Estradiol & Breast Cancer

Friend or Foe?

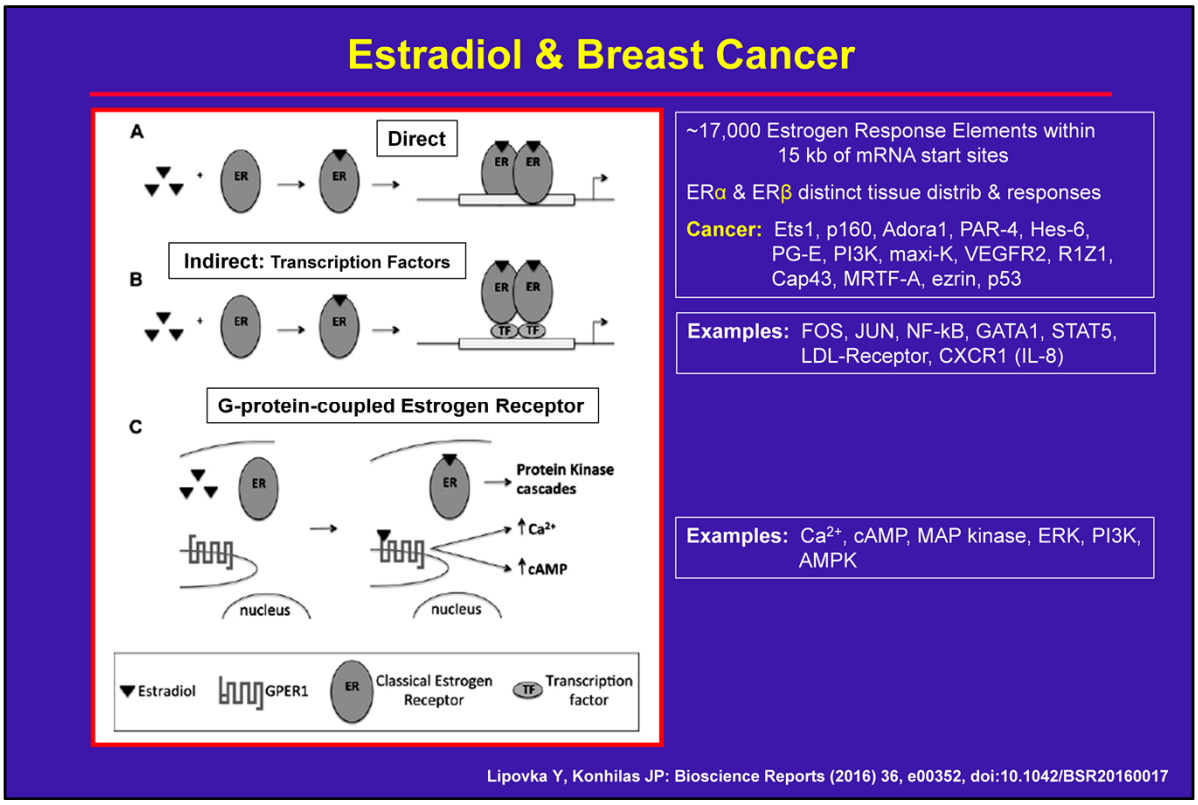
Disclosures:

1. No financial conflicts
2. The opinions expressed in this discussion are my own and do not represent those of any group or organization

Objectives:

1. How does estradiol impact normal cell metabolism?
2. How does estradiol impact cancer cell metabolism?
3. Review available clinical trials concerning breast cancer
4. Briefly review estradiol effects on osteoporosis, CNS, & CVD

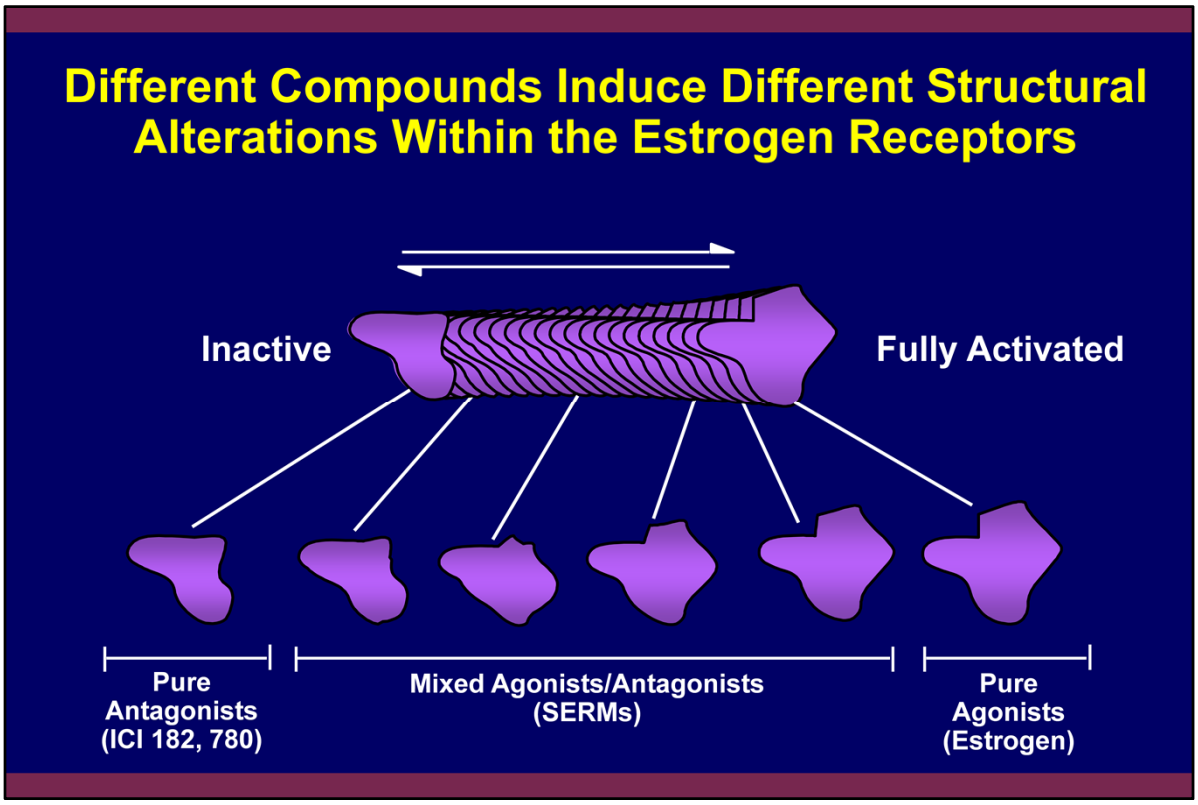
Thomas A. Hughes, M.D.
Professor of Medicine, Retired
Division of Endocrinology, Metabolism, and Diabetes
University of Tennessee Health Science Center
HughesEndo.com



(A) The direct transcriptional pathway involves interaction of ER dimers with EREs within the DNA sequence to modulate gene regulation.

(B) The indirect transcriptional pathway involves protein–protein interaction of the ER dimers with transcription factors (TF) to regulate gene transcription.

(C) The non-transcriptional pathway involves a subclass of classical ERs and GPER1 to trigger signal transduction cascades in response to E2 stimulation.

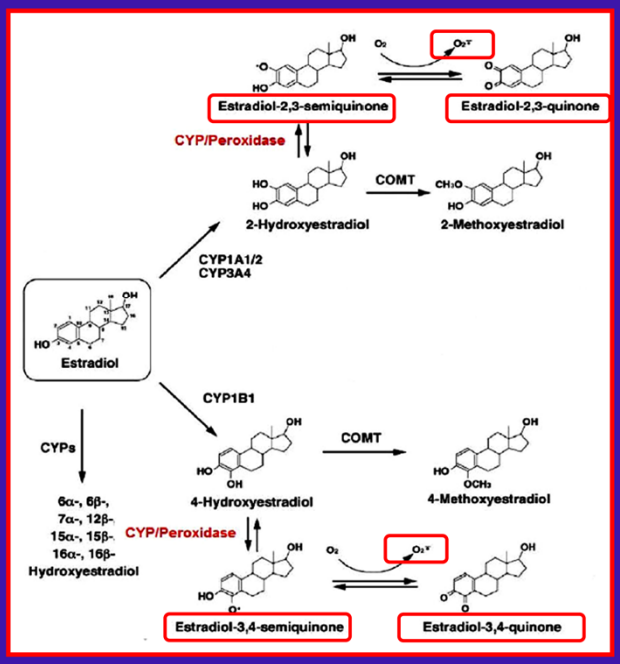

Different Compounds Induce Different Structural Alterations Within the Estrogen Receptors
Inactive
Fully Activated
Pure Antagonists (ICI 182, 780)
Mixed Agonists/Antagonists (SERMs)
Pure Agonists (Estrogen)

Estradiol & Breast Cancer

Is Estrogen carcinogenic?

Estrogen semi-quinones & quinones are reactive and carcinogenic metabolites which can cause DNA damage.

There is no clinical evidence that these compounds are produced in significant amounts “in vivo”.

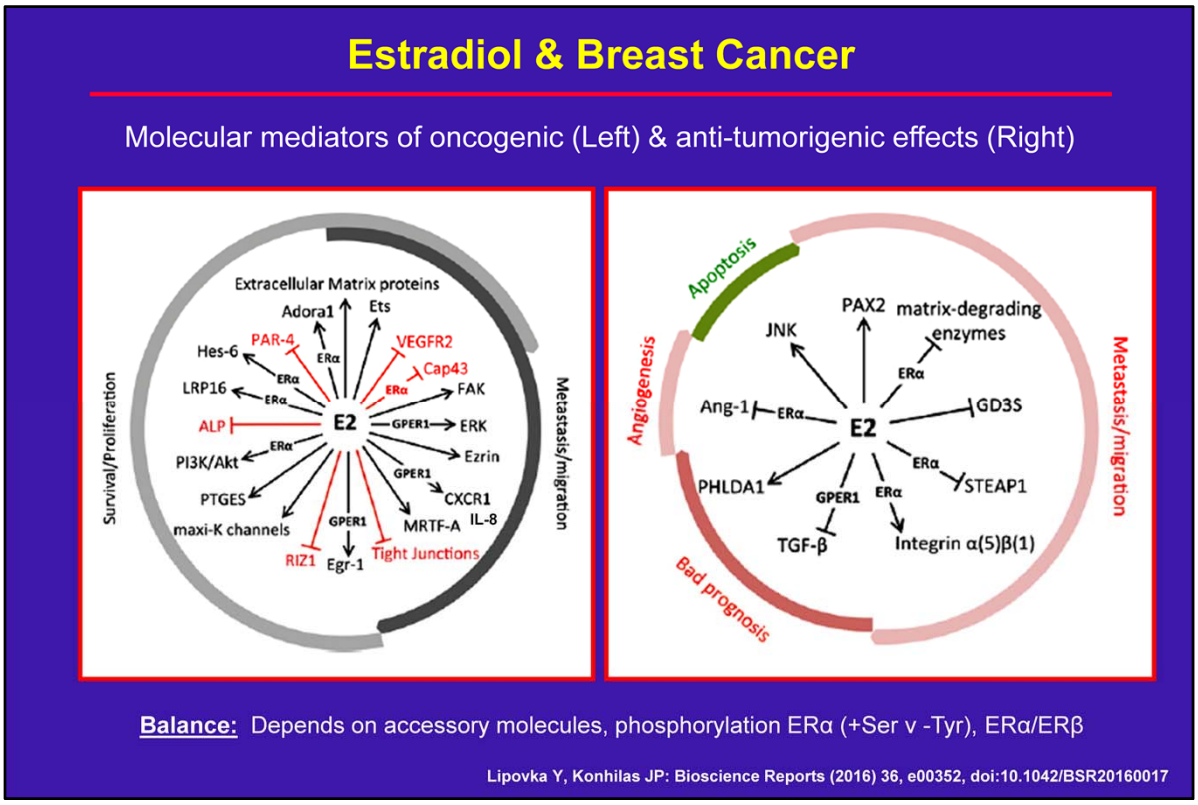


Samavat H & Kurzer MS:
Cancer Lett. 2015 January 28; 356(2 0 0): 231–243
doi:10.1016/j.canlet.2014.04.018

There is currently accumulating evidence that endogenous estrogens play a critical role in the development of breast cancer. Estrogens and their metabolites have been studied in both pre- and postmenopausal women with more consistent results shown in the latter population, in part because of large hormonal variations during the menstrual cycle and far fewer studies having been performed in premenopausal women. In this review we describe in detail estrogen metabolism and associated genetic variations, and provide a critical review of the current literature regarding the role of estrogens and their metabolites in breast cancer risk.

Figure 3. Estradiol metabolism and DNA adduct formation
Estradiol catechol estrogens, including 2-hydroxyestradiol and 4-hydroxyestradiol, can go through reductive-oxidative cycling and produce mutagenic free radicals. These reactions are catalyzed by CYP and peroxidase enzymes. Estrogen semiquinones and quinones are reactive and carcinogenic intermediate metabolites of redox cycling pathways and can cause DNA damage.
Abbreviations: CYP, cytochrome P-450 enzyme; COMT, catechol-O-methyltransferase

The role of estrogen metabolites in the etiology of breast cancer has been studied, but available data are mixed and no firm conclusion can be drawn in either pre- or postmenopausal women.



Abstract: The pleiotropic nature of oestradiol, the main oestrogen found in women, has been well described in the literature. Oestradiol is positioned to play a unique role since it can respond to environmental, genetic and non-genetic cues to affect genetic expression and cellular signalling. In breast cancer, oestradiol signalling has a dual effect, promoting or inhibiting cancer growth. The potential impact of oestradiol on tumorigenesis depends on the molecular and cellular characteristics of the breast cancer cell. In this review, we provide a broad survey discussing the cellular and molecular consequences of oestrogen signalling in breast cancer. First, we review the structure of the classical oestrogen receptors and resultant transcriptional (genomic) and non-transcriptional (non-genomic) signalling. We then discuss the nature of oestradiol signalling in breast cancer including the specific receptors that initiate these signalling cascades as well as potential outcomes, such as cancer growth, proliferation and angiogenesis. Finally, we examine cellular and molecular mechanisms underlying the dimorphic effect of oestrogen signalling in breast cancer.

Figure 3 Mediators of oestrogen oncogenic effects. A diagram of oestrogen targeted effectors, discussed in this review, that mediate its oncogenic effects leading to proliferation, metastasis or both. Wherever known, the involvement of ER*α* or GPER1 is indicated.

Figure 4 Molecular mediators of anti-tumorigenic oestrogen signalling. A diagram of oestrogen targeted effectors, discussed in this review, that mediate its apoptotic,

anti-metastatic, anti-angiogenic effects, or improve bad prognosis. Processes shown in green are potentiated, whereas processes shown in red are inhibited. Wherever known, the involvement of ER*α* or GPER1 is indicated.

Summary: Taken together, all the information presented above suggests that E2 signalling in breast cancer is very complex and cannot be categorized as detrimental or beneficial without prior knowledge of function. It is the particular combination of molecular assets within the cancer cell that helps fine-tune the course of molecular events triggered by oestrogen. The overall response to oestrogen stimulation can be modulated at different levels within the cells. These regulatory levels can be classified as receptor-dependent or receptor-independent. The first one refers to ER expression status, presence of post-translational modifications and formation of functional dimers. The latter includes alternative metabolic processing of oestrogen and the unique pool of intracellular effectors that can be found in each cell type.

Estradiol & Breast Cancer

Estrogen utilizes RAS & Akt pathways to modulate gene expression

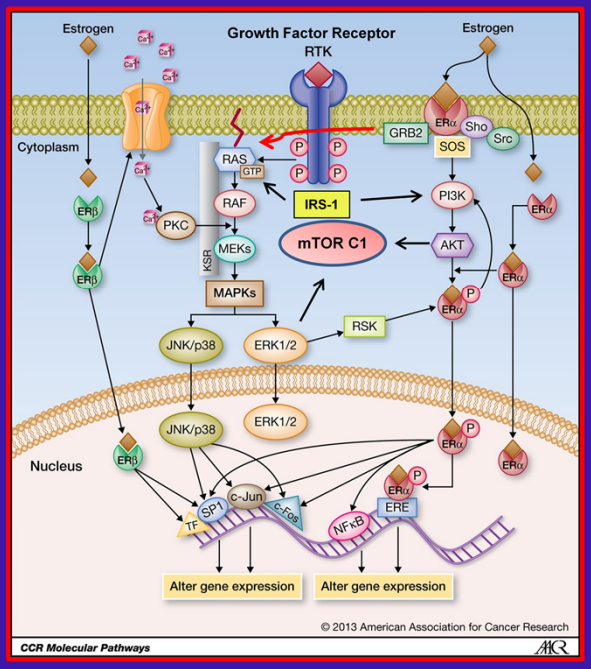
Both activate mTOR C1 which controls growth, proliferation, & survival

GPER also activates Ca^{++}, PI3K, cAMP
(G-protein-coupled estrogen receptor)

Epidermal Growth Factor Receptor Family (EGFR)
(ERBs / HERS / FGFR)
Primary Ligands: EGF & TGFα
Total Ligands: ~13

Ligands are membrane or stromal anchored but released by metalloproteases (ADAMs & MMPs) to bind EGFR.
Activated by:
PK-C & MAPK → Cytokines
Wnt Ligands
Ligands & EGFR over-expressed in cancer

AREG (amphiregulin) induced by **E2** via ADAM17
Released from ductal epithelium to stimulate stromal cells
ADAM17, AREG, TGFα up-regulated in Cancer
Suppression of AREG reduces tumorigenesis
Phosphorylation of ER bypasses Estrogen



Eccles, S: Int. J. Dev. Biol. 55: 685-696, 2011
doi: 10.1387/ijdb.113396se

Fig. 2. EGFR/Erb-B signaling linked to epithelial cell-stromal cell interactions in ductal development in the mammary gland. The Figure illustrates the reciprocal interactions between stromal cells and mammary epithelial cells (MEC) during normal development. ADAM sheddases, regulated by estrogens, release ligands such as AREG which stimulate EGFR on neighboring stromal cells. Additional proteases (e.g MMP2 activated by MT1-MMP) release growth factors which reciprocally stimulate MECs to proliferate. MMPs are also required for ductal 'invasion' into the fat pad. Adapted from (Sternlicht and Sunnarborg, 2008).

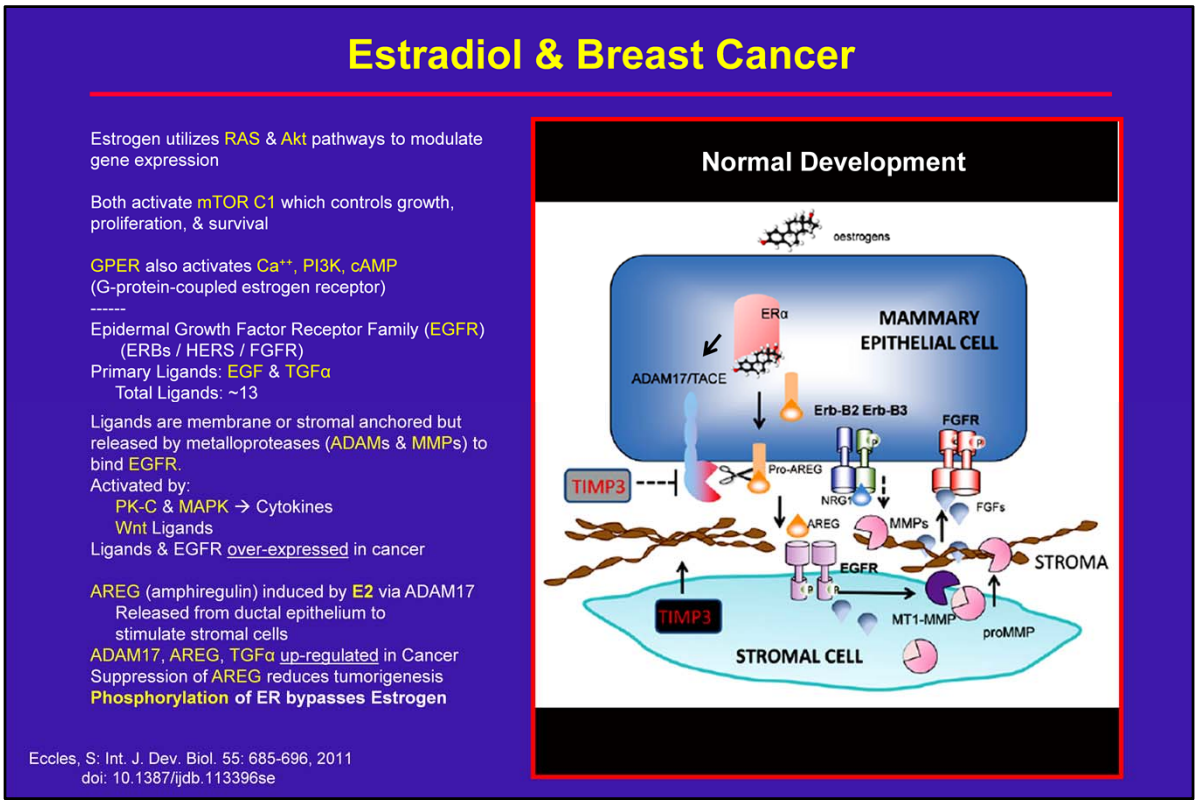


Fig. 2. EGFR/Erb-B signaling linked to epithelial cell-stromal cell interactions in ductal development in the mammary gland. The Figure illustrates the reciprocal interactions between stromal cells and mammary epithelial cells (MEC) during normal development. ADAM sheddases, regulated by estrogens, release ligands such as AREG which stimulate EGFR on neighboring stromal cells. Additional proteases (e.g MMP2 activated by MT1-MMP) release growth factors which reciprocally stimulate MECs to proliferate. MMPs are also required for ductal 'invasion' into the fat pad. Adapted from (Sternlicht and Sunnarborg, 2008).

