=hazard ratio.

Many observational studies report increased breast cancer risk with oestrogen alone in normal weight but not overweight or obese women.[4,5,8] We noted no effect modification with body-mass index; oestrogen hazard ratios were less than one in normal weight, overweight, and obese women.

Use of mammography is a potential source of confounding in observational studies.[35] In clinical practice, women who take hormone therapy have mammograms more regularly than untreated women[36] and screened populations have an increased rate of breast cancer detection,[37,38] potentially explaining the increased breast cancer risk in oestrogen users noted in previous studies that did not assess screening. Controlling for ongoing screening in observational studies is unusual and not straightforward because it depends on adequate data collection and modelling, and the assumption that mammography use is not an intermediate variable of exposure and disease.[39,40]

Detection bias is an unlikely explanation for our results. Mammography rates were protocol-defined and very similar between randomisation groups.[17,22] Furthermore, oestrogen use has little effect on breast density[41] or detection of breast cancer.[22] Finally, the significant reduction in breast cancer mortality reported with oestrogen use provides strong evidence against the possibility that the risk reduction noted was an artifact of oestrogen effects on screening,[40] because a delay in detection would be expected to increase mortality.

Favourable effects of use of oestrogen alone on breast cancer survival, measured from cancer diagnosis, have been seen in some[10–12] but not all[2,7,13–16] reports. Measurement of survival by time since diagnosis might introduce lead-time bias in hormone therapy studies if screening rates varied between hormone users and non-users. Studies of mostly oestrogen alone use on breast cancer mortality, measured from start of hormone therapy have provided mixed results with favourable,[10–12] neutral,[13–15] and unfavourable[2,7] associations reported. Our results, measured from randomisation (although still imprecise) provide important new evidence that oestrogen use for about 5 years reduces breast cancer mortality, supporting a favourable association with breast cancer incidence.

A reduction in breast cancer incidence with conjugated equine oestrogen is biologically plausible. Although such oestrogen is a recognised mitogen that usually stimulates mammary cell proliferation and inhibits apoptosis through activation of the oestrogen receptor,[1] both preclinical[42–46] and clinical[45] findings suggest that after long-term oestrogen deprivation, adaptive changes in mammary tumour gene expression profiles render tumours paradoxically susceptible to oestrogen-induced apoptosis.[43,44] Although the mechanisms are complex and not wholly understood,[46] preclinical studies suggest involvement of the Fas/Fas L extrinsic (receptor-mediated) death regulatory pathway[47] and the intrinsic (mitochondrial) apoptotic pathway, mediated through increased expression of several proapoptotic proteins including P53-unregulated modulator of apoptosis.[48,49]

Efforts to reconcile the original findings of this trial[17] with observational study results suggested that the time from menopause to first hormone use (gap time) is a potential modulator of hormone therapy's influence on breast cancer risk.[28] In the parallel WHI randomised clinical trial[29] and the observational Million Women Study,[5] women starting oestrogen plus progesterone use with a short gap time were at increased breast cancer risk. For use of oestrogen alone, the Million Women Study investigators reported no increased incidence in breast cancer with oestrogen use starting 5 years or more from menopause but an increased risk with a shorter gap time.[5] In these analyses, oestrogen hazard ratios were lower than 1 for early initiation (gap time <5 years) and late initiation (≥5 years). We noted a somewhat greater influence in women starting oestrogen therapy 5 years or more after menopause, but the interaction with gap time was not statistically significant.

Although breast cancer incidence and related mortality were lower for women who took oestrogen alone than for controls, our findings do not support oestrogen use for breast cancer risk reduction because subgroup analyses suggest the benefit might not apply to populations at increased breast cancer risk. Additionally, other hormone-targeted drugs have a substantially greater influence on breast cancer than does oestrogen.[33,34,50,51] However, our findings, together with a relatively balanced risk–benefit profile for clinical events,[18,52] provide reassurance about breast cancer safety for postmenopausal women with previous hysterectomy who receive unopposed oestrogen to reduce climacteric symptoms for durations equivalent to those reported in this trial.

Tamoxifen, raloxifene, and exemestane all provide greater breast cancer risk reduction than oestrogen, but have important limitations. The selective oestrogen receptor modulators tamoxifen and raloxifene reduce risk of breast cancer and fractures but increase climacteric symptoms and have adverse effects on stroke, blood clots, and endometrial cancer, leading to an unfavourable risk–benefit profile in most postmenopausal women.[33,53] Exemestane, an aromatase inhibitor that lowers oestrogen concentrations, also substantially reduces breast cancer risk.[34] However, aromatase inhibitors cause bone loss and increase climacteric symptoms and arthralgias,[54] exerting a greater influence when started soon after menopause.[55] The mechanisms through which exogenous oestrogens, tamoxifen, raloxifene, and aromatase inhibitors all reduce breast cancer risk are not known but clearly warrant further study.

Study strengths include the randomised, double-masked, placebo-controlled prospective design with breast cancer as the designated primary safety outcome, a large sample size, high-quality outcomes ascertainment, and protocol-required mammography throughout most of the follow-up period. Median time to non-adherence to intervention was only 3·5 years, which was much shorter than that noted in studies reporting increased risks. However, any bias arising from poor adherence would probably dilute the differences between randomisation groups over the duration of follow-up, as the adherence-adjusted analyses confirmed. Small numbers of breast cancer deaths, some attrition associated with re-consenting for extended follow-up, and a median of only 4·7 years of post-intervention follow-up should also be noted. The trial assessed only one dose and schedule of oral conjugated equine oestrogens; whether these findings apply to lower doses, other oestrogen preparations, or longer durations of use is not known.

Major differences exist in WHI trial findings between oestrogen only therapy in women with previous hysterectomy and those of the parallel WHI randomised trial of oestrogen plus medroxyprogesterone acetate in women with an intact uterus. Whereas oestrogen-alone treatment was associated with reduced breast cancer incidence and reduced breast cancer mortality, combined hormone therapy increased breast cancer incidence,[29,52] delayed breast cancer diagnosis,[56] and increased breast cancer mortality.[48] The biological basis for this difference is unknown. The comparability of breast cancer incidence rates for the placebo groups in the two trials (figure 4) suggests that differences in hormone therapy, rather than hysterectomy, is the primary determinant. Changes in the serum proteome in response to oestrogen and combined hormone therapies are generally quite similar but differences have been identified that could influence breast cancer risk, including those in NOTCH2 and some IGF binding proteins.[57]

Contributors

GLA, RTC, and AKA conceived the study design. AKA did the statistical analysis. GLA and RTC drafted the initial manuscript. GLA, RTC, LHK, JEM, MG, EB, FAH, DL, LM, JO, RS, and JW-W were responsible for data collection and acquired funding. All authors participated in data interpretation and in critical review of the manuscript. GLA and AKA had full access to the data.

Conflicts of interest

RTC has been a consultant for AstraZeneca, Novartis, Amgen, and Pfizer, received funding support from Amgen, and served on speaker's bureaus for AstraZeneca and Novartis. All other authors declare that they have no conflicts of interest.

Acknowledgments

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