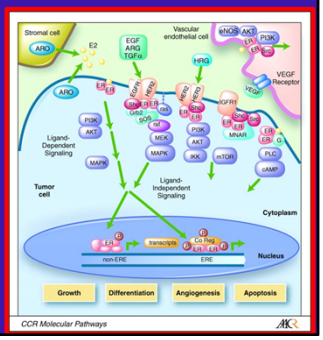
Estradiol & Breast Cancer

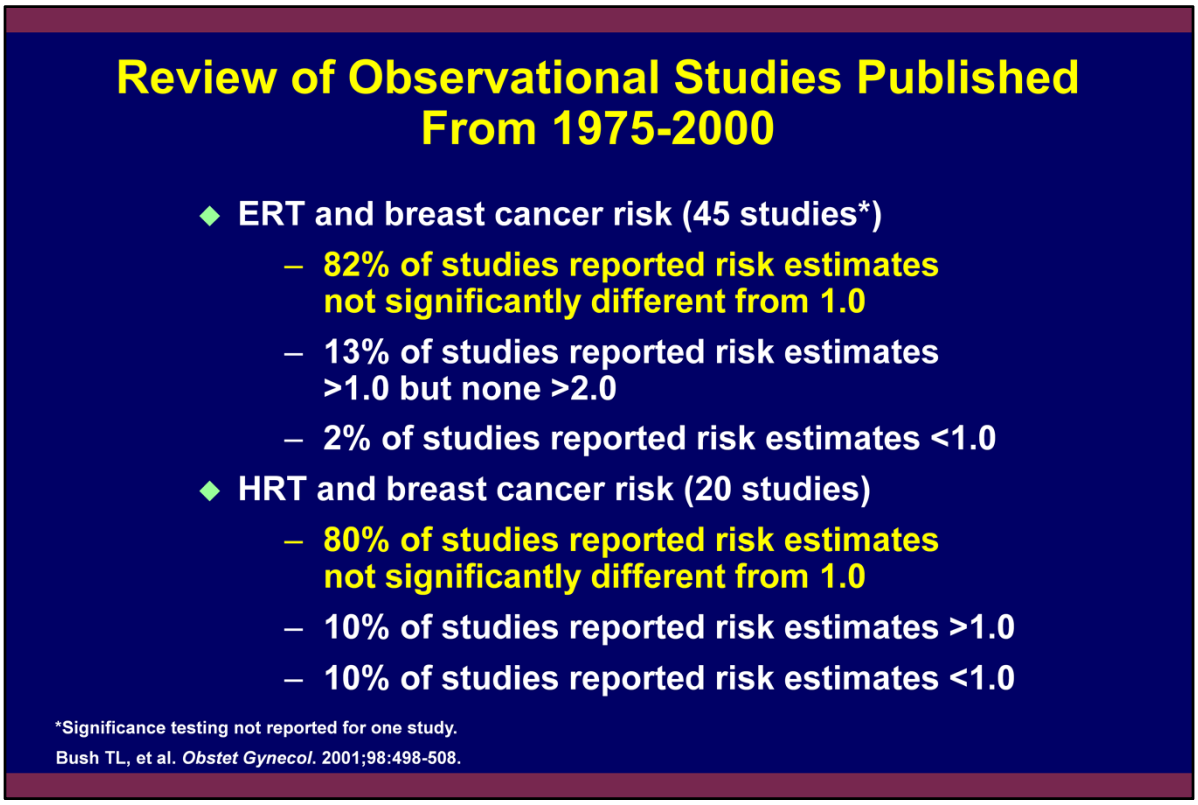
Problem:

Observation: Estrogen can both inhibit or promote various pathways for breast growth, differentiation, involution, tumorigenesis, & metastasis.

Question: Which effects dominate?

Solution: Clinical Trails are required to answer this question.





In the past 25 years, over **50 epidemiologic studies** and six meta-analyses have examined the association between ERT/HRT and breast cancer risk. Bush et al[1] compiled the results from these studies and found a lack of consistency among the findings. Among studies that examined ERT use, 82% found no effect of ERT use on breast cancer risk; 13% reported a modest increase in risk (>1.0 but not >2.0); and 2% reported a reduced risk of breast cancer with ERT use. Preliminary data from the ERT arm of the WHI, which is ongoing, also indicate no increased risk of breast cancer with an average of 5.2 years of ERT use.[2]

Results from **observational studies** on HRT followed a similar pattern: 80% of the studies found no effect of HRT use on breast cancer risk; 10% of the studies reported an elevated risk; and 10% of the studies reported a reduction in breast cancer risk. Preliminary data from the HRT arm of the WHI showed a small increase in risk that was not statistically significant. However, the risk estimate crossed the predetermined stopping boundary for breast cancer and, as a result, the HRT arm of the WHI was stopped.[2] In summary, while the large majority of observational studies have failed to show any difference in breast cancer risk between ERT/HRT users and nonusers, preliminary results from the WHI suggest a small increase in risk with long-term use (>4 years) of HRT.

[1]Bush TL, Whiteman M, Flaws J. Hormone replacement therapy and breast cancer: a qualitative review. *Obstet Gynecol*. 2001;98:498-508.

[2]Writing Group for the Women's Health Initiative Investigators. 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.

Breast Cancer Risk and Estrogen-Progestin Therapy

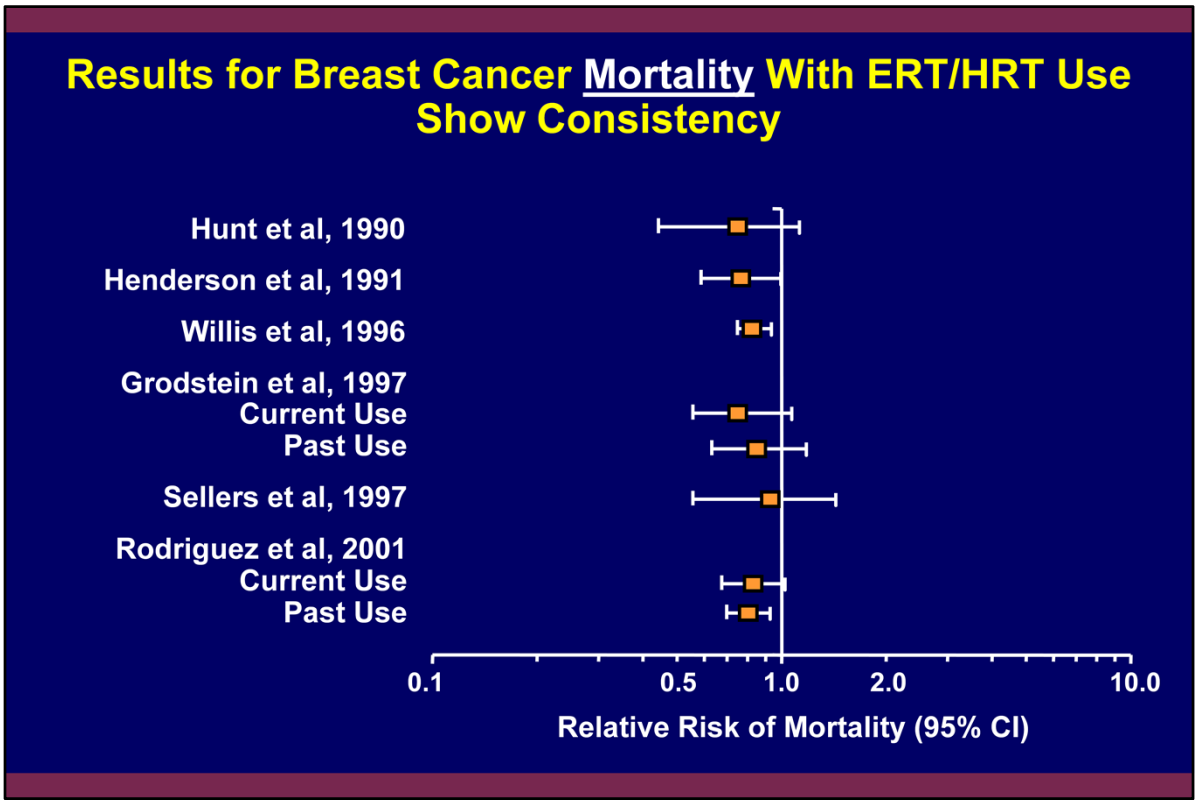
Summary of Findings

Author	OR or RR (95% CI)	HRT Use
Writing Group for WHI, 2002	1.26 (1.0–1.6)	5.2 years
Hulley et al, 2002	1.27 (0.84–1.94)	6.8 years
Schairer et al, 2000	1.3 (1.0–1.6)	Ever
Ross et al, 2000	1.2 (1.1–1.5)*	Per 5 years use
Magnusson et al, 1999	1.6 (1.4–1.9)*	Ever
Brinton et al, 1998	1.0 (0.7–1.3)	Ever
Collaborative Group, 1997	1.5 (not reported)	≥5 years
Stanford et al, 1995	0.9 (0.7–1.3)	Ever
Newcomb et al, 1995	1.0 (0.8–1.3)	Ever
Yang et al, 1992	1.2 (0.6–2.2)	Ever

*$P < .05$.

This slide summarizes the results of the most recent studies which have examined whether use of a progestin with estrogen increases breast cancer risk.[1-10]
Ross et al[4] and Magnusson et al[5] reported a statistically significant increased risk with HRT use.
The remaining studies did not find a statistically significant increase in breast cancer risk with HRT use.[1-3,6-10]

A complete list of references for this slide can be found in the accompanying document titled “Breast Health References.”



The body of research on breast cancer risk and ERT/HRT use demonstrates little, if any, consistency of findings.[1-6] However, what is consistent in the literature is the observation that mortality from breast cancer is decreased among ERT/HRT users. A summary of the literature from 1990-2001 shows the relative risk of mortality consistently to be <1.0 with ERT/HRT use. The studies in this slide[1-6] all found a relative risk of <1.0 for mortality from breast cancer with ERT/HRT use. The largest of these studies assessed 422,737 female participants from the Cancer Prevention Study II for 9 years and analyzed breast cancer deaths and their association to estrogen use.[3] In this study, there was a significant decreased risk of fatal breast cancer (RR, 0.84; 95%CI, 0.75–0.94) in ever-users of ERT. Former users of HRT also showed a significant decreased risk of fatal breast cancer (RR, 0.78; 95% CI, 0.68–0.89). All studies show that women who take hormones and ultimately develop breast cancer live longer.

A complete list of references for this slide can be found in the accompanying document titled “Breast Health References.”

Estrogen: Breast Cancer

Nachtigall et al., Obstet Gynecol 80:827, 1992

Prospective, double-blind, placebo controlled for 10 yrs
84 matched pairs of hospitalized women (age, smoking Hx, Dx)
Premarin 2.5 mg/d + Provera 10 mg/d x 7 days/mth
(after 10 yrs changed to .625 and 10 mg/d x 10 days/mth)

Pts were allowed to switch after 10 yrs and were monitored for 12 more yrs
32 'controls' started E and 33 'treated' stopped E

Breast Ca after 10 yrs: Controls - 4 Treated - 0 (p=.042)

Breast Ca after 22 yrs: Never Treated - 6 (n=52) Ever Treated - 0 (n=116) (p=.001)
Expected - 4.8

HERS Secondary Noncardiovascular Outcomes

2,763 Women with CVD → CEE 0.625 + MPA 2.5 for 4.1 yrs

Outcome	*HRT (n)*	*Placebo (n)*	*Relative Hazard (95% CI)*
Venous thrombotic events			
Deep venous thrombosis	25	8	3.18 (1.43–7.04)*
Pulmonary embolism	11	4	2.79 (0.89–8.75)
Any thromboembolic event	34	12	2.89 (1.50–5.58)*
Cancer			
Breast	32	25	1.30 (0.77–2.19)
Endometrial	2	4	0.49 (0.09–2.68)
Any cancer	96	87	1.12 (0.84–1.50)
Fracture			
Hip	12	11	1.10 (0.49–2.50)
Any fracture	130	138	0.95 (0.75–1.21)
Gall bladder disease	84	62	1.38 (1.00–1.92)

*$P < .05$.

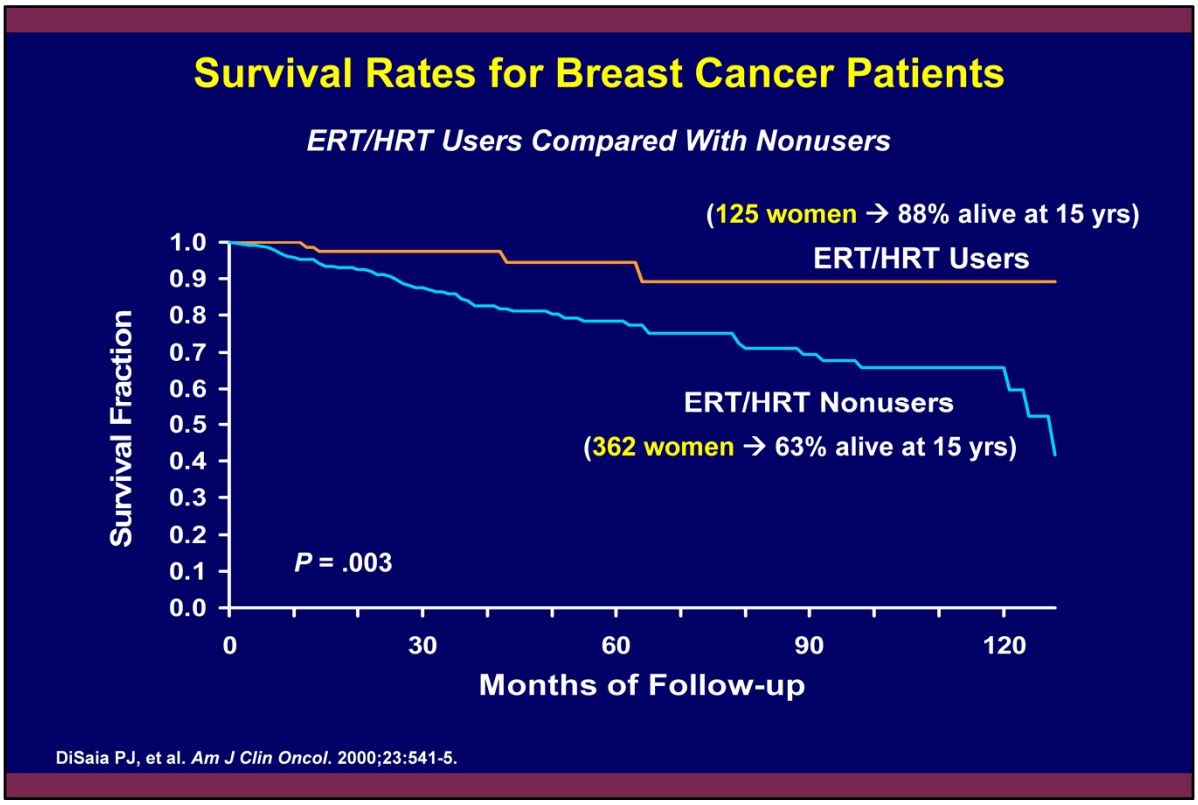
Hulley S, et al. *JAMA*. 1998;280:605-13.

Estrogen: Breast Cancer

Eden et al., Menopause 2:67, 1995

Case-Control Study:

- 901 breast cancer survivors
- 90 women took continuous estrogen and progesterone for relief of menopausal symptoms
- Matched for age and disease-free interval prior to starting ERT
- 7% recurrence among users, 17% recurrence among non-users



If ERT/HRT increases the risk of breast cancer, one would expect breast cancer survivors who use ERT/HRT to have a higher rate of recurrence than survivors not using ERT/HRT, and possibly increased mortality.

DiSaia et al conducted a follow-up study of 125 breast cancer survivors who used ERT/HRT, matched with 362 breast cancer survivors who did not use ERT/HRT, to compare mortality rates between the two groups.

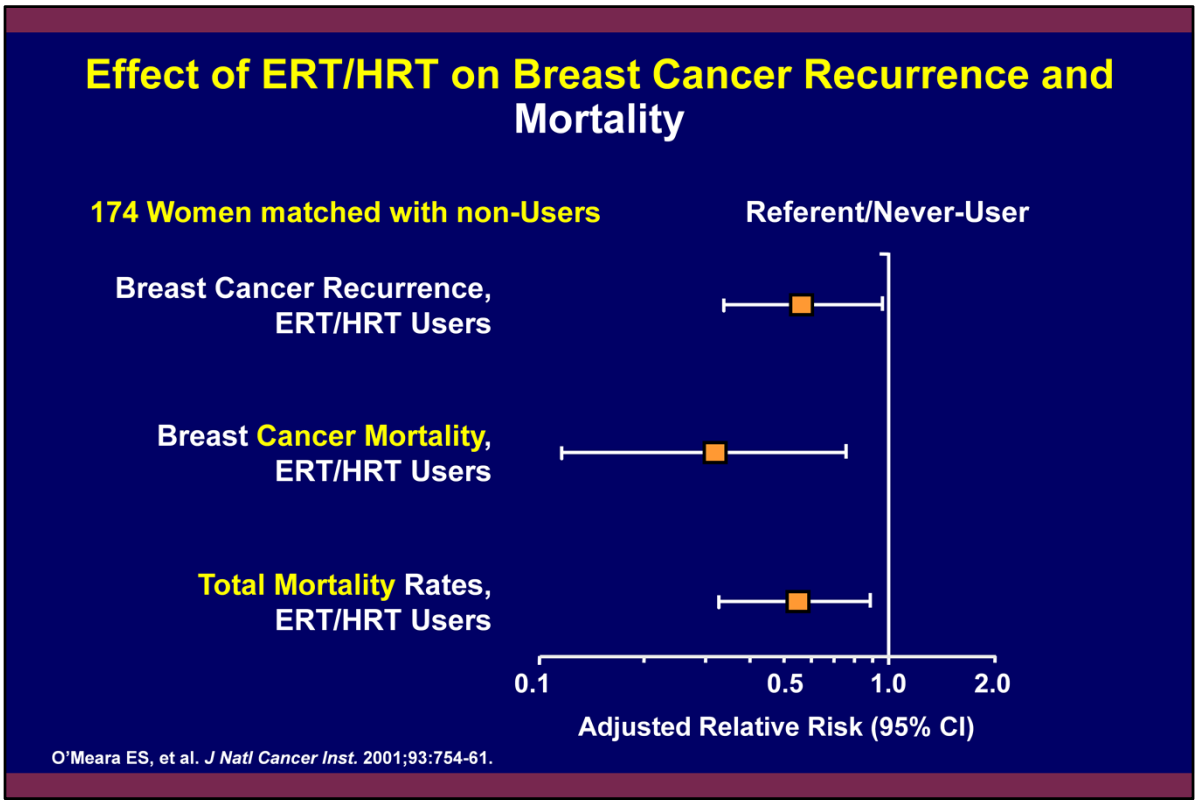
These investigators found that the mortality rate among breast cancer survivors who used ERT/HRT was improved over those patients who were nonusers.

This graph shows the magnitude of the survival advantage for ERT/HRT users versus nonusers over the course of 15 years after breast cancer diagnosis.

Analysis of death from all causes found 88% of women taking ERT/HRT alive at 15 years compared with 63% (P = .003) of women not using ERT/HRT.

These results amount to a 70% reduction in the risk of death for the women choosing to use ERT/HRT. This risk reduction was observed in all ERT/HRT users regardless of stage, receptor status, and nodal involvement.

DiSaia PJ, Brewster WR, Ziogas A, Anton-Culver H. Breast cancer survival and hormone replacement therapy: a cohort analysis. *Am J Clin Oncol*. 2000;23:541-545.

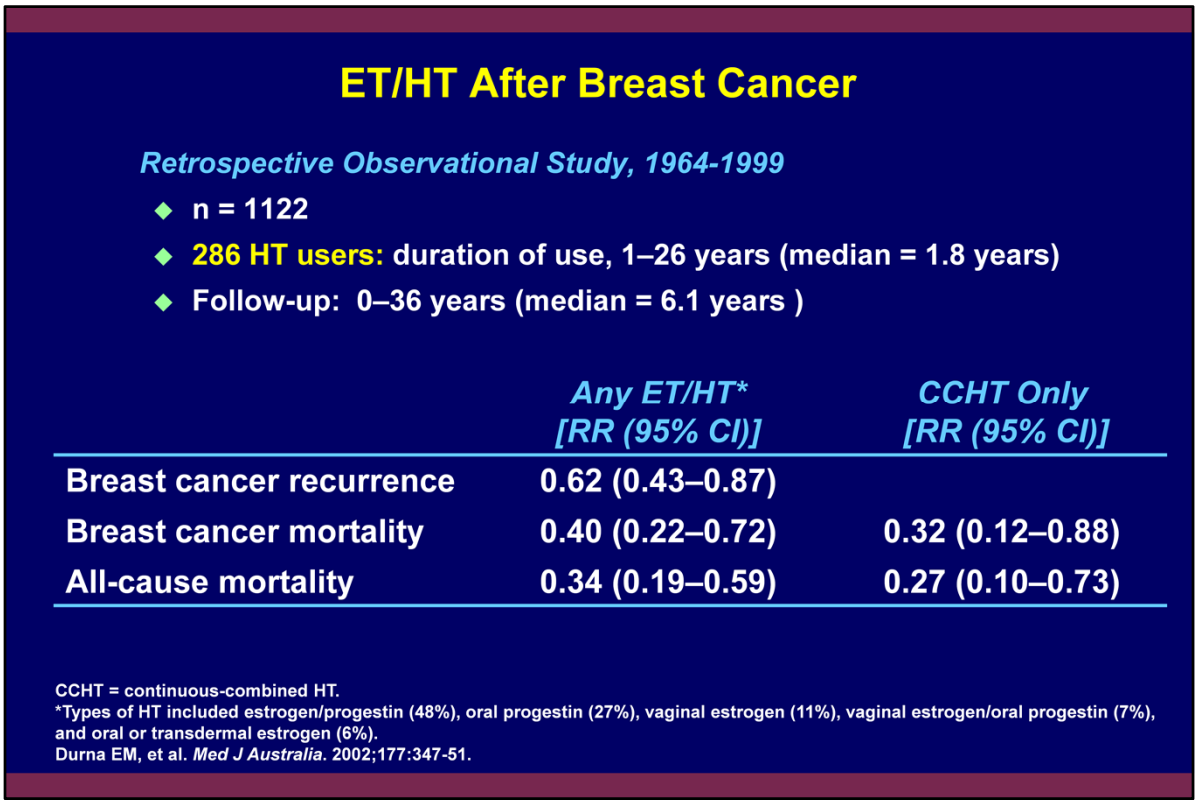


O'Meara et al further investigated ERT/HRT use and breast cancer recurrence/survival in a study of women aged 35 to 74 years diagnosed with invasive breast cancer. In this trial, 174 women using ERT/HRT were matched with nonusers for age, stage, and diagnosis date. Rates of recurrence and mortality were assessed and adjusted relative risks were calculated.

Adjusted relative rates for recurrence in ERT/HRT users was 0.5.
Adjusted relative rates for breast cancer mortality was 0.34.
Adjusted relative rates for total mortality was 0.48.

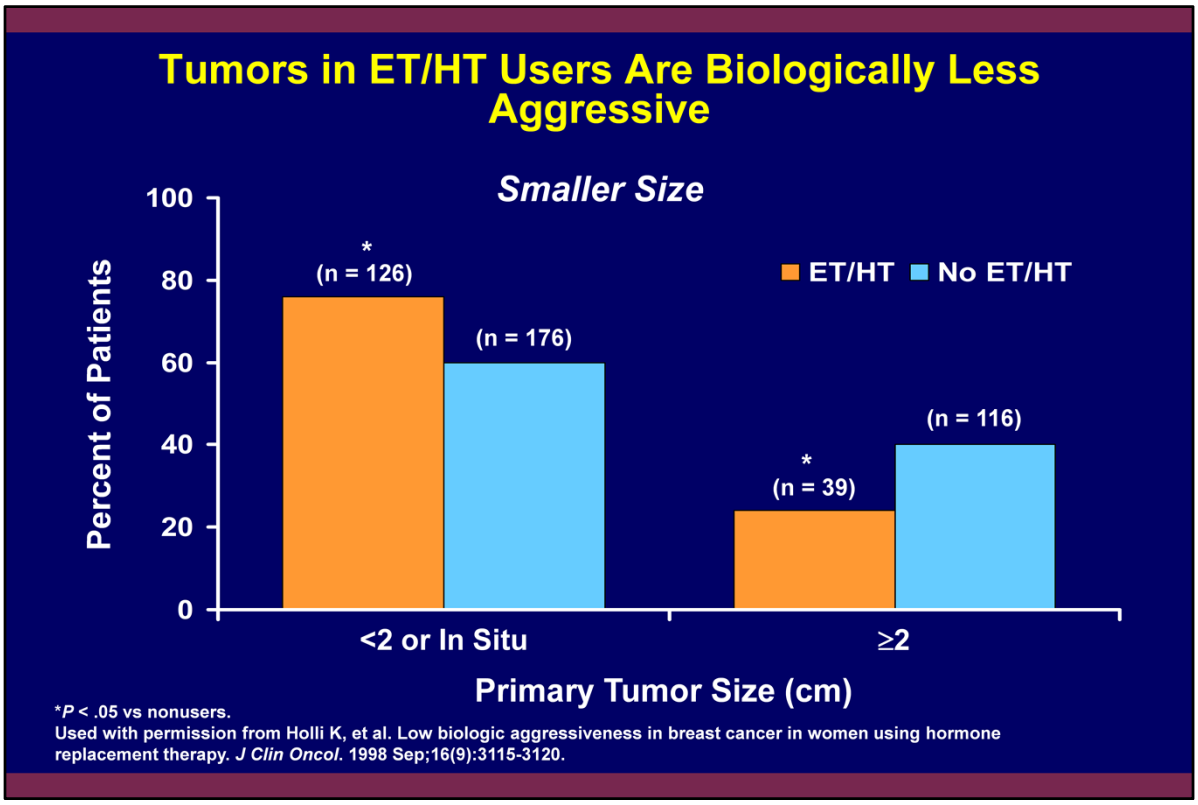
Thus, women taking ERT/HRT had less breast cancer recurrence, breast cancer mortality, and total mortality than nonusers.

O'Meara ES, Rossing MA, Daling JR, Elmore JG, Barlow WE, Weiss NS. Hormone replacement therapy after a diagnosis of breast cancer in relation to recurrence and mortality. *J Natl Cancer Inst*. 2001;93:754-761.



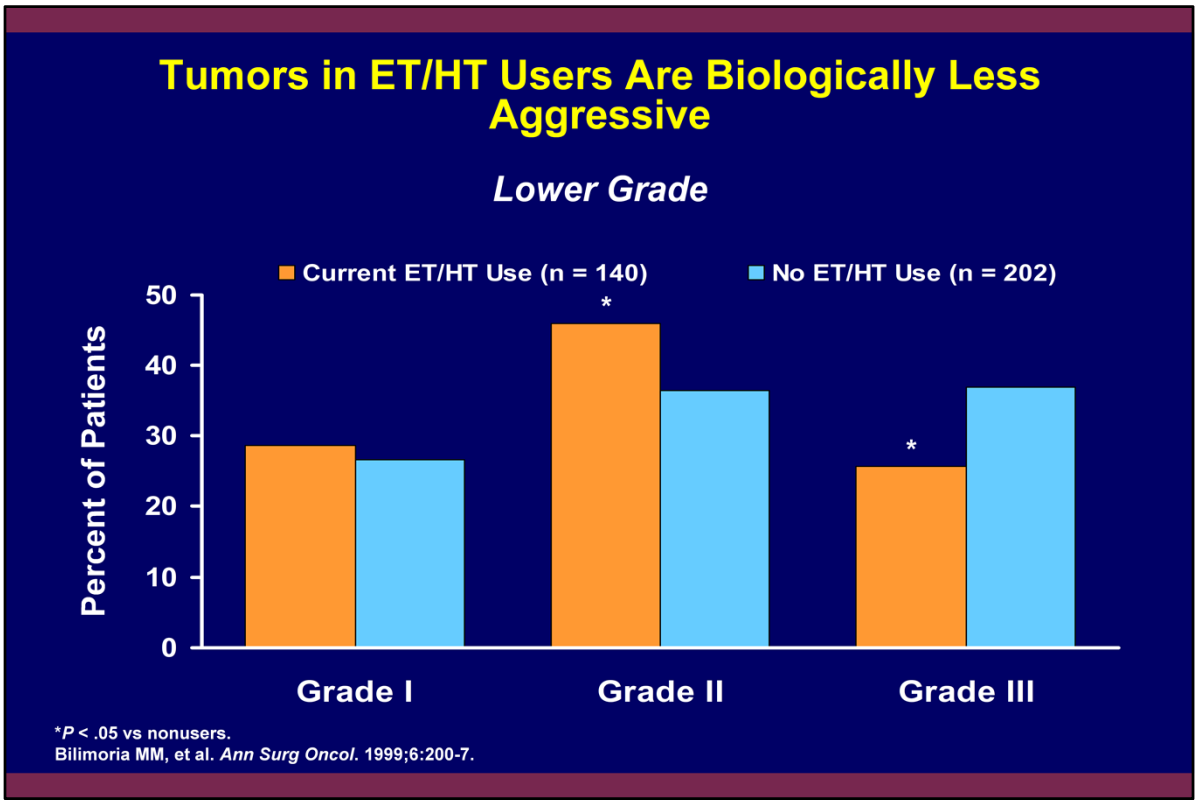
This **retrospective, observational** study evaluated whether HT in breast cancer patients is associated with increased risk for breast cancer recurrence or mortality. Participants were 1,122 breast cancer patients in Australia, diagnosed between 1964 and 1999. Investigators measured time from diagnosis to cancer recurrence, detection of new breast tumors, or death among HT users and compared these numbers to those for nonusers. Compared with nonusers, HT users had a 38% reduced risk of cancer recurrence, a 60% reduced risk of death from primary breast cancer, and a 66% reduced risk of all-cause mortality. Continuous combined HT was associated with reduced mortality from cancer and reduced all-cause mortality. It should be noted that this was not a randomized, controlled trial, and also that women in this study with Stage I or Stage II breast cancer were more likely to use HT than were women with Stage III or Stage IV cancer, which could have contributed to more favorable outcomes for HT users.

Durna EM, Wren BG, Heller GZ, Leader LR, Sjoblom P, Eden JA. Hormone replacement therapy after a diagnosis of breast cancer: cancer recurrence and mortality. *Med J Aust*. 2002;177:347-351.



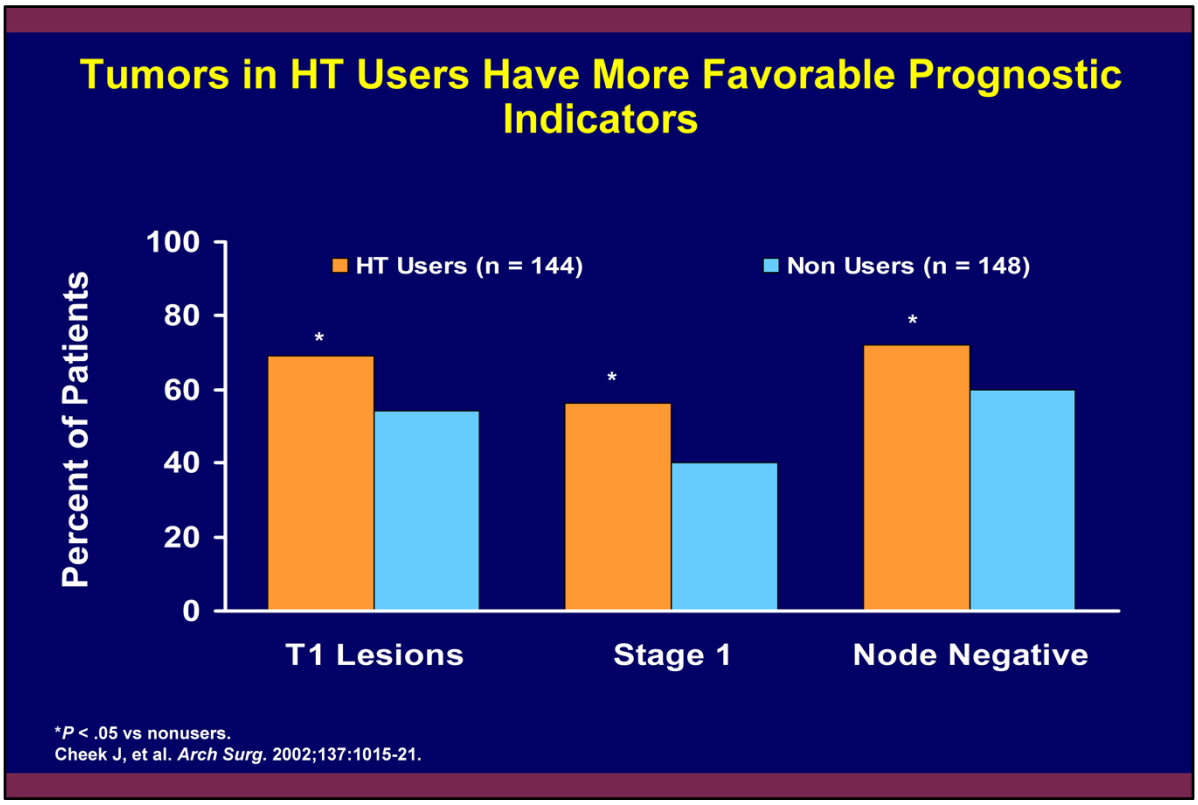
One hypothesis to explain why ET/HT decreases mortality from breast cancer is that ET/HT may promote the development of slow-growing tumors or discourage the development of more aggressive tumors. Better screening of these women also contributes to the lower mortality. Holli et al reported that women who received ET/HT had biologically less aggressive breast cancers than those not taking ET/HT. In this study, 477 postmenopausal women with breast cancer had indicators of tumor aggressiveness measured. Results showed tumors in women taking ET/HT were smaller, had a better histologic differentiation, and had a lower cell-proliferation rate than tumors from nonusers. Seventy-six percent of women in the ET/HT group had tumors <2 cm in size or in situ tumors, whereas only 60% of women not taking ET/HT had these smaller tumors.

Holli K, Isola J, Cuzick J. Low biologic Holli K, Isola J, Cuzick J. Low biologic aggressiveness in breast cancer in women using hormone replacement therapy. *J Clin Oncol*. 1998;16:3115-3120.



Bilimoria et al evaluated tumor characteristics at the time of breast cancer diagnosis in 140 patients receiving ET/HT and 202 patients with no history of estrogen use. Compared with nonusers with breast cancer, breast cancer patients receiving ET/HT at the time of diagnosis had more grade II tumors (45.9% vs. 36.5%, $P < .05$) and fewer grade III tumors (25.6% vs. 37.0%, $P < .05$). Similar to the effect seen with endometrial tumors, HT may promote less aggressive tumors.

Bilimoria MM, Winchester DJ, Sener SF, Motykie G, Sehgal UL, Winchester DP. Estrogen replacement therapy and breast cancer: analysis of age of onset and tumor characteristics. *Ann Surg Oncol*. 1999;6:200-207.



Retrospective study of 292 postmenopausal women with breast cancer: tumors diagnosed in HT users had better prognostic indicators compared to those diagnosed in non-users. Although the number of Stage II cancers was not reported, cancers in HT users were more likely to be diagnosed as Stage I, compared with cancers in non-users (P = .02). Tumors in HT users were also more likely to be node-negative, and there was a higher incidence of T1 lesions among HT users. 58% of the cancers were detected by mammography among HT users, compared with 43% among non-users (P = .01). The 6-yr survival rate was 92% for HT users, compared with an 80% rate for non-users (P = .05). For patients w/ tumors detected by mammogram, 6-year survival was 100% for HT users and 87% for non-users (P = .03).

Cheek J, Lacy J, Toth-Fejel S, Morris K, Calhoun K, Pommier RF. The impact of hormone replacement therapy on the detection and stage of breast cancer. *Arch Surg*. 2002;137:1015-1021.

Women's Health Initiative (WHI)

Baseline Characteristics

Characteristic	HRT n = 8506	Placebo n = 8102
Age at screening, years*	63.2 (7.1)	63.3 (7.1)
Prior hormone use, %	26.1	25.6
Body mass index, kg/m²*	28.5 (5.8)	28.5 (5.9)
Never smokers, %	49.6	50.0
Diabetes, %	4.4	4.4
Hypertension, %	35.7	36.4
Statin use at baseline, %	6.9	6.8
Family history of breast cancer, %	16.0	15.3
History of MI,† %	1.6	1.9
History of CABG/PTCA,† %	1.1	1.5‡

*Values are means (SD). †Overall incidence of prior cardiovascular disease = 7.7%. ‡*P* = .04 vs HRT. CABG = coronary artery bypass graft; PTCA = percutaneous transluminal coronary angioplasty.

Writing Group for the Women's Health Initiative Investigators. *JAMA.* 2002;288:321-33.

This table summarizes characteristics of the WHI HRT and placebo groups at baseline.[1]
The mean age of the WHI subjects (63 years) was older than the typical age of most women who present to physicians and clinics with menopausal symptoms, and similar to the mean age of the HERS participants (67 years).[2] Because the WHI subjects were on average more than 10 years beyond the mean age for the onset of menopause, it seems possible that, at study initiation, some of the subjects already had early breast cancer or CHD that was undetected.
At study outset, there were no substantive differences between groups for most of the parameters above, including age, family history of breast cancer, and prior hormone use.

[1]Writing Group for the Women's Health Initiative Investigators. 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.

[2]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;288:58-66.

WHI Results

Absolute and Relative Risk or Benefit of CEE/MPA

Health Event	*Overall Hazard Ratio*	*Confidence Interval*		*Increased Absolute Risk per 10,000 Women/Year*	*Increased Absolute Benefit per 10,000 Women/Year*
		Nominal 95%	*Adjusted 95%*		
CHD	1.29	1.02–1.63	0.85–1.97	7	
Strokes	1.41	1.07–1.85	0.86–2.31	8	
Breast cancer	1.26	1.00–1.59	0.83–1.92	8	0.08% annually or 1 in 1,250 women
VTED	2.11	1.58–2.82	1.26–3.55	18	
Colorectal cancer	0.63	0.43–0.92	0.32–1.24		6
Hip fractures	0.66	0.45–0.98	0.33–1.33		5
Total fractures	0.76	0.69–0.85	0.63–0.92		44

CHD = coronary heart disease; VTED = venous thromboembolic disease.
Writing Group for the Women's Health Initiative Investigators. *JAMA.* 2002;288:321-33.

For the clinical outcomes listed here, the overall HRs at 5.2 years for CEE/MPA users versus placebo are shown in the black box. For each of these ratios, two different CIs were calculated, nominal and adjusted, and the intervals which reached statistical significance, that is, those that did not cross 1.0, are highlighted in yellow.

Reporting of both types of CIs in the same publication is rare as they tend to make interpretation of the data difficult.

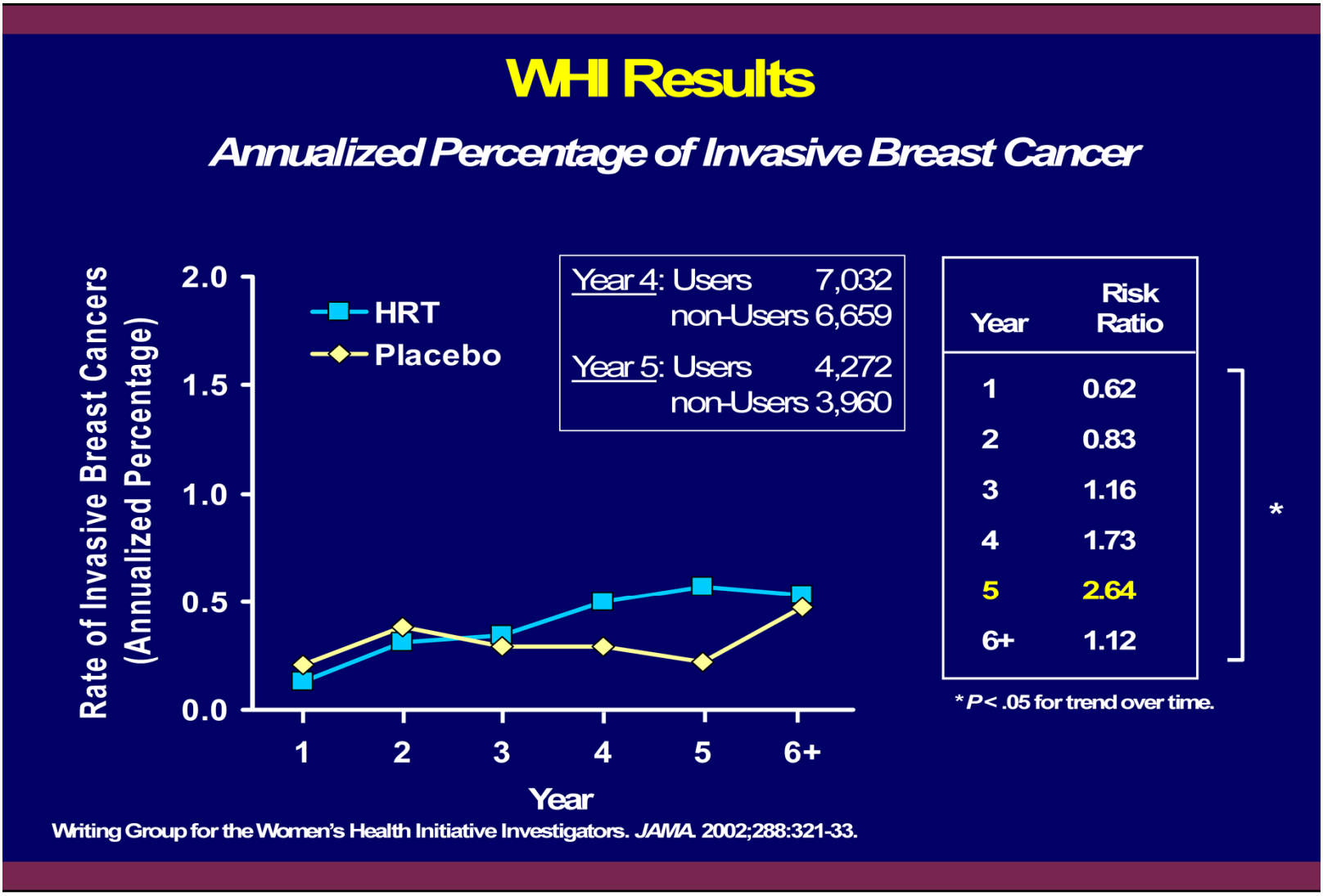
The nominal CI for CHD and the adjusted CI for stroke indicate statistically significant increases in risk among CEE/MPA users. Both the nominal and adjusted CIs for venous thromboembolic disease (VTED; the combination of deep vein thrombosis and pulmonary embolism) indicate a statistically significant increase in risk among CEE/MPA users.

Both the nominal and adjusted CIs for total fractures indicate statistically significant decreased risk among CEE/MPA users. The nominal CIs for colorectal cancer and hip fracture indicate statistically significant decreases in risk among CEE/MPA users.

Examining either the nominal or adjusted CI, the HR for invasive breast cancer was not significantly different from 1.0.

The absolute excess risk or benefit attributable to CEE/MPA was low. The WHI findings predict that over 1 year, 10,000 women taking CEE/MPA compared with placebo might be expected to experience 7 more CHD events, 8 more strokes, 8 more invasive breast cancers, 18 more pulmonary embolisms, 6 fewer colorectal cancers, 5 fewer hip fractures, and 44 fewer total fractures.

Writing Group for the Women's Health Initiative Investigators. 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.

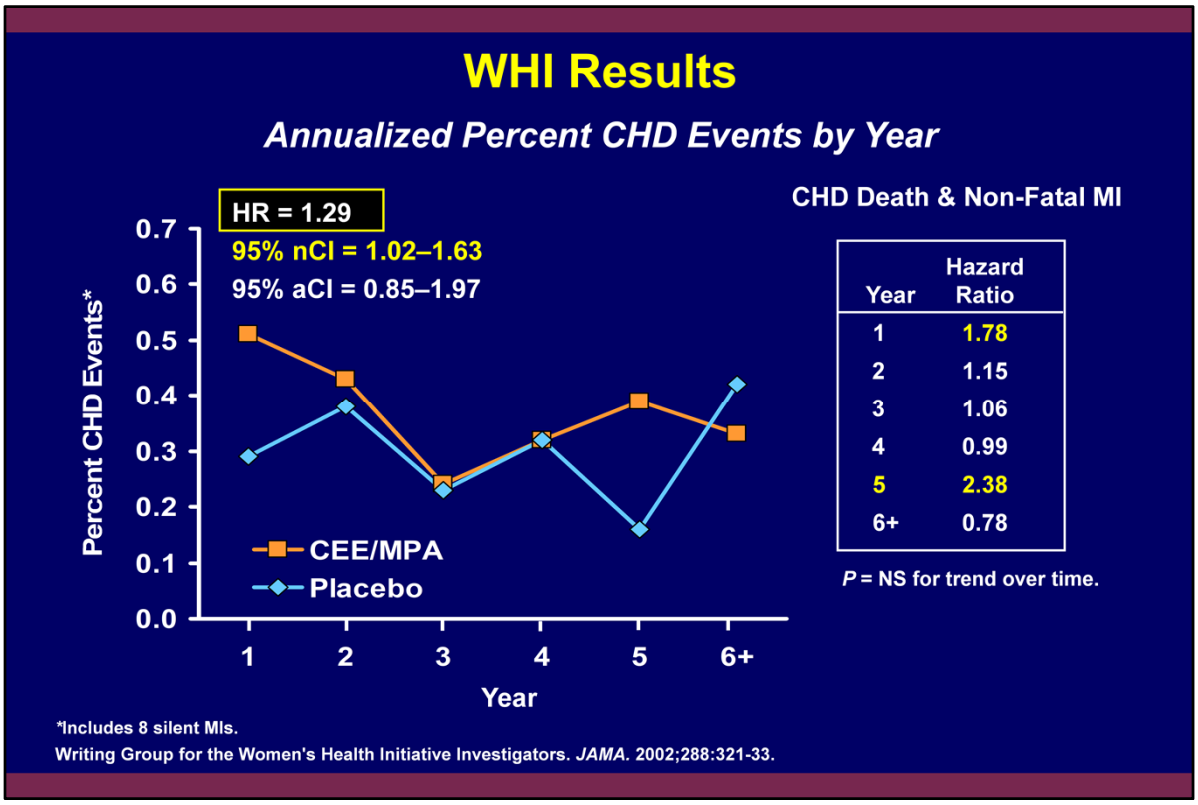


This figure compares yearly rates of invasive breast cancer in the HRT and placebo groups. Yearly rates, expressed as annualized percentages, were very low in both groups and did not exceed 1.0% for either group:

	HRT	Placebo
Year 1	0.13%	0.21%
Year 2	0.31%	0.38%
Year 3	0.34%	0.29%
Year 4	0.50%	0.29%
Year 5	0.57%	0.22%
Year 6	0.53%	0.47%

Thus, in any given year of follow-up, the total number of breast cancer cases was very small in both the HRT and placebo groups. On average, breast cancer was diagnosed each year in 0.30% of the women (ie, in less than 1/3 of 1% of the women) in the placebo group, and in 0.38% of the women (ie, in slightly more than 1/3 of 1% of the women) in the HRT group. The table to the right of the line graph lists the HRs for breast cancer with HRT use on a year-by-year basis. Statistical analysis for a linear trend with time detected an increasing risk of breast cancer over time associated with HRT use ($P < .05$). However, the investigators note that these time trend results should be viewed cautiously because the number of breast cancer cases in each year was small, the data for later years are incomplete, and risk comparisons in later years are limited to women who were not diagnosed with breast cancer in previous years.

Writing Group for the Women's Health Initiative Investigators. 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.



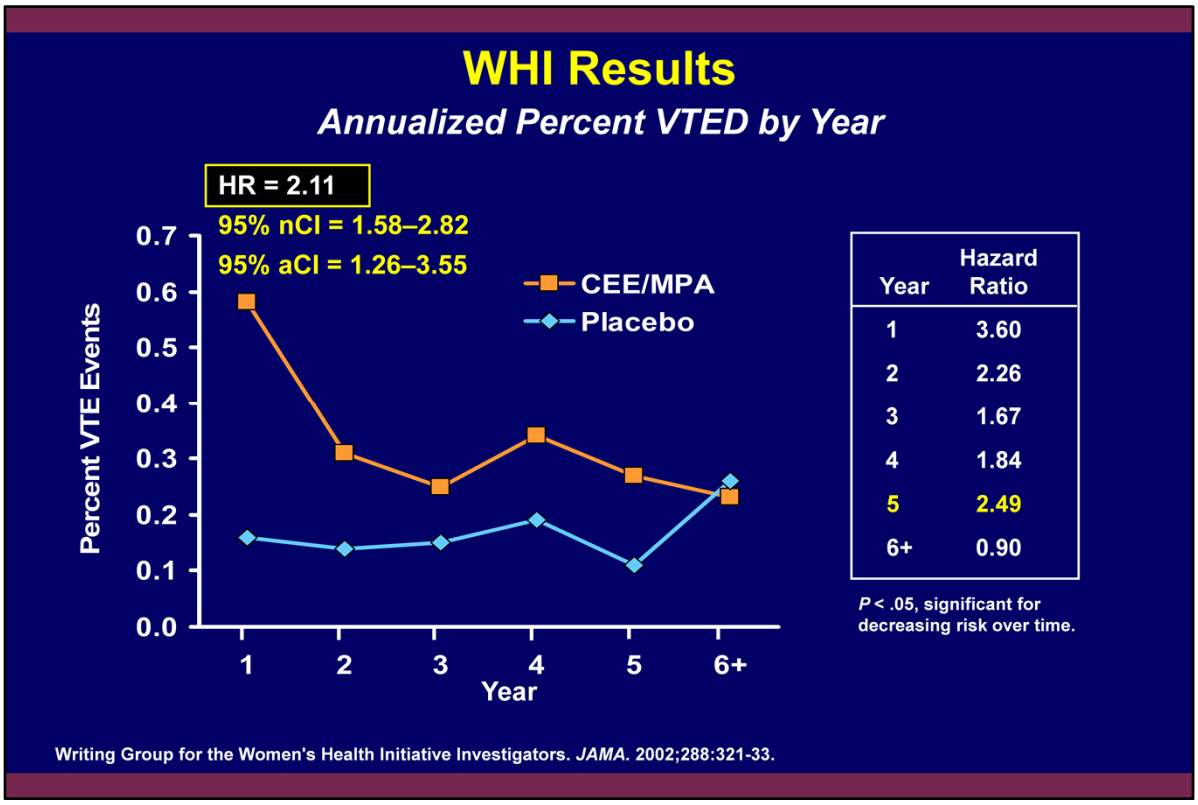
This figure compares yearly rates (annualized percent) of CHD events in the CEE/MPA and placebo groups. Annualized percent represents the percent of women experiencing first time CHD events in the patient group during that particular year. For example, an annualized percent of 0.29 represents 29% of women in that treatment group who had not previously had a CHD event having one during that year.

The annualized percent of CHD events in both the CEE/MPA and placebo group were variable, with a notable decrease in events in the placebo group at year 5.

The table to the right of the line graph lists the HRs for total CHD events with CEE/MPA use on a year-to-year basis. Statistical tests for linear trends with time since randomization detected no significant trend over time for CHD; however, the WHI investigators note that these time-trend results should be viewed cautiously because the number of CHD cases in each year were small, the data for later years are incomplete, and risk comparisons in later years are limited to women who were not diagnosed with CHD in previous years

For the entire follow-up period examined, overall HR for CHD was 1.29, with a significant 95% nominal CI of 1.02–1.63.

Writing Group for the Women's Health Initiative Investigators. 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.



This figure compares yearly rates of VTED (deep vein thromboembolism and pulmonary embolism) in the CEE/MPA and placebo groups.[1]

The table to the right of the line graph lists the HRs for VTED on a year-to-year basis. CIs were not reported for the yearly HRs.

Following an average of 5.2 years follow-up, the overall HR for VTED was 2.11 (95% aCI, 1.26–3.55), indicating a significantly increased risk in CEE/MPA users.

Statistical analysis for a linear trend with time detected a decreasing risk of VTED with time (*z* score = –2.45) in the CEE/MPA users; a similar trend for decreasing risk of VTED over time is observed among oral contraceptive users.[2]

The WHI investigators note that the results from time-trend analyses in this first WHI report should be viewed cautiously because the number of events in each year were small, the data for later years are incomplete, and risk comparisons in later years are limited to women who were not diagnosed with VTED in previous years.

[1]Writing Group for the Women's Health Initiative Investigators. 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.

[2]Lidegaard O, Edstrom B, Kreiner S. Oral contraceptives and venous thromboembolism: a five-year national case-control study. *Contraception*. 2002;65:187-196.

WHI Results: Venous Thromboembolic Disease (VTED)

Summary by Year

Year	*CEE/MPA n (%)*	*Placebo n (%)*	*Hazard Ratio**
1	49 (0.58)	13 (0.16)	3.60
2	26 (0.31)	11 (0.14)	2.26
3	21 (0.25)	12 (0.15)	1.67
4	27 (0.34)	14 (0.19)	1.84
5	16 (0.27)	6 (0.11)	2.49
6+	12 (0.23)	11 (0.26)	0.90

Overall HR = 2.11; 95% nCI, 1.58–2.82; 95% aCI, 1.26–3.55
n = number of patients; % = annualized % calculated from average exposure over ~60 months.
**P* < .05, significant for decreasing risk over time.
Writing Group for the Women's Health Initiative Investigators. *JAMA*. 2002;288:321-33.

In WHI VTED was defined as the combination of deep vein thromboembolism and pulmonary embolism events.
This figure illustrates the yearly number of patients (annualized percentage in brackets) who experienced VTED outcomes in the CEE/MPA arm of the WHI. In addition, the year-to-year HRs for VTED are provided. CIs were not reported for the individual year-to-year HRs.
Statistical analysis for a linear trend with time detected a decreasing risk of VTED with time (*z* score = –2.45) in the CEE/MPA users; however, the WHI investigators note that these time-trend results should be viewed cautiously because the number of VTED cases in each year were small, the data for later years are incomplete, and risk comparisons in later years are limited to women who were not diagnosed with VTED in previous years.
Following an average of 5.2 years follow-up, the overall HR for VTED was 2.11 (95% aCI, 1.26–3.55), indicating a significantly increased risk in CEE/MPA users.

Writing Group for the Women's Health Initiative Investigators. 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.

WHI Results: CVD Events

Outcome	CEE/MPA n (%)	Placebo n (%)	Hazard Ratio	Nominal 95% CI	Adjusted 95% CI
CHD	164 (0.37)	122 (0.30)	1.29	1.02–1.63	0.85–1.97
CHD death	33 (0.07)	26 (0.06)	1.18	0.70–1.97	0.47–2.98
Nonfatal MI	133 (0.30)	96 (0.23)	1.32	1.02–1.72	0.82–2.13
CABG/PTCA	183 (0.42)	171 (0.41)	1.04	0.84–1.28	0.71–1.51
Stroke	127 (0.29)	85 (0.21)	1.41	1.07–1.85	0.86–2.31
Fatal	16 (0.04)	13 (0.03)	1.20	0.58–2.50	0.32–4.49
Nonfatal	94 (0.21)	59 (0.14)	1.50	1.08–2.08	0.83–2.70
VTED	151 (0.34)	67 (0.16)	2.11	1.58–2.82	1.26–3.55
Deep vein thrombosis	115 (0.26)	52 (0.13)	2.07	1.49–2.87	1.14–3.74
Pulmonary embolism	70 (0.16)	31 (0.08)	2.13	1.39–3.25	0.99–4.56
Total CVD	694 (1.57)	546 (1.32)	1.22	1.09–1.36	1.00–1.49

n = number of patients; % = annualized % calculated from average exposure over ~60 months. Nominal = variability based on simple trial for single outcome; Adjusted = corrects variability for multiple analyses over time.
CABG/PTCA = coronary artery bypass grafting/percutaneous transluminal coronary angioplasty.
Writing Group for the Women's Health Initiative Investigators. *JAMA*. 2002;288:321-33.

This figure illustrates the HRs with 95% CIs for CVD outcomes evaluated in the CEE/MPA arm of the WHI. These results are considered preliminary as not all outcomes were centrally adjudicated at the time of publication.
Although both nominal and adjusted CIs are presented for comparison purposes, the WHI investigators specified that they applied the nominal 95% CI to evaluate risk for primary outcomes (CHD) and the adjusted 95% CI for the evaluation of risk for secondary outcomes.
Overall CHD rates in WHI were low. The rate of women experiencing CHD events was increased by 29% for women taking HRT relative to placebo. Most of the excess for CEE/MPA users was in nonfatal MI.
No significant differences were observed in CHD deaths or revascularization procedures (coronary artery bypass grafting or percutaneous transluminal coronary angioplasty).
Stroke rates were higher in women taking CEE/MPA, with most of the difference occurring in nonfatal events. In addition, women using CEE/MPA had a 2-fold greater rate of VTED (both deep vein thrombosis and pulmonary embolism). Total CVD, including other events requiring hospitalization, was increased by 22% in the HRT group.

Writing Group for the Women's Health Initiative Investigators. 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.

WHI Results

*Causes of Death**

Outcome	*CEE/MPA (n = 8506) n (%)*	*Placebo (n = 8102) n (%)*
Total deaths	231 (0.52)	218 (0.53)
Adjudicated deaths	215 (0.49)	201 (0.49)
Cardiovascular	65 (0.15)	55 (0.13)
Breast cancer	3 (0.01)	2 (<0.01)
Other cancer	104 (0.24)	86 (0.21)
Other known cause	34 (0.08)	41 (0.10)
Unknown cause	9 (0.02)	17 (0.04)

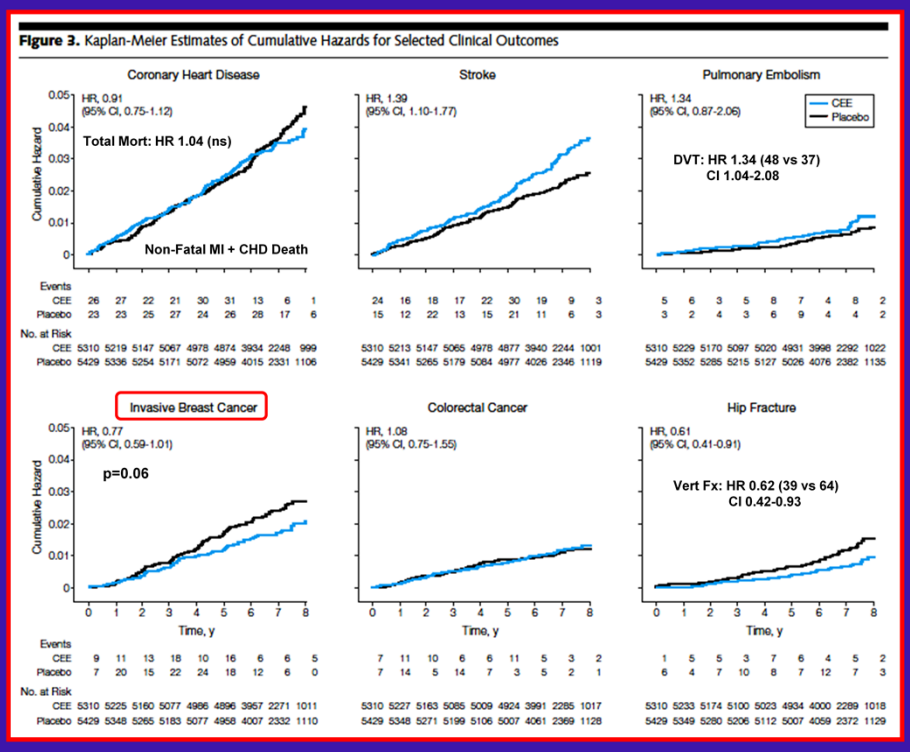
*Causes of death for placebo versus HRT were not statistically different
n = number of patients; % = annualized % calculated from average exposure over ~60 months.
Writing Group for the Women's Health Initiative Investigators. *JAMA.* 2002;288:321-33.

This table details the causes of deaths that occurred in the CEE/MPA arm of the WHI.
There were no significant differences in mortality or cause of death between the CEE/MPA and placebo groups during the observational period of 5.2 years. Longer-term changes in mortality could not be determined.
Of the adjudicated deaths, only 30% were due to cardiovascular events.

Writing Group for the Women's Health Initiative Investigators. 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.

WHI - II

Estrogen Only Group



JAMA 291(14): 1701-1712, 2004 (doi:10.1001/jama.291.14.1701)

Context Despite decades of use and considerable research, the role of estrogen alone in preventing chronic diseases in postmenopausal women remains uncertain.

Objective To assess the effects on major disease incidence rates of the most commonly used postmenopausal hormone therapy in the United States.

Design, Setting, and Participants A randomized, double-blind, placebo-controlled disease prevention trial (the estrogen-alone component of the Women's Health Initiative [WHI]) conducted in 40 US clinical centers beginning in 1993. Enrolled were 10739 postmenopausal women, aged 50-79 years, with prior hysterectomy, including 23% of minority race/ethnicity.

Intervention Women were randomly assigned to receive either 0.625 mg/d of conjugated equine estrogen (CEE) or placebo.

Main Outcome Measures The primary outcome was coronary heart disease (CHD) incidence (nonfatal myocardial infarction or CHD death). Invasive breast cancer incidence was the primary safety outcome. A global index of risks and benefits, including these primary outcomes plus stroke, pulmonary embolism (PE), colorectal cancer, hip fracture, and deaths from other causes, was used for summarizing overall effects.

Results In February 2004, after reviewing data through November 30, 2003, the

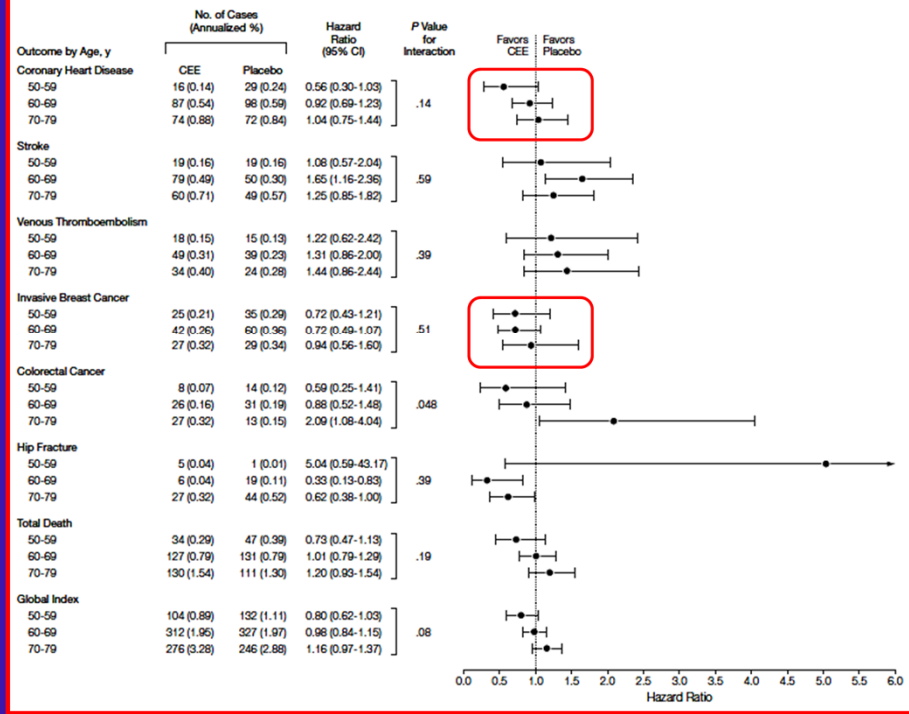
National Institutes of Health (NIH) decided to end the intervention phase of the trial early. Estimated hazard ratios (HRs) (95% confidence intervals [CIs]) for CEE vs placebo for the major clinical outcomes available through February 29, 2004 (average follow-up 6.8 years), were: CHD, 0.91 (0.75-1.12) with 376 cases; breast cancer, 0.77 (0.59-1.01) with 218 cases; stroke, 1.39 (1.10-1.77) with 276 cases; PE, 1.34 (0.87-2.06) with 85 cases; colorectal cancer, 1.08 (0.75-1.55) with 119 cases; and hip fracture, 0.61 (0.41-0.91) with 102 cases. Corresponding results for composite outcomes were: total cardiovascular disease, 1.12 (1.01-1.24); total cancer, 0.93 (0.81-1.07); total fractures, 0.70 (0.63-0.79); total mortality, 1.04 (0.88-1.22), and the global index, 1.01 (0.91-1.12). For the outcomes significantly affected by CEE, there was an absolute excess risk of 12 additional strokes per 10,000 person-years and an absolute risk reduction of 6 fewer hip fractures per 10,000 person-years. The estimated excess risk for all monitored events in the global index was a nonsignificant 2 events per 10,000 person-years.

Conclusions The use of CEE increases the risk of stroke, decreases the risk of hip fracture, and does not affect CHD incidence in postmenopausal women with prior hysterectomy over an average of 6.8 years. A possible reduction in breast cancer risk requires further investigation. The burden of incident disease events was equivalent in the CEE and placebo groups, indicating no overall benefit. Thus, CEE should not be recommended for chronic disease prevention in postmenopausal women.

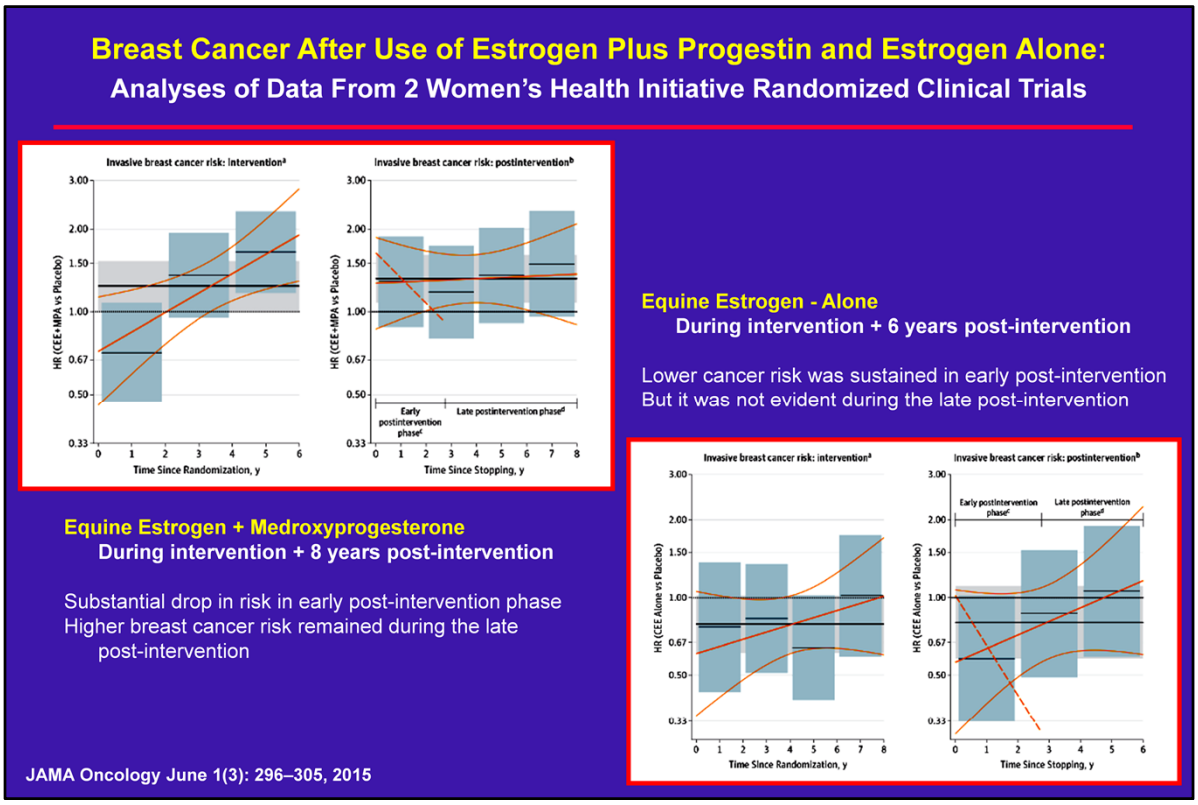
JAMA. 2004;291:1701-1712

WHI - II

Figure 5. Selected Clinical Outcomes by Participant Age and Randomization Assignment



JAMA 291(14): 1701-1712, 2004 (doi:10.1001/jama.291.14.1701)



Abstract

IMPORTANCE—The use of menopausal hormone therapy (HT) continues in clinical practice, but reports are conflicting concerning the longer-term breast cancer effects of relatively short-term use.

OBJECTIVE—To report the longer-term influence of menopausal HT on breast cancer incidence in the 2 Women's Health Initiative (WHI) randomized clinical trials.

DESIGN, SETTING, AND PARTICIPANTS—A total of 27,347 postmenopausal women aged 50 to 79 years were enrolled at 40 US centers from 1993 to 1998 and followed up for a median of 13 years through September 2010.

INTERVENTIONS—A total of 16,608 women with a uterus were randomized to conjugated equine estrogens (0.625 mg/d [estrogen]) plus medroxyprogesterone acetate (2.5 mg/d [progestin]) (E + P) or placebo with a median intervention duration of 5.6 years, and 10 739 women with prior hysterectomy were randomized to conjugated equine estrogens alone (0.625 mg/d) or placebo with a median intervention duration of 7.2 years.

MAIN OUTCOMES AND MEASURES—Time-specific invasive breast cancer incidence rates and exploratory analyses of breast cancer characteristics by intervention and post-intervention phases in the 2 HT trials.

RESULTS—In the E + P trial, hazard ratios (HRs) for the influence of combined HT on breast

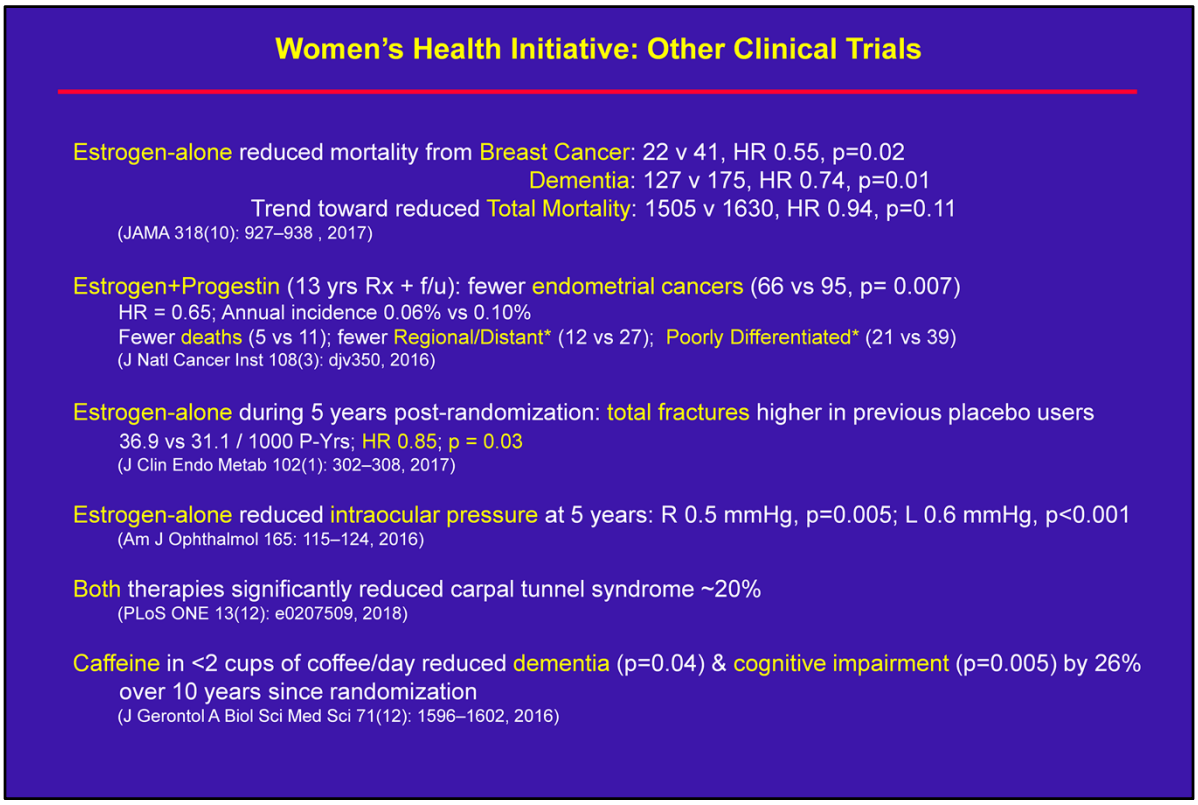
cancer were lower than 1 for 2 years (HR, 0.71; 95% CI, 0.47–1.08) and steadily increased throughout intervention, becoming significantly increased for the entire intervention phase (HR, 1.24; 95% CI, 1.01–1.53). In the early post-intervention phase (within 2.75 years from intervention), there was a sharp decrease in breast cancer incidence in the combined HT group, though the HR remained higher than 1 (HR, 1.23; 95% CI, 0.90–1.70). During the late post-intervention phase (requiring patient re-consent), the HR for breast cancer risk remained higher than 1 through 5.5 years (median) of additional follow-up (HR, 1.37; 95% CI, 1.06–1.77). In the estrogen alone trial, the HR for invasive breast cancer risk was lower than 1 throughout the intervention phase (HR, 0.79; 95% CI, 0.61–1.02) and remained lower than 1 in the early post-intervention phase (HR, 0.55; 95% CI, 0.34–0.89), but risk reduction was not observed during the late post-intervention follow-up (HR, 1.17; 95% CI, 0.73–1.87). Characteristics of breast cancers diagnosed during early and late post-intervention phases differed in both trials.

CONCLUSIONS AND RELEVANCE—In the E + P trial, the higher breast cancer risk seen during intervention was followed by a substantial drop in risk in the early post-intervention phase, but a higher breast cancer risk remained during the late post-intervention follow-up. In the estrogen alone trial, the lower breast cancer risk seen during intervention was sustained in the early post-intervention phase but was not evident during the late post-intervention follow-up.

Figure 1.
Effects Over Time of Estrogen Plus Progestin on the Incidence of Breast Cancer in the Women's Health Initiative Clinical Trial
Overall hazard ratio (HR) and 95% CI (black line and gray-shaded region, respectively) are shown for the effect of conjugated equine estrogens plus medroxyprogesterone acetate (CEE + MPA) on the risk of invasive breast cancer compared with placebo during the intervention period (left panel) and the overall post-intervention phase (right panel). A reference line (dotted black) at unity corresponds to no differential risk between randomization groups. Hashmarks (bottom of right panel) indicate the early and late post-intervention periods. Time-varying linear HR and 95% CI (orange lines) are also displayed for the intervention period (left panel) and overall post-intervention period (right panel), as well as a time-varying linear HR for the early post-intervention phase (dashed orange line). Biennial HRs and 95%CIs (solid blue lines and blue-shaded regions, respectively) are presented as an alternate description for time-varying risk. The biennial HR (95% CI) were 0.71 (0.47–1.08), 1.36 (0.95–1.94), 1.65 (1.17–2.32) during the intervention, and 1.29 (0.88–1.88), 1.18 (0.80–1.74), 1.36 (0.91–2.02), and 1.49 (0.96–2.33) for the postintervention phase. Significance tests of the time-varying linear HR for the primary (adherence adjusted) analysis were conducted and yielded P = .008 (.007) for linear trend during the intervention; P = .28 (.04) for linear trend during the early postintervention phase; P = .07 (.006) for difference between linear trends of intervention and early postintervention phase; P = .86 (.65) for linear trend during the overall postintervention phase; and P = .04 (.02) for difference between linear trends of intervention and overall postintervention phase. Time-varying linear HR is not shown for the late postintervention phase because significance test results were not suggestive of a trend:

P = .96 (.55) for a linear trend during the late postintervention phase.
a HR, 1.24 (95% CI, 1.01–1.53).
b HR, 1.32 (95% CI, 1.08–1.61).
c HR, 1.23 (95% CI, 0.90–1.70).
d HR, 1.37 (95% CI, 1.06–1.77).

Figure 2.
Overall hazard ratio (HR) and 95% CI (black line and gray-shaded region, respectively) are shown for the effect of conjugated equine estrogens (CEE) alone on the risk of invasive breast cancer compared with placebo during the intervention period (left panel) and the overall post-intervention phase (right panel). A reference line (dotted black) at unity corresponds to no differential risk between randomization groups. Hashmarks (top of right panel) indicate the early and late post-intervention periods. Time-varying linear HR and 95% CI (orange lines) are also displayed for the intervention period (left panel) and overall post-intervention period (right panel), as well as a time-varying linear HR for the early post-intervention phase (dashed orange line). Biennial HRs and 95% CIs (solid blue lines and blue-shaded regions, respectively) are presented as an alternate description for time-varying risk. Significance tests of the time-varying linear HR for the primary (adherence adjusted) analysis were conducted and yielded P = .29 (.97) for linear trend during the intervention; P = .14 (.63) for linear trend during the early post-intervention phase; P = .10 (.64) for difference in these linear trends; P = .20 (.27) for linear trend during the overall post-intervention phase; and P = .61 (.34) for the difference between linear trends of intervention and overall post-intervention phase. Time-varying linear HR is not shown for the late post-intervention phase because significance test results were not suggestive of a trend: P = .62 (.46) for a linear trend during the late post-intervention phase.
a HR, 0.79 (95% CI, 0.61–1.02).
b HR, 0.80 (95% CI, 0.58–1.11).
c HR, 0.55 (95% CI, 0.34–0.89).
d HR, 1.17 (95% CI, 0.73–1.87).



Menopausal Hormone Therapy and Long-term All-Cause and Cause-Specific Mortality - Abstract

Importance—Health outcomes from the Women's Health Initiative Estrogen Plus Progestin and Estrogen-Alone Trials have been reported, but previous publications have generally not focused on all-cause and cause-specific mortality.

Objective—To examine total and cause-specific cumulative mortality, including during the intervention and extended postintervention follow-up, of the 2 Women's Health Initiative hormone therapy trials.

Design, Setting, and Participants—Observational follow-up of US multiethnic postmenopausal women aged 50 to 79 years enrolled in 2 randomized clinical trials between 1993 and 1998 and followed up through December 31,2014.

Interventions—Conjugated equine estrogens (CEE, 0.625 mg/d) plus medroxy progesterone acetate (MPA, 2.5 mg/d) (n = 8506) vs placebo (n = 8102) for 5.6 years (median) or CEE alone (n = 5310) vs placebo (n = 5429) for 7.2 years (median).

Main Outcomes and Measures—All-cause mortality (primary outcome) and cause-specific mortality (cardiovascular disease mortality, cancer mortality, and other major causes of mortality) in the 2 trials pooled and in each trial individually, with prespecified analyses by 10-year age group based on age at time of randomization.

Results—Among 27 347 women who were randomized (baseline mean [SD]age, 63.4 [7.2] years; 80.6% white), mortality follow-up was available for more than 98%. During the cumulative 18-year follow-up, 7489 deaths occurred (1088 deaths during the intervention phase and 6401 deaths during postintervention follow-up). All-cause mortality was 27.1% in the hormone therapy group vs 27.6% in the placebo group (hazard ratio [HR], 0.99 [95% CI, 0.94-1.03]) inthe overall pooled cohort; with CEE plus MPA, the HR was 1.02 (95% CI, 0.96-1.08); and with CEE alone, the HR was 0.94 (95% CI, 0.88-1.01). In the pooled cohort for cardiovascular mortality, the HR was 1.00 (95% CI, 0.92-1.08 [8.9 % with hormone therapy vs 9.0% with placebo]); for total cancer mortality, the HR was 1.03 (95% CI, 0.95-1.12 [8.2% with hormone therapy vs 8.0% with placebo]); and for other causes, the HR was 0.95 (95% CI, 0.88-1.02 [10.0% with hormone therapy vs 10.7% with placebo]), and results did not differ significantly between trials. When examined by 10-year age groups comparing younger women (aged 50-59 years) to older women (aged 70-79 years) in the pooled cohort, the ratio of nominal HRs for all-cause mortality was 0.61 (95% CI, 0.43-0.87) during the intervention phase and the ratio was 0.87 (95% CI, 0.76-1.00) during cumulative 18-year follow-up, without significant heterogeneity between trials.

Conclusions and Relevance—Among postmenopausal women, hormone therapy with CEE plus MPA for a median of 5.6 years or with CEE alone for a median of 7.2 years was not associated with risk of all-cause, cardiovascular, or cancer mortality during a cumulative follow-up of 18 years.

Continuous Combined Estrogen Plus Progestin and Endometrial Cancer - Abstract
Background: While progestin addition to estrogen mitigates endometrial cancer risk, the magnitude of the effect on incidence, specific endometrial cancer histologies, and endometrial cancer mortality remains unsettled. These issues were assessed by analyses after extended follow-up of the Women's Health Initiative (WHI) randomized clinical trial evaluating
continuous combined estrogen plus progestin use.

Methods: The WHI enrolled 16 608 postmenopausal women into a randomly assigned, double-blind, placebo-controlled trial. Women age 50 to 79 years with intact uteri with normal endometrial biopsy at entry were randomly assigned to once daily 0.625 mg conjugated equine estrogen plus 2.5 mg medroxyprogesterone acetate (n = 8506) as a single pill or matching
placebo (n = 8102). Follow-up beyond the original trial completion date required reconsent, obtained from 12 788 (83%) of surviving participants. Analyses were by intent-to-treat. All statistical tests were two-sided.

Results: After 5.6 years' median intervention and 13 years' median cumulative follow-up, there were fewer endometrial cancers in the combined hormone therapy compared with the placebo group (66 vs 95 case patients, yearly incidence, 0.06% vs 0.10%; hazard ratio [HR] = 0.65, 95% confidence interval [CI] = 0.48 to 0.89, *P* = .007). While there were somewhat

fewer endometrial cancers during intervention (25 vs 30, respectively; HR = 0.77, 95% CI = 0.45 to 1.31), the difference became statistically significant postintervention (41 vs 65, respectively; HR = 0.59, 95% CI = 0.40 to 0.88, *P* = .008), but hazard ratios did not differ between phases (*P*difference = .46). There was a statistically nonsignificant reduction in deaths from endometrial cancer in the estrogen plus progestin group (5 vs 11 deaths, HR = 0.42, 95% CI = 0.15 to 1.22).

Conclusion: In postmenopausal women, continuous combined estrogen plus progestin decreases endometrial cancer incidence.

No Increase in Fractures After Stopping Hormone Therapy - Abstract

Context: The Women's Health Initiative (WHI) hormone therapy (HT) trials showed protection against hip and total fractures, but a later observational report suggested loss of benefit and a rebound increased risk after cessation of HT.

Objective: The purpose of this study was to examine fractures after discontinuation of HT.

Design and Setting: Two placebo-controlled randomized trials served as the study setting.

Patients: Study patients included WHI participants (N = 15,187) who continued active HT or placebo through the intervention period and who did not take HT in the postintervention period.

Interventions: Trial interventions included conjugated equine estrogen (CEE) plus medroxyprogesterone acetate (MPA) in naturally menopausal women and CEE alone in women with prior hysterectomy.

Main Outcome Measures: Total fractures and hip fractures through 5 years after discontinuation of HT were recorded.

Results: Hip fractures were infrequent (~2.5 per 1000 person-years); this finding was similar between trials and in former HT and placebo groups. There was no difference in total fractures in the CEE + MPA trial for former HT vs former placebo users (28.9 per 1000 person-years and 29.9 per 1000 person-years, respectively; hazard ratio [HR], 0.97; 95% confidence interval [CI], 0.87 to 1.09; *P* = 0.63); however, in the CEE-alone trial, total fractures were higher in former placebo users (36.9 per 1000 person-years) compared with the former active group (31.1 per 1000 person-years), a finding that was suggestive of a residual benefit of CEE against total fractures (HR, 0.85; 95% CI, 0.73 to 0.98; *P* = 0.03).

Conclusions: We found no evidence for increased fracture risk, either sustained or transient, for former HT users compared with former placebo users after stopping HT. There was residual benefit for total fractures in former HT users from the CEE-alone study.

Effects of Hormone Therapy on Intraocular Pressure - Abstract

Purpose—Previous studies suggest that hormone therapy favorably affects intraocular pressure (IOP). Here, we examined the association between hormone therapy use and IOP in the context of a large randomized trial.

Design—Secondary data analysis from a randomized-control trial

Methods—We used data from the Women's Health Initiative-Sight Exam (WHISE). Women with prior hysterectomy received oral conjugated equine estrogen (0.625 mg/day) or placebo. Women with a uterus received estrogen plus progestin (medroxyprogesterone acetate 2.5 mg/day) or placebo. IOP was measured five years after randomization. Adjusted linear regression models were used to assess the association between hormone therapy and IOP.

Results—The WHISE included 1,668 women in the estrogen-alone trial (aged 63–86, mean 72 years) and 2,679 women in the estrogen-plus-progestin trial (aged 63–87, mean 72 years). In multivariate analyses, compared to placebo treatment, treatment with estrogen alone was associated with a 0.5-mmHg reduction of the IOP in the right eye (95% CI; −0.8, −0.1, $p = 0.005$) and a 0.6 mmHg (95% CI; −0.9, −0.3, $p < 0.001$) reduction of the IOP in the left eye. In the estrogen-plus-progestin trial, there was no significant difference in IOP between the treatment and placebo groups ($p = 0.30$ right eye and $p = 0.43$ left eye).

Conclusions—This study represents an IOP analysis in the largest hormone trial available. Estrogen-alone therapy in postmenopausal women is associated with a small but significant IOP reduction of 0.5 mmHg. The clinical significance of this small decrease remains to be determined.

Menopausal hormone therapy and the incidence of carpal tunnel syndrome in postmenopausal women - Abstract

Importance - Carpal tunnel syndrome (CTS) is a common and debilitating condition that commonly affects postmenopausal women.

Objective - To determine the effect of menopausal hormone therapy (HT) in healthy postmenopausal women on CTS risk.

Design - We conducted a secondary analysis of the Women's Health Initiative's (WHI) HT trials linked to Medicare claims data. Separate intention-to-treat analyses were performed for the two trials; the conjugated equine estrogens alone (CEE alone) and the trial of CEE plus medroxyprogesterone acetate (MPA) trial. (ClinicalTrials.gov, NCT **number**): NCT00000611.

Setting - Two randomized, double-blind, placebo-controlled trials conducted at 40 US clinical centers.

Participants - The sample size included in the analysis was 16,053 community-dwelling women aged ~65 years at study entry or those who later aged into Medicare eligibility, and who were enrolled in Medicare (including Part A and/or Part B coverage).

Intervention - Women with hysterectomy were randomized to 0.625 mg/d of conjugated equine estrogens (CEE) or placebo (n = 8376). Women without hysterectomy were randomized to estrogen plus progestin (E+P), given as CEE plus 2.5 mg/d of medroxyprogesterone acetate (n = 14203).

Main outcome(s) - The primary outcome was incident CTS and the secondary outcome was therapeutic CTS procedure occurring during the intervention phases of the trials.

Results - A total of 16,053 women were randomized in both trials. During mean follow up of 4.5 ± 2.8 years in the CEE trial (n = 6,833), there were 203 incident CTS cases in the intervention and 262 incident CTS cases in the placebo group (HR, 0.78; 95% CI, 0.65–0.94; P = 0.009). The CEE+MPA trial (n = 9,220) followed participants for a mean of 3.7 ± 2.3 years. There were 173 incident CTS cases in the intervention compared to 203 cases in the placebo group (HR, 0.80, 95% CI, 0.65–0.97; P = 0.027).

Conclusions - These findings suggest a protective effect of menopausal HT on the incidence of CTS among postmenopausal women. A potential therapeutic role for other forms of estrogen
therapy in the management of CTS warrants future research.

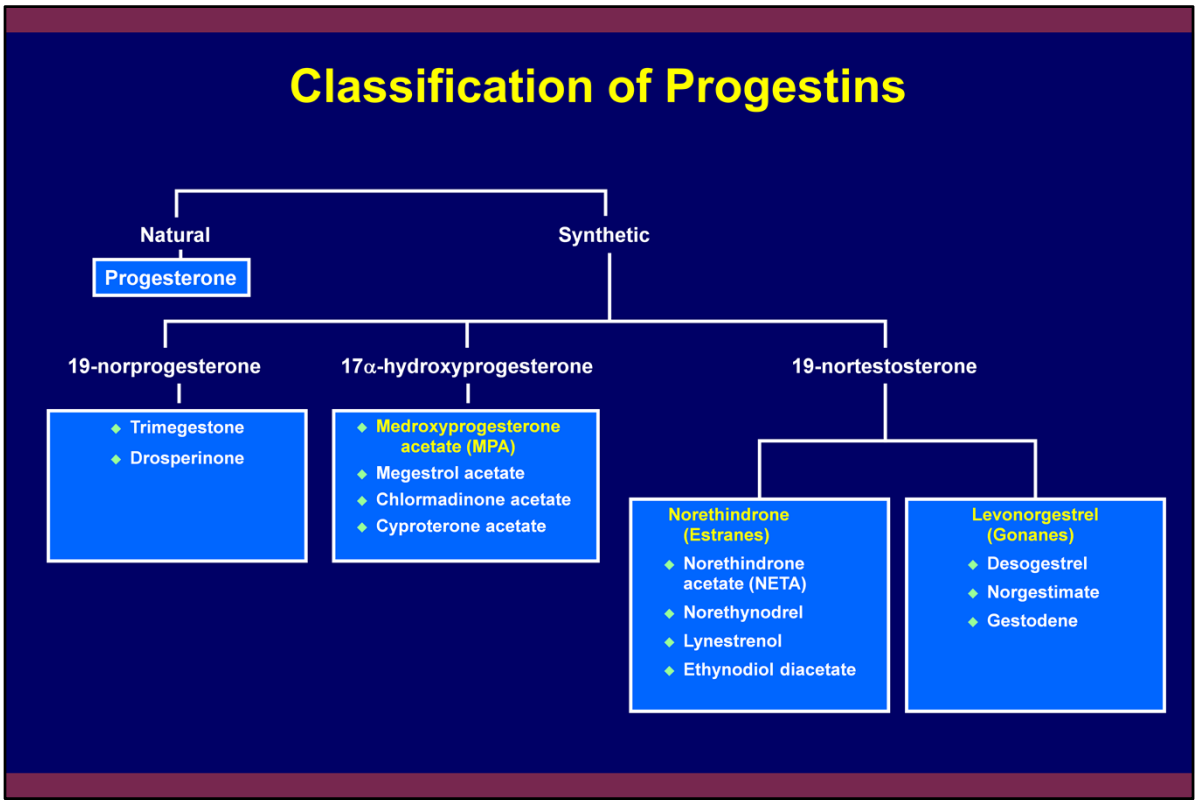
Relationships Between Caffeine Intake and Risk for Probable Dementia or Global Cognitive Impairment – Abstract

Background: Nonhuman studies suggest a protective effect of caffeine on cognition. Although human literature remains less consistent, reviews suggest a possible favorable relationship between caffeine consumption and cognitive impairment or dementia. We investigated the relationship between caffeine intake and incidence of cognitive impairment or probable dementia in women aged 65 and older from the Women's Health Initiative Memory Study.

Methods: All women with self-reported caffeine consumption at enrollment were included (*N* = 6,467). In 10 years or less of follow-up with annual assessments of cognitive function, 388 of these women received a diagnosis of probable dementia based on a 4-phase protocol that included central adjudication. We used proportional hazards regression to assess differences in the distributions of times until incidence of probable dementia or composite cognitive impairment among women grouped by baseline level of caffeine intake, adjusting for risk factors (hormone therapy, age, race, education, body mass index, sleep quality, depression, hypertension, prior cardiovascular disease, diabetes, smoking, and alcohol consumption).

Results: Women consuming above median levels (mean intake = 261 mg) of caffeine intake for this group were less likely to develop incident dementia (hazard ratio = 0.74, 95% confidence interval [0.56, 0.99], p = .04) or any cognitive impairment (hazard ratio = 0.74, confidence interval [0.60, 0.91], p = .005) compared to those consuming below median amounts (mean intake = 64 mg) of caffeine for this group.

Conclusion: Our findings suggest lower odds of probable dementia or cognitive impairment in older women whose caffeine consumption was above median for this group and are consistent with the existing literature showing an inverse association between caffeine intake and age-related cognitive impairment.



In addition to estrogen, any discussion of ovarian hormone action requires careful consideration of progestins. Although progestin action likely is as complex as estrogen action, to date there is less known regarding progesterone receptor structure-function relationships.

Progestins can be classified into natural (ie, progesterone) and synthetic progestins.[1-6] Synthetic progestins are structurally related to either progesterone or testosterone as depicted in the chart on this slide. As with the ER, the progesterone receptors (PRs) react with either synthetic or natural progestins the same way.

The classification that has been adopted for progestins is not based on their biological activity, but rather reflects the method of synthesis for the compounds.

A complete list of references for this slide can be found in the accompanying document titled “Ovarian Hormone Action References.”

Risk of Breast Cancer by Type of Menopausal Hormone Therapy: a Case-Control Study among Post-Menopausal Women in France (PLOS ONE 8(11):e78016, 2013)

Menopausal women: 1,232 cases compared to 1,317 controls without cancer
Risk increased by benign breast disease, Fam Hx, early menarche, low parity, late preg

Table 2. Odds ratios for breast cancer by type of menopausal hormone therapy and duration of use in current and past users.

	Current users				Past users			
Duration of MHT use	**Cases**	**Controls**	**OR[a]**	**95% CI**	**Cases**	**Controls**	**OR[a]**	**95% CI**
Never	311	357	1	ref	311	357	1	ref
Estrogen-only therapy								
Any duration	34	31	1.19	[0.69-2.04]	72	93	0.83	[0.57-1.21]
< 4 years	14	10	1.58	[0.67-3.75]	26	32	0.90	[0.51-1.59]
≥ 4 years	20	20	1.01	[0.51-2.02]	39	53	0.77	[0.48-1.24]
Combined EP therapy								
Any duration	92	82	1.33	[0.92-1.92]	133	171	0.78	[0.57-1.05]
< 4 years	17	26	0.86	[0.43-1.73]	25	38	0.65	[0.37-1.14]
≥ 4 years	73	56	**1.55**	**[1.02-2.36]**	101	129	0.80	[0.57-1.12]
Tibolone								
Any duration	17	8	2.42	[0.96-6.10]	10	15	0.55	[0.22-1.36]
< 4 years	7	5	2.04	[0.59-7.07]	5	11	0.46	[0.15-1.42]
≥ 4 years	10	3	3.09	[0.79-12.0]	4	4	0.66	[0.13-3.26]

a. Odds Ratios adjusted for Study area / Age at reference date/ Age at menarche / Parity / Age at first full-term pregnancy / Breast feeding /History of benign breast disease / Family history of breast cancer in first-degree relatives / BMI / Oral contraceptive use

doi: 10.1371/journal.pone.0078016.t002

Background: There is extensive epidemiological evidence that menopausal hormone therapy (MHT) increases breast cancer risk, particularly combinations of estrogen and progestagen (EP). We investigated the effects of the specific formulations and types of therapies used by French women. Progestagen constituents, regimen (continuous or sequential treatment by the progestagen), and time interval between onset of menopause and start of MHT were examined.

Methods: We conducted a population-based case-control study in France in 1555 menopausal women (739 cases and 816 controls). Detailed information on MHT use was obtained during in-person interviews. Odds ratios and 95% confidence interval adjusted for breast cancer risk factors were calculated.

Results: We found that breast cancer risk differed by type of progestagen among current users of EP therapies. No increased risk was apparent among EP therapy users treated with natural micronized progesterone. Among users of EP therapy containing a synthetic progestin, the odds ratio was 1.57 (0.99-2.49) for progesterone-derived and 3.35 (1.07-10.4) for testosterone-derived progestagen. Women with continuous regimen were at greater risk than women treated sequentially, but regimen and type of progestagen could not be investigated independently, as almost all EP combinations containing a testosterone-derivative were administered continuously and vice-versa. Tibolone was also associated with an increased risk of breast cancer. Early users of MHT after onset of menopause were at greater risk than users who delayed treatment.

Conclusion: This study confirms differential effects on breast cancer risk of progestagens and regimens specifically used in France. Formulation of EP therapies containing natural progesterone, frequently prescribed in France, was not associated with increased risk of breast cancer but may poorly protect against endometrial cancer.

Risk of Breast Cancer by Type of Menopausal Hormone Therapy: a Case-Control Study among Post-Menopausal Women in France (PLOS ONE 8(11):e78016, 2013)

Menopausal women: 1,232 cases compared to 1,317 controls without cancer
Risk increased by benign breast disease, Fam Hx, early menarche, low parity, late preg

Table 3. Odds ratios for breast cancer among current users of combined MHT by type of treatment and duration of use.

	Any duration				Duration < 4 years				Duration ≥ 4 years			
	Cases	Controls	OR[a]	95% CI	Cases	Controls	OR[a]	95% CI	Cases	Controls	OR[a]	95% CI
Never MHT use	311	357	1	ref	311	357	1	ref	311	357	1	ref
Estrogen + natural progesterone	25	34	0.80	[0.44-1.43]	10	17	0.69	[0.29-1.68]	14	17	0.79	[0.37-1.71]
Estrogen + synthetic progestagen	67	48	**1.72**	**[1.11-2.65]**	11	14	1.17	[0.48-2.86]	55	34	**2.07**	**[1.26-3.39]**
By type of synthetic progestagen												
Estrogen + Progesterone Der.	55	43	**1.57**	**[0.99-2.49]**	10	13	1.02	[0.40-2.58]	45	30	**1.92**	**[1.13-3.27]**
Estrogen + Testosterone Der.	11	5	**3.35**	**[1.07-10.4]**	4	4	1.64	[0.38-7.15]	7	1	**9.47**	**[1.09-82.6]**
By regimen												
Continuous	9	5	2.52	[0.77-8.32]	3	2	2.41	[0.36-16.1]	6	3	2.70	[0.60-12.2]
Sequential	56	40	**1.75**	**[1.09-2.79]**	11	10	1.40	[0.54-3.65]	45	30	**2.00**	**[1.18-3.41]**

a. Odds Ratios adjusted for Study area/ Age at reference date / Age at menarche / Parity / Age at first full-term pregnancy / Breast feeding /History of benign Breast disease / Family history of breast cancer in first-degree relatives / BMI / Oral contraceptive use

doi: 10.1371/journal.pone.0078016.t003

Results similar for all receptor classes
Numbers relatively small
No randomized, controlled studies comparing progestins

Testosterone Derivatives:
Norethindrone
Levonorgestrel

HABITS (hormonal replacement therapy after breast cancer—is it safe?), a randomized comparison (*Lancet* 363:453–55, 2004)

Estimated 5-year relapse rate: 20%
Goal: **1,300** women → **345** analyzed
Completed therapy w/o recurrence
Up to Stage II (<4 positive nodes)
72% E+P 21% E-alone 2.1 yrs

	HRT	no HRT
Death:	5	4
Cancer Death:	3	4
Local Recur:	16	3
Distant Mets:	10	5
Serious Events:	8	4
Rapid Progress:	2	2
Pulm Embol:	1	1
DVT:	1	1
Lung Cancer:	1	0
Endomet Ca:	1	0
Suicide:	1	0
Thrombophleb:	1	0

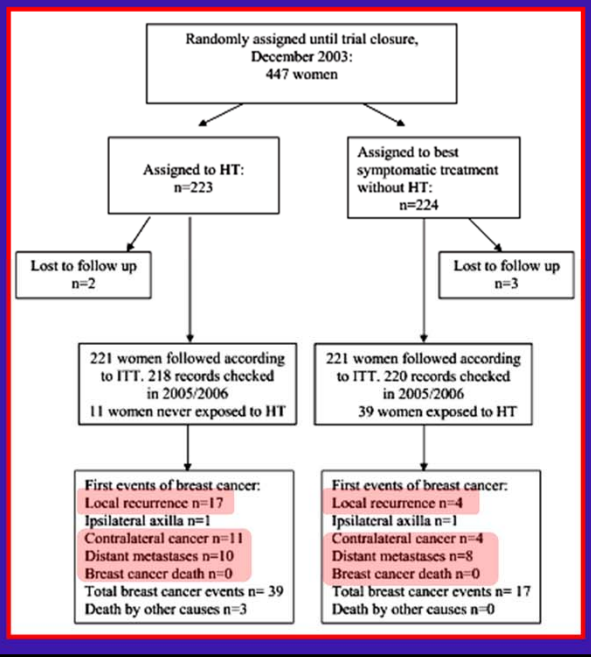
	HRT (219 randomised)	No HRT (215 randomised)
Number with follow-up	174	171
Median follow-up in years (range)	2·1 (0·1–5·3)	2·1 (0·1–5·6)
Median time in years between primary treatment and randomisation (range)	2·6 (0·1–23·3)	2·7 (0·2–26·5)
Median number of follow-up reports (range)	3 (1–15)	3 (1–12)
Mean age in years (range)	55·5 (42–75)	55·0 (40–74)
Node-positive*	38 of 147 (26%)	31 of 145 (21%)
Hormone-receptor positive*	86 of 154 (56%)	73 of 151 (48%)
Hormone receptor status unknown*	41 of 154 (27%)	33 of 151 (22%)
Breast preserved*	100 of 162 (62%)	96 of 167 (57%)
On HRT before diagnosis*	86 of 165 (52%)	91 of 163 (56%)
On adjuvant tamoxifen*	36 of 174 (21%)	36 of 171 (21%)

*Data are number (%).

	RH (95% CI)	Number of events (number of women in subset)
All women	3·5 (1·5–8·1)	33 (345)
All women, adjusted	3·5 (1·5–8·1)	33 (345)
Hormone-receptor positive	4·8 (1·1–21·4)	14 (159)
Hormone-receptor negative	1·9 (0·4–9·6)	6 (72)
Tamoxifen	2·8 (0·3–27·4)	4 (72)
No tamoxifen	3·7 (1·5–9·0)	29 (273)
HRT before diagnosis	6·9 (1·6–31·1)	14 (177)
No HRT before diagnosis	2·1 (0·8–5·9)	19 (168)

In the 1990s, two randomised clinical trials started in Scandinavia addressing whether hormone replacement therapy (HRT) is safe for women with previous breast cancer. We report the findings of the safety analysis in HABITS (hormonal replacement therapy after breast cancer—is it safe?), an open randomised clinical trial with allocation to either HRT or best treatment without hormones. The main endpoint was any new breast cancer event. All analyses were done according to intention-to-treat. Until September, 2003, 434 women were randomised; 345 had at least one follow-up report. After a median follow-up of 2·1 years, 26 women in the HRT group and seven in the non-HRT group had a new breast-cancer event. All women with an event in the HRT group and two of those in the non-HRT group were exposed to HRT and most women had their event when on treatment. We decided that these findings indicated an unacceptable risk for women exposed to HRT in the HABITS trial, and the trial was terminated on Dec 17, 2003.

Increased Risk of Recurrence After Hormone Replacement Therapy in Breast Cancer Survivors (HABITS: 4 yr F/U) (J Natl Cancer Inst 100:475–482, 2008)



Background Hormone replacement therapy (HT) is known to increase the risk of breast cancer in healthy women, but its effect on breast cancer risk in breast cancer survivors is less clear. The randomized HABITS study, which compared HT for menopausal symptoms with best management without hormones among women with previously treated breast cancer, was stopped early due to suspicions of an increased risk of new breast cancer events following HT. We present results after extended follow-up.

Methods HABITS was a randomized, non – placebo-controlled noninferiority trial that aimed to be at a power of 80% to detect a 36% increase in the hazard ratio (HR) for a new breast cancer event following HT. Cox models were used to estimate relative risks of a breast cancer event, the maximum likelihood method was used to calculate 95% confidence intervals (CIs), and 2 tests were used to assess statistical significance, with all *P* values based on two-sided tests. The absolute risk of a new breast cancer event was estimated with
the cumulative incidence function. Most patients who received HT were prescribed continuous combined or sequential estradiol hemihydrate and norethisterone.

Results Of the 447 women randomly assigned, 442 could be followed for a median of 4 years. Thirty-nine of the 221 women in the HT arm and 17 of the 221 women in the control arm experienced a new breast cancer event (HR = 2.4, 95% CI = 1.3 to 4.2). Cumulative incidences at 5 years were 22.2% in the HT arm and 8.0% in the control arm. By the end of follow-up, six women in the HT arm had died of breast

cancer and six were alive with distant metastases. In the control arm, five women had died of breast cancer and four had metastatic breast cancer (*P* = .51, log-rank test).

Conclusion After extended follow-up, there was a clinically and statistically significant increased risk of a new breast cancer event in survivors who took HT.
J Natl Cancer Inst 2008;100: 475 – 482

Menopausal Hormone Therapy After Breast Cancer: The Stockholm Randomized Trial (J Natl Cancer Inst 97:533–535, 2005)

Terminated because of HABITS
Follow-up longer
Time from diagnosis shorter
More treated with tamoxifen (52% v 21%)

Used Medroxyprogesterone spacing:
20 mg for 14 days q3 months (50%)
23% Estrogen-alone
22% MP monthly (10 mg for 10 days)

	HRT	non-HRT
Deaths:	4	9
Breast Ca Death:	2	4

Discussion:
Breast cell proliferation during luteal phase
More proliferation during Combined Rx
than Estrogen-alone (monkeys)

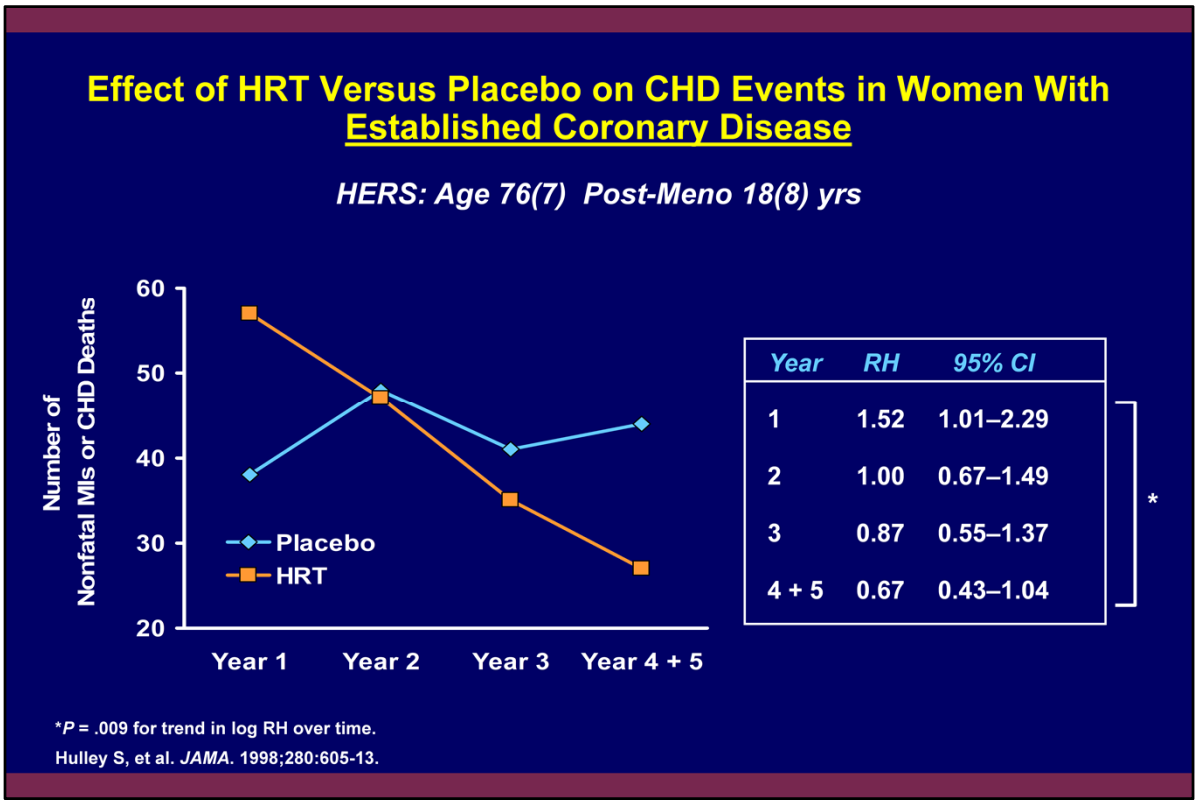
Table 1. Clinical characteristics of patients with available follow-up information in the Stockholm trial

	HT*	No HT
Total No. randomly assigned	188	190
No. with follow-up available	175	184
Median follow-up, y (range)	4.1 (0.2–7)	4.2 (0.3–6)
Time from primary breast cancer diagnosis to randomization, y (range)	1.3 (0.1–16)	1.4 (0.1–20)
Median age, y (range)	56.9 (42–69)	57.5 (44–70)
Lymph node–positive disease, No./No. tested (%)	28/172 (16)	37/183 (20)
Estrogen receptor–positive disease, No./No. tested (%)	113/175 (65)	103/184 (56)
Estrogen receptor status unavailable/unknown, No./No. tested (%)	40/175 (23)	54/184 (29)
Breast-conserving surgery, No./No. tested (%)	123/175 (70)	135/184 (73)
HT before diagnosis, No./No. tested (%)	132/174 (76)	129/177 (73)
Concomitant adjuvant tamoxifen, No./No. tested (%)	91/175 (52)	98/184 (53)

Table 2. Clinical data on the 24 patients with breast cancer recurrence (median follow-up = 4.1 years)

	HT*	No HT
Total No. with recurrence	11	13
Recurrence-free interval†, No.		
<1 y	3	3
1–2 y	2	6
>2 y	6	4
Type of recurrence, No.		
Locoregional	5	5
Distant	3	5
Contralateral breast	3	3
Axillary lymph node status at primary surgery, No.		
Positive	4	5
Unavailable/unknown	2	2
Concomitant tamoxifen, No.	5	4
Estrogen receptor–positive disease, No.	7	8
Previous HT, No.	8	8

In 1997 two independent randomized clinical trials, Hormonal Replacement Therapy After Breast Cancer — Is It Safe? (HABITS; 434 patients) and the Stockholm trial (378 patients), were initiated in Sweden to compare menopausal hormone therapy with no menopausal hormone therapy after diagnosis of early-stage breast cancer. Much of the design of both studies was similar; however, a goal of the Stockholm protocol, not shared with the HABITS trial, was to minimize the use of progestogen combined with estrogen. The HABITS trial was prematurely stopped in December 2003, because, at a median follow-up of 2.1 years, the risk for recurrence of breast cancer among patients receiving menopausal hormone therapy was statistically significantly higher (relative hazard [RH] = 3.3, 95% confidence interval [CI] = 1.5 to 7.4) than among those receiving no treatment. In the Stockholm trial, however, at a median follow-up of 4.1 years, the risk of breast cancer recurrence was not associated with menopausal hormone therapy (RH = 0.82, 95% CI = 0.35 to 1.9). Statistically significant heterogeneity in the rate of recurrence was observed (*P* = .02; two-sided likelihood-ratio test) between the two studies, indicating that chance may not be the only explanation. Doses of estrogen and progestogen and treatment regimens for menopausal hormone therapy may be associated with the recurrence of breast cancer. [J Natl Cancer Inst 2005:97: 533–5]



This graph shows the results from a post hoc analysis of the results from the HERS trial. This post hoc analysis was conducted to explore if there were any time trends in the data which explained the neutral outcome of the study.[1]

A statistically significant (*P* = .009) time trend was noted for decreased CHD risk.

It is noteworthy that the risk hazard for years 4 and 5 represents a 33% reduction in events, which is comparable to the 35% to 50% reduction in risk that has been consistently reported in observational studies of HRT use and CVD risk.

In summary, the results of the HERS trial showed a null overall effect of HRT on risk of CHD events, an increased risk in year 1, and a trend toward a decreased risk in years 4 and 5.

[1]Hulley S, Grady D, Bush T, et al. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. *JAMA*. 1998;280:605-613.

Estrogen Effects on Atherogenesis

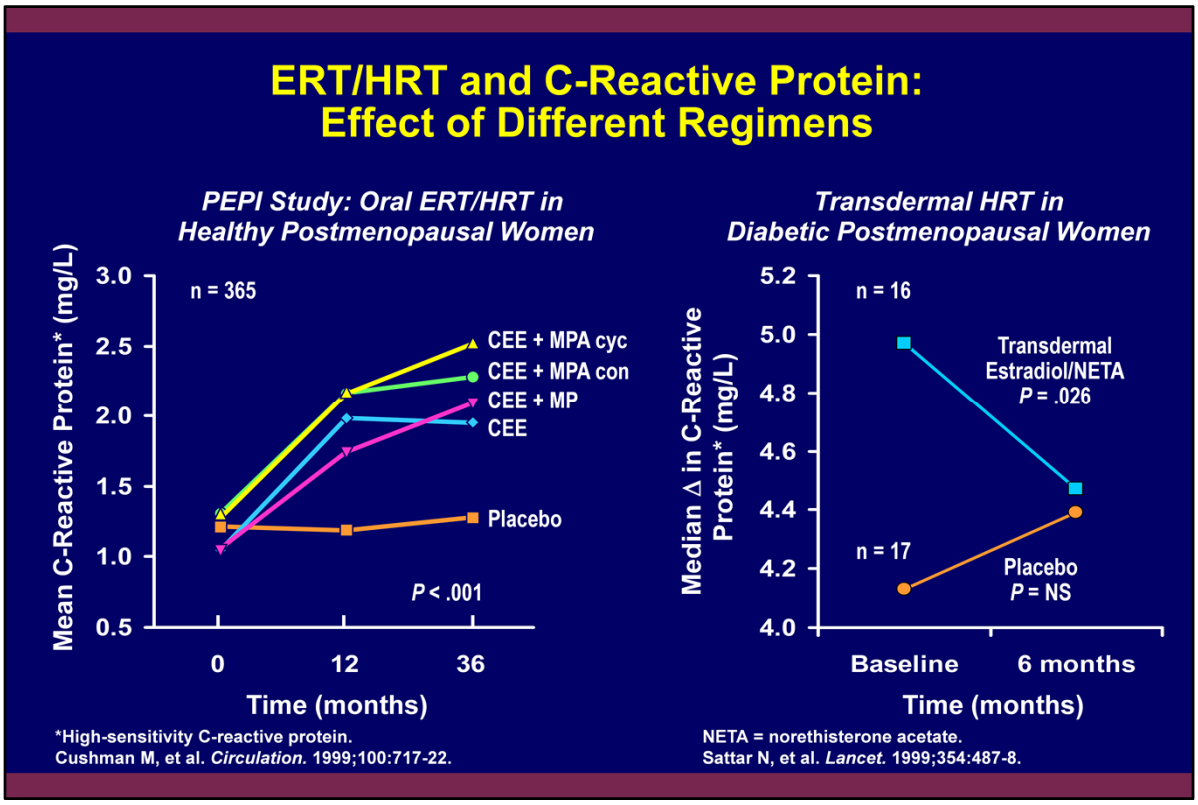
Inhibit:

Lipoprotein penetration through endothelium
Lipoprotein oxidation
IL-1, TNFa, endothelin, & AT1 receptors
Adhesion molecules (ICAM, VCAM, E-selectin)
& inflammatory cell invasion
LDL uptake by macrophages (foam cells)
Smooth muscle cell proliferation and migration
Tissue factor, Factor VII (?), fibrinogen, PAI-1, thromboxane, Lp(a),
(AT-III, Protein S)

Stimulate (oral):

Nitric Oxide & hsCRP
Protein C, prostacyclin, t-PA

High dose estrogen causes venous thromboses



Oral administration of estrogen has been shown to increase levels of high-sensitivity CRP. CRP is an acute phase protein that generally indicates the presence of inflammation. However, it is questionable whether the estrogen-induced increase in CRP reflects an inflammatory response or a first-pass liver effect because estrogen is known to *decrease* the levels of a number of other inflammatory markers including IL-6, the cytokine MCP-1, and certain CAMs (E-selectin, P-selectin, VCAM-1, and ICAM-1). In inflammation, the liver production of CRP is induced by cytokines such as IL-6.

The data on this slide provide further information to question whether the estrogen-induced increase in CRP reflects an inflammatory response. These data show that while oral administration of HRT increases CRP levels, transdermal administration does not. These contrasting findings suggest that increased CRP levels observed with oral HRT may be due to first-pass liver metabolism, rather than an inflammatory effect.

The graph on the left shows that oral administration of CEE alone (blue line), or CEE with cyclical or continuous progestins to healthy postmenopausal women resulted in an increase in CRP values at 1 year. This increase persisted throughout the remainder of the study.[1]

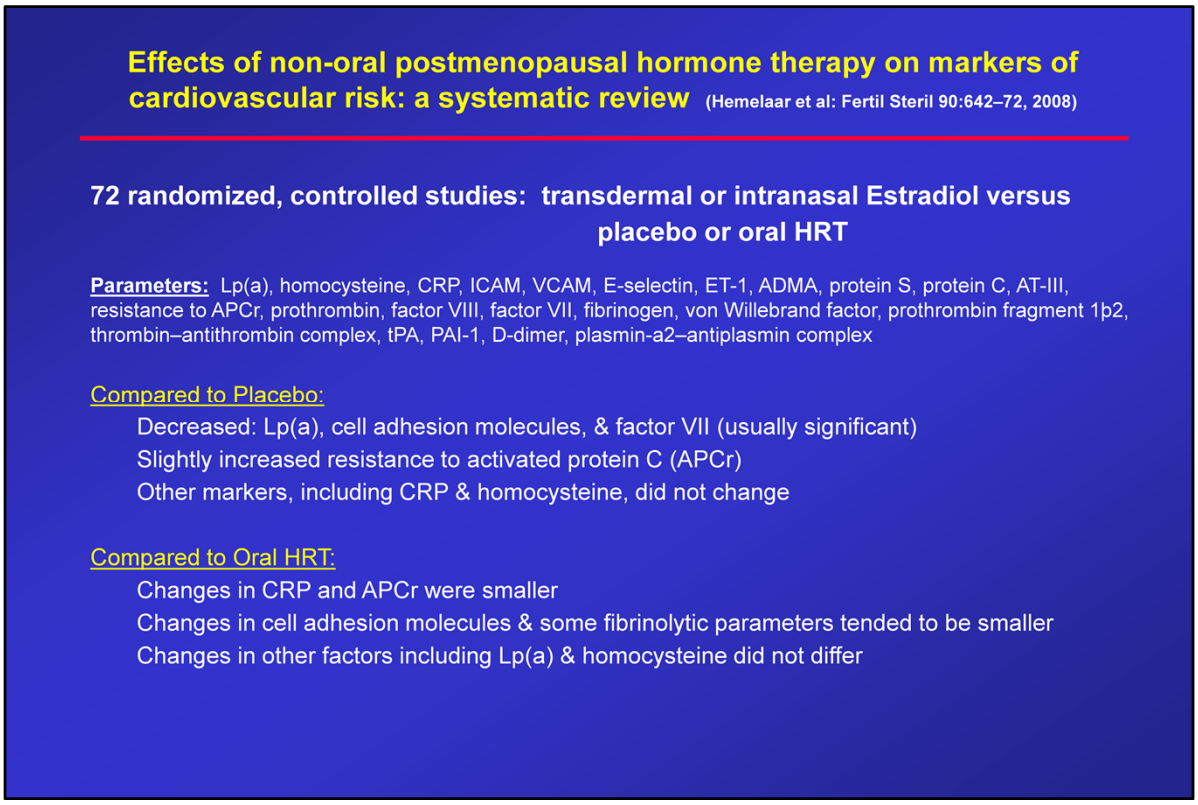
The graph on the right is from a double-blind, parallel group study by Sattar et al[2] that evaluated the effect of transdermal HRT on CRP. The study included 33 postmenopausal women with type 2 diabetes (mean age, 62 years). As reported in other studies, the baseline values of CRP in the diabetic women were higher (by almost four-fold) than those of the healthy PEPI subjects.

These data show that transdermal delivery of estradiol (80 µg) with continuous norethisterone acetate (1 mg) significantly reduced CRP levels by about 10% within 6 months in the diabetic women.[2]

In summary, the effect of oral HRT on CRP levels appears to be a first-pass liver effect rather than a sign of inflammation.

[1]Cushman M, Legault C, Barrett-Connor E, et al. Effect of postmenopausal hormones on inflammation-sensitive proteins. The Postmenopausal Estrogen/Progestin Interventions (PEPI) Study. *Circulation.* 1999;100:717-722.

[2]Sattar N, Perera M, Small M, Lumsden M-A. Hormone replacement therapy and sensitive C-reactive protein concentrations in women with type-2 diabetes [letter]. *Lancet.* 1999;354:487-488.



Objective: To review the effects of non-oral administration of postmenopausal hormone therapy (HT) on risk markers for atherosclerotic and venous thromboembolic disease.Non-oral postmenopausal HT appears not to increase venous thromboembolic risk, whereas the effect on coronary heart disease risk is less clear.

Design: Systematic review of literature obtained from MEDLINE, EMBASE, and CENTRAL databases from 1980 until and including April 2006. Terms for ‘‘postmenopausal hormone therapy’’ and for ‘‘non-oral administration’’ were combined in the search.

Setting: Randomized clinical trials.

Patient(s): Postmenopausal women, both healthy and with established cardiovascular disease or specified cardiovascular risk factors

Intervention(s): Non-oral HT (e.g., transdermal or intranasal) compared with oral HT or no treatment/placebo.

Main Outcome Measure(s): Lipoprotein(a), homocysteine, C-reactive protein (CRP), cell adhesion molecules, markers of endothelial dysfunction, coagulation, and fibrinolysis.

Result(s): Seventy-two studies investigating either transdermal or intranasal administration were included. For non-oral HT, decreases in lipoprotein(a), cell adhesion molecules, and factor VII generally were significant, resistance to activated protein C (APCr) was slightly increased, and other markers including CRP and homocysteine did not change. Compared with oral HT, changes in CRP and APCr were smaller, changes in cell adhesion molecules and some fibrinolytic parameters tended to be smaller, whereas changes in other factors including lipoprotein(a) and homocysteine did not differ.

Conclusion(s): Potentially unfavorable changes seen with oral HT on two important markers, CRP and APCr, are substantially smaller with non-oral HT. Non-oral HT has minor effects on the other cardiovascular risk markers studied. Therefore, compared with oral HT, non-oral HT appears be safer with respect to atherosclerotic and venous thromboembolic disease risk. (Fertil Steril 2008;90:642–72. 2008 by American Society for Reproductive Medicine.)

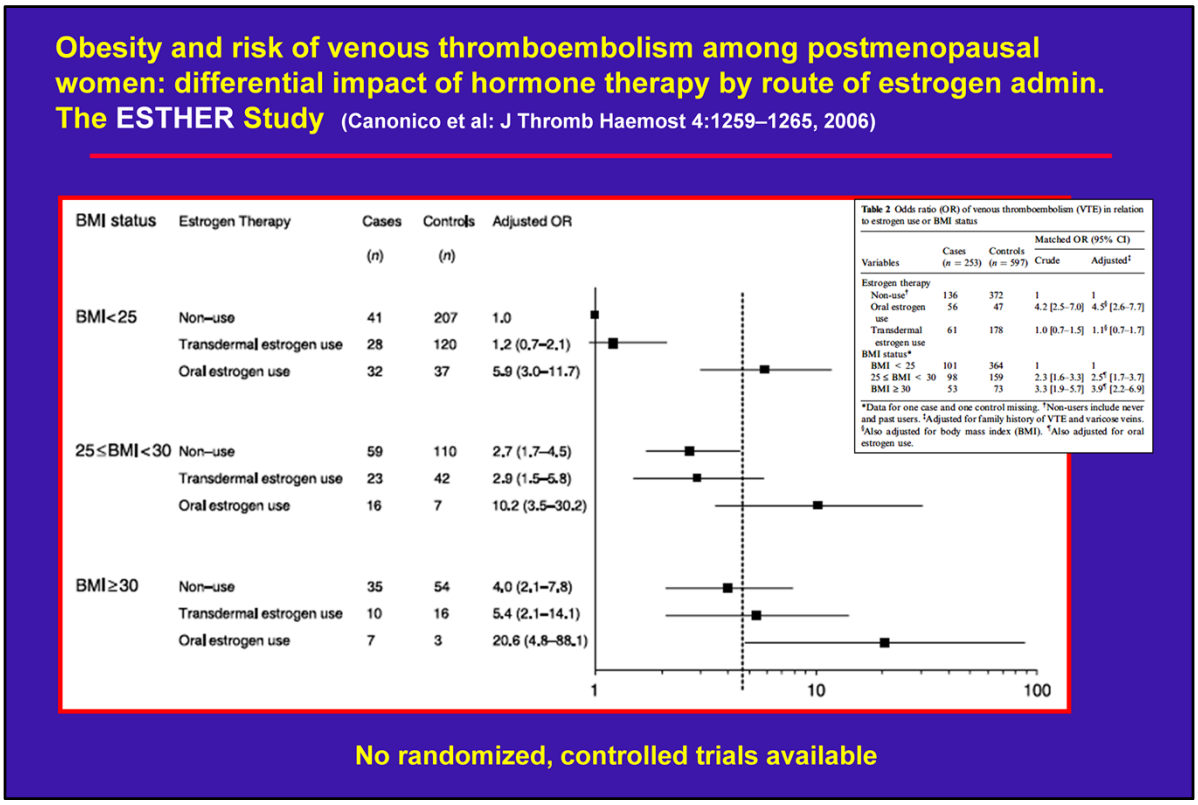


Fig. 1. Risk of venous thromboembolism (VTE) in relation to body mass index by route of estrogen administration. Values are ORs (95%CI) adjusted for family history of VTE and varicose veins. Dotted vertical line indicates the OR of VTE associated with oral estrogen use in the whole population (odds ratio ¼ 4.5; 95% CI: 2.6–7.7).

Background: Oral estrogen use and elevated body mass index (BMI) increase the risk of venous thromboembolism (VTE). Recent data suggest that transdermal estrogen might be
safe with respect to thrombotic risk. However, the impact of transdermal estrogen on the association between overweight (25 kg m)2 < BMI £ 30 kg m)2) or obesity (BMI >30 kg m)2) and VTE risk has not been investigated.

Methods: We carried a multicenter case–control study of VTE among postmenopausal women aged 45–70 years, between 1999 and 2005, in France. Case population consisted of women with a first documented idiopathic VTE. We recruited 191 hospital cases matched with 416 hospital controls and 62 outpatient cases matched with 181 community controls. **Results:** The odds ratio (OR) for VTE was 2.5 [95% confidence interval (CI):1.7–3.7] for overweight and 3.9 (95% CI: 2.2–6.9) for obesity. Oral, not transdermal, estrogen was associated with an increased VTE risk (OR = 4.5; 95% CI: 2.6–7.7 and OR = 1.1; 95% CI: 0.7–1.7, respectively). Compared with non-users with normal weight, the combination of oral

estrogen use and overweight or obesity further enhanced VTE risk (OR = 10.2; 95% CI: 3.5–30.2 and OR = 20.6;95%CI: 4.8–88.1, respectively). However, transdermal users with increased BMI had similar risk as nonusers with increased BMI (OR = 2.9; 95% CI: 1.5–5.8 and OR = 2.7; 95% CI: 1.7–4.5 respectively for overweight; OR ¼ 5.4; 95% CI: 2.1–14.1 and OR = 4.0; 95% CI: 2.1–7.8 respectively for obesity).

Conclusions: In contrast to oral estrogen, transdermal estrogen does not confer an additional risk of idiopathic VTE in women with increased BMI. The safety of transdermal estrogen on thrombotic risk has to be confirmed.

Hormone Therapy and Venous Thromboembolism Among Postmenopausal Women - Impact of the Route of Estrogen Administration and Progestogens: The ESTHER Study (Canonico et al: *Circulation* 115:840-845, 2007)

TABLE 2. Impact of Hormone Therapy on VTE Risk by Route of Estrogen Administration and Type of Progestogens

	Cases (n=259)	Controls (n=603)	Matched OR (95% CI) Crude	Adjustment 1	Adjustment 2
Nonuse	146	384	1	1	1
Oral estrogen use	45	39	3.6 (1.5–8.8)	4.0 (1.6–10.1)	4.2 (1.5–11.6)
Transdermal estrogen use	67	180	0.8 (0.4–1.6)	0.8 (0.4–1.8)	0.9 (0.4–2.1)
No progestogens	14	40	...	...	...
Micronized progesterone	19	63	1.0 (0.4–2.3)	0.9 (0.4–2.2)	0.7 (0.3–1.9)
Pregnane derivatives	39	79	1.0 (0.4–2.3)	0.9 (0.4–2.2)	0.9 (0.4–2.3)
Norpregnane derivatives	40*	37†	3.8 (1.6–8.7)	4.0 (1.7–9.4)	3.9 (1.5–10.0)

Users of oral estrogen combined with nortestosterone derivatives (12 cases, 7 controls) were excluded (OR, 6.7; 95% CI, 2.1 to 21.9 vs nonusers). Estrogen-by-progestogen interaction terms were not significant. Adjustment 1: adjustment for obesity status, familial history of VTE, and history of varicose veins. Adjustment 2: adjustment for obesity status, familial history of VTE, history of varicose veins, education, age at menopause, hysterectomy, and cigarette smoking.

*Twenty-two cases received nomegestrol acetate, and 18 cases received promogestone.

†Nineteen controls received nomegestrol acetate, and 18 controls received promogestone.

Norpregnane Derivatives:

Demegestone
Gestonorone
Nomegestrol
Norgestomet
Promegestone
Segesterone
Trimegestone

Oral but not transdermal estrogen is associated with an increased VTE risk. In addition, our data suggest that norpregnane derivatives may be thrombogenic, whereas micronized progesterone and pregnane derivatives appear safe with respect to thrombotic risk.

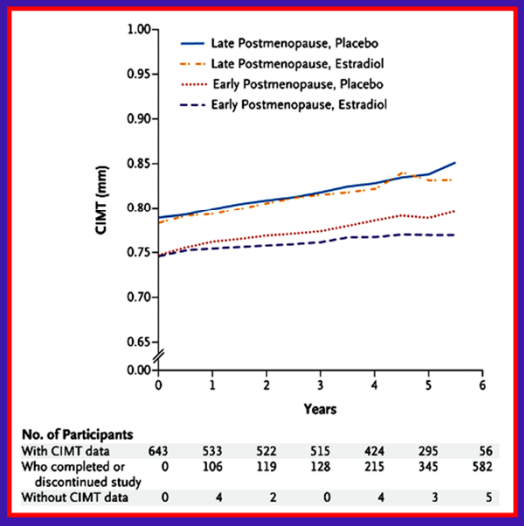
Background—Oral estrogen therapy increases the risk of venous thromboembolism (VTE) in postmenopausal women. Transdermal estrogen may be safer. However, currently available data have limited the ability to investigate the wide variety of types of progestogen.

Methods and Results—We performed a multicenter case–control study of VTE among postmenopausal women 45 to 70 years of age between 1999 and 2005 in France. We recruited 271 consecutive cases with a first documented episode of idiopathic VTE (208 hospital cases, 63 outpatient cases) and 610 controls (426 hospital controls, 184 community controls) matched for center, age, and admission date. After adjustment for potential confounding factors, odds ratios (ORs) for VTE in current users of oral and transdermal estrogen compared with nonusers were 4.2 (95% CI, 1.5 to 11.6) and 0.9 (95% CI, 0.4 to 2.1), respectively. There was no significant association of VTE with micronized progesterone and pregnane derivatives (OR, 0.7; 95% CI, 0.3 to 1.9 and OR, 0.9; 95% CI, 0.4 to 2.3, respectively). In contrast, norpregnane derivatives were associated with a 4-fold-increased VTE risk (OR, 3.9; 95% CI, 1.5 to 10.0).

Conclusions—Oral but not transdermal estrogen is associated with an increased VTE risk. In addition, our data suggest that norpregnane derivatives may be thrombogenic, whereas micronized progesterone and pregnane derivatives appear safe with respect to thrombotic risk. If confirmed, these findings could benefit women in the management of their menopausal symptoms with respect to the VTE risk associated with oral estrogen and use of progestogens. **(*Circulation*. 2007;115:840-845.)**

Vascular Effects of Early versus Late Postmenopausal Treatment with Estradiol

(Hodis et al: N Engl J Med 374(13): 1221–1231, 2016)



Carotid-Artery Intima–Media Thickness (CIMT) Progression and Baseline CIMT.*

Measure and Postmenopause Stratum	Placebo (N = 299)	Estradiol (N = 297)	P Value†	P Value for Postmenopause Stratum Interaction
Mean rate of change in CIMT (95% CI) — mm/yr‡				0.007
Early postmenopause	0.0078 (0.0060–0.0096)	0.0044 (0.0026–0.0061)	0.008	
Late postmenopause	0.0088 (0.0073–0.0103)	0.0100 (0.0085–0.0115)	0.29	
Mean baseline CIMT (95% CI) — mm				
Early postmenopause	0.75 (0.73–0.76)	0.75 (0.73–0.76)		
Late postmenopause	0.79 (0.77–0.81)	0.78 (0.77–0.80)		

Oral Estradiol 1 mg daily
Vaginal Progesterone 45 mg 10 days monthly
for women with a uterus

<6 years (median 3.5 yrs) ~15% Chol Rx, LDL ~136
>10 years (median 14.5 yrs) ~42% Chol Rx, LDL ~132
since menopause

Normal BP
Age ~55 v ~64 years

Figure 2. CIMT Progression According to Study Group and Postmenopause Stratum
At 5 years, the mean absolute CIMT values were as follows: in the late-postmenopause stratum, placebo group (83 participants), 0.838 mm (95% confidence interval [CI], 0.810 to 0.866), and in the estradiol group (72 participants), 0.831 mm (95% CI, 0.805 to 0.857); in the early-postmenopause stratum, placebo group (65 participants), 0.789 mm (95% CI, 0.763 to 0.814), and in the estradiol group (75 participants), 0.770 mm (95% CI, 0.746 to 0.793). The effect of hormone therapy on the absolute value of CIMT at 5 years differed significantly between the postmenopause strata (P = 0.03 for the interaction). In the early-postmenopause stratum, the mean 5-year CIMT was significantly lower in the estradiol group than in the placebo group (P = 0.04); in the late-postmenopause stratum, the mean 5-year CIMT did not differ significantly between the estradiol and placebo groups. Shown at the bottom of the figure for each time point are the numbers of participants for whom CIMT data were available, participants who had completed or discontinued participation in the study, and participants who were still in the study but did not have CIMT data available.

Abstract:
BACKGROUND—Data suggest that estrogen-containing hormone therapy is associated with beneficial effects with regard to cardiovascular disease when the therapy is initiated temporally close to menopause but not when it is initiated later. However, the hypothesis that the cardiovascular effects of postmenopausal hormone therapy vary with the timing of therapy initiation (the hormone-timing hypothesis) has not been tested.

METHODS—A total of 643 healthy postmenopausal women were stratified according to

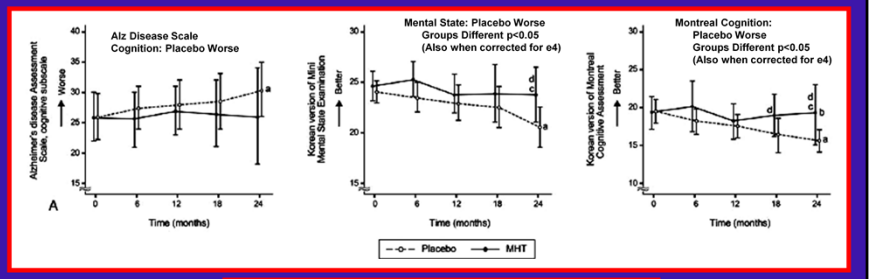
time since menopause (<6 years [early postmenopause] or ≥10 years [late postmenopause]) and were randomly assigned to receive either oral 17β-estradiol (1 mg per day, plus progesterone [45 mg] vaginal gel administered sequentially [i.e., once daily for 10 days of each 30-day cycle] for women with a uterus) or placebo (plus sequential placebo vaginal gel for women with a uterus). The primary outcome was the rate of change in carotid-artery intima– media thickness (CIMT), which was measured every 6 months. Secondary outcomes included an assessment of coronary atherosclerosis by cardiac computed tomography (CT), which was performed when participants completed the randomly assigned regimen.

RESULTS—After a median of 5 years, the effect of estradiol, with or without progesterone, on CIMT progression differed between the early and late postmenopause strata (P = 0.007 for the interaction). Among women who were less than 6 years past menopause at the time of randomization, the mean CIMT increased by 0.0078 mm per year in the placebo group versus 0.0044 mm per year in the estradiol group (P = 0.008). Among women who were 10 or more years past menopause at the time of randomization, the rates of CIMT progression in the placebo and estradiol groups were similar (0.0088 and 0.0100 mm per year, respectively; P = 0.29). CT measures of coronary-artery calcium, total stenosis, and plaque did not differ significantly between the placebo group and the estradiol group in either postmenopause stratum.

CONCLUSIONS—Oral estradiol therapy was associated with less progression of subclinical atherosclerosis (measured as CIMT) than was placebo when therapy was initiated within 6 years after menopause but not when it was initiated 10 or more years after menopause. Estradiol had no significant effect on cardiac CT measures of atherosclerosis in either postmenopause stratum. (Funded by the National Institute on Aging, National Institutes of Health; ELITE ClinicalTrials.gov number, NCT00114517.)

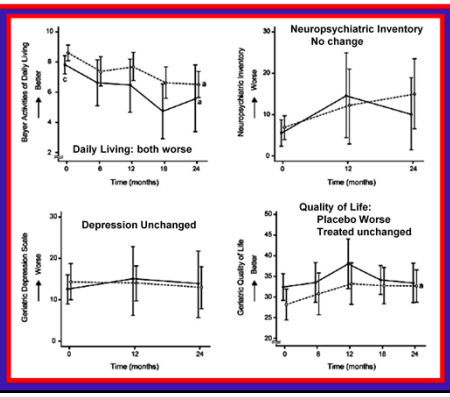
Menopausal hormone therapy and mild cognitive impairment: a randomized, placebo-controlled trial (Yoon et al: *Menopause* 25(8):870-876, 2018)

21 women (8 MHT)
Treatment: 2 years
Topical Estradiol
2 mg/day +
Progesterone
100 mg/day



Progression to dementia
reduced 52.9% v 44.4%

Very small study
Model for future studies



Objective: The aim of the study was to explore the therapeutic potential of menopausal hormone therapy (MHT) in women with mild cognitive impairment (MCI).

Methods: Thirty-seven postmenopausal women (age range: 57-82 y) with multiple-domain, amnestic subtype MCI were randomly assigned to either placebo (n¼18) or MHT (n¼19) for 24 months (percutaneous estradiol [E2] gel [0.1%, 2 mg/d] and oral micronized progesterone [MP4] [100 mg/d]). All participants received donepezil, and apolipoprotein E genotype was determined. The primary endpoint was general cognitive function: Alzheimer's disease Assessment Scale, cognitive subscale, the Korean version of Mini-Mental State Examination (K-MMSE),
and the Korean version of the Montreal Cognitive Assessment (MoCA_K) were performed in-person every 6 months.

Results: Twenty-one participants (placebo 13, MHT 8) completed the trial (56.8%). Progression rates to dementia were 52.9% (9/17) in the placebo group and 44.4% (8/18) in the MHT group. Within-group analysis showed that all three tests significantly worsened during the trial in the placebo, but not the MHT groups. Analysis adjusted for e4 allele demonstrated that MHT significantly reduced deterioration of MoCA_K score, a sensitive tool for assessing global cognition in MCI (P=0.0261). Compared with the control group, both MoCA_K (P=0.043; mean difference, 3.85; 95% CI, 0.46 to 8.16) and K-MMSE (P=0.0319; mean difference, 3.26; 95% CI, 0.04-6.48) scores were significantly better at 24 months in the MHT group.

Conclusions: Long-term MHT using percutaneous E2 gel and oral MP4 might attenuate cognitive decline in postmenopausal women with MCI.

FIG. 2. Mean changes in outcome measures, examined with a Generalized Estimating Equation. (A) General cognition. (B) Activities of daily living, abnormal behavior, depression symptomatology, and quality of life. NPI was analyzed after data were log transformed due to a skewed distribution.
'a' Significant time trend within group
'b' Significant difference in time trend compared with placebo, after controlling for e4 allele (P=0.0261)
'c' P<0.05 versus control
'd' P<0.05 versus control after adjusting for e4 allele
I bars,95%Cis
MHT, menopausal hormone therapy; NPI, Neuropsychiatric Inventory.

Estradiol & Breast Cancer

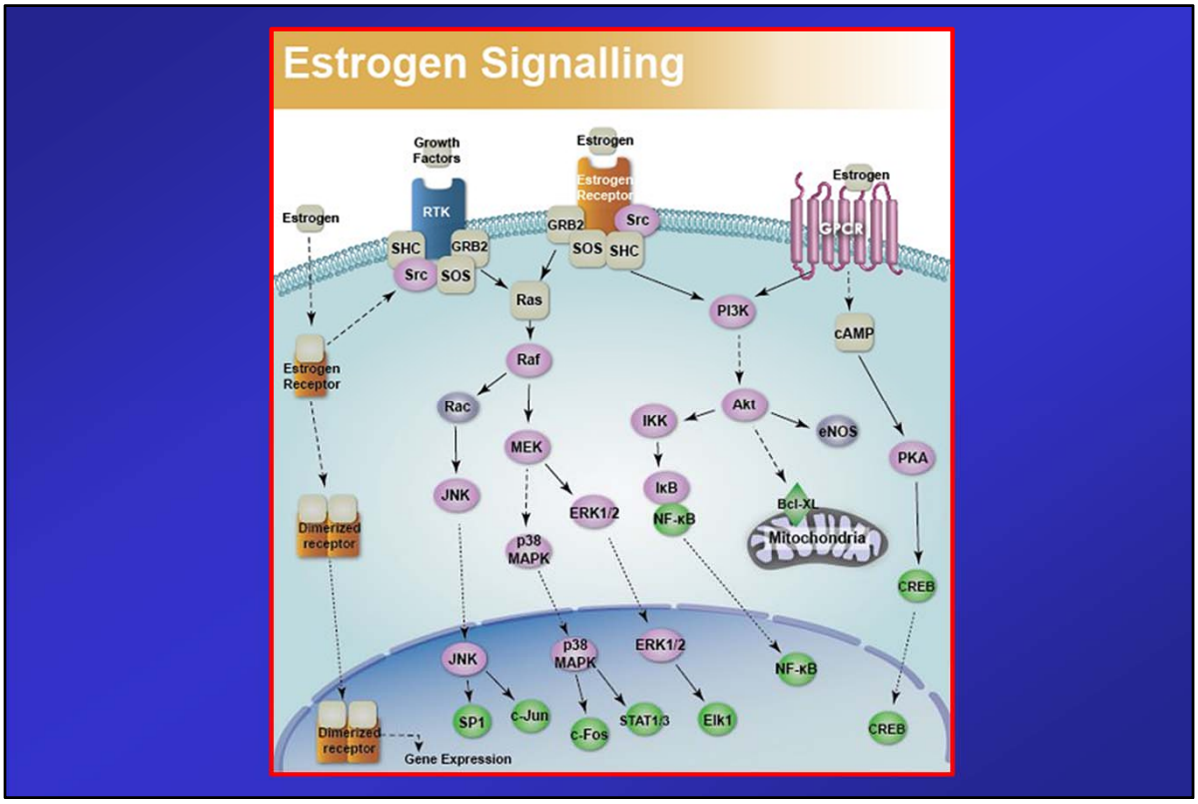
Summary:

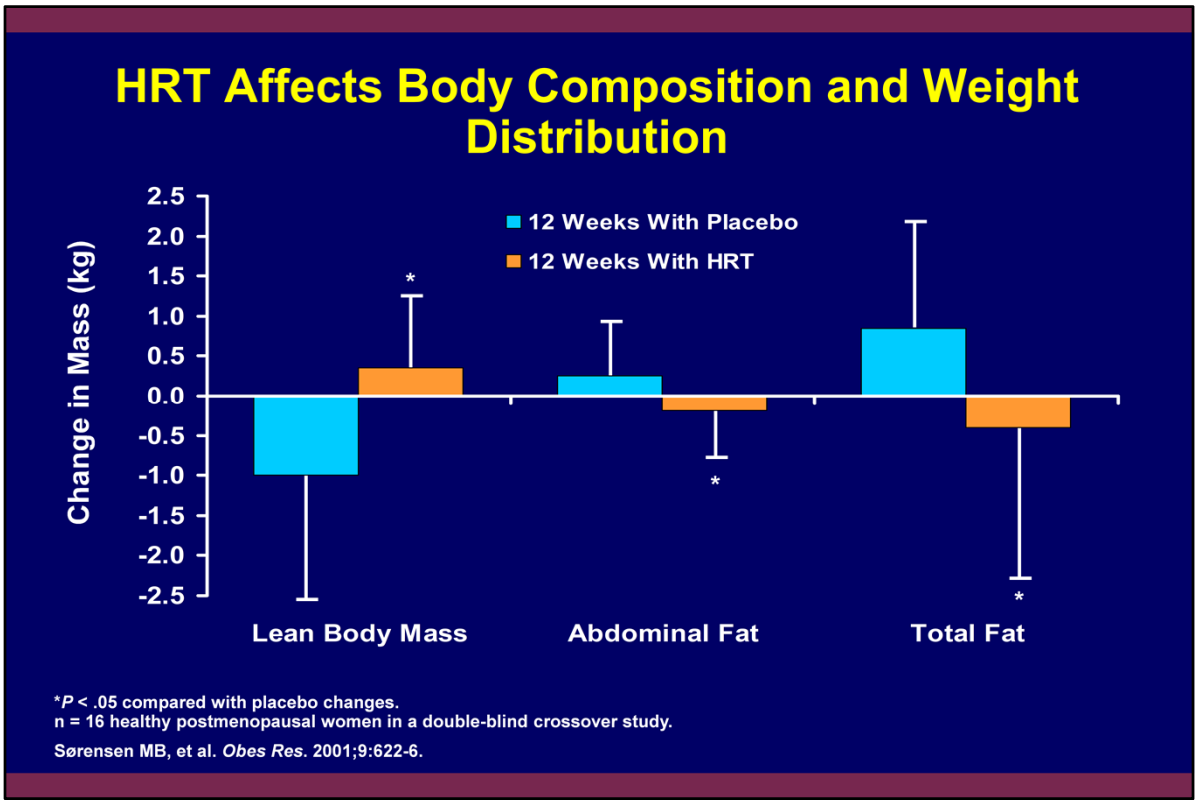
1. Estrogen impacts thousands of genes, many involved in growth, differentiation, metastasis, & apoptosis.
2. Theoretically, these effects could inhibit or promote cancer growth & metastasis.
3. There is currently no evidence that estrogen-only therapy increases the incidence of breast cancer or promotes its spread. In fact, there are strong trends toward inhibiting breast cancer incidence, growth, and metastasis while mortality is significantly reduced.
4. Some progestins may promote breast cancer but only during their active use.
5. Progesterone does not appear to promote breast cancer growth or mortality.
6. Estrogen-only therapy does not increase the recurrence rate of breast cancer but progestins might (again, only during their active use).
7. Oral Estrogen does increase coagulation & thrombotic events but transdermal estrogen does not. Obesity and, perhaps, some progestins worsen this effect.

Estradiol & Breast Cancer

My Conclusions:

1. "Hormone Replacement Therapy" has very different effects on cancer depending on which hormone receptor ligand(s) is(are) used. Therefore, this term is too imprecise to be used in this context.
2. Hormones do maintain certain growth pathways that can be utilized by cancers but there is little evidence that hormones are carcinogens.
3. If these pathways are blocked by "anti-hormones", then there can be a temporary suspension or regression of cancer growth. However, cancers usually eventually bypass this pathway by a variety of means and resume growing.
4. A cancer that is hormone-receptor positive indicates that the cells have not de-differentiated to the point that they no longer respond to hormones. Therefore, they may be manipulated by hormone therapy.
5. Transdermal estradiol with or without natural progesterone appears to have almost no adverse effects ("break-through" bleeding) and very likely has several beneficial effects.


Estrogen Signalling
Growth Factors
Estrogen
Estrogen Receptor
Estrogen
RTK
GPCR
Estrogen
SHC
GRB2
GRB2
Src
Src
SOS
SOS
SHC
Ras
PI3K
cAMP
Raf
Estrogen Receptor
Rac
Akt
IKK
eNOS
PKA
MEK
IkB
JNK
ERK1/2
NF-κB
Bcl-XL
Dimerized receptor
p38 MAPK
Mitochondria
CREB
JNK
p38 MAPK
ERK1/2
NF-κB
SP1
c-Jun
c-Fos
STAT1/3
Elk1
CREB
Dimerized receptor
Gene Expression



Menopause has been linked to a reduction in lean body mass.[1] Menopause-related sarcopenia (ie, decreased skeletal muscle mass) has been suggested to be linked directly to reduced ovarian hormone output.[2] Estrogen loss is also associated with a shift in adipose tissue distribution toward central or abdominal obesity.[3]

The study shown here demonstrates that postmenopausal HRT can be protective against shifts in body mass and composition associated with estrogen loss. In this placebo-controlled, crossover study, 16 healthy postmenopausal women were randomized to either HRT (17b-estradiol plus NETA) or placebo in two 12-week periods separated by a 3-month washout.

Total and regional body composition was measured by dual X-ray absorptiometry at baseline and in the 10th treatment week in both periods. Significant differences were found in changes in lean body mass, abdominal fat mass, and total fat mass between the placebo treatment and the HRT treatment.

Although the number of participants in this study was fairly small (n = 12), these findings support the hypothesis that estrogen loss is linked to abdominal obesity.[4] The increase in lean body mass seen with HRT might be linked to a gain in skeletal muscle, as reported elsewhere.[5]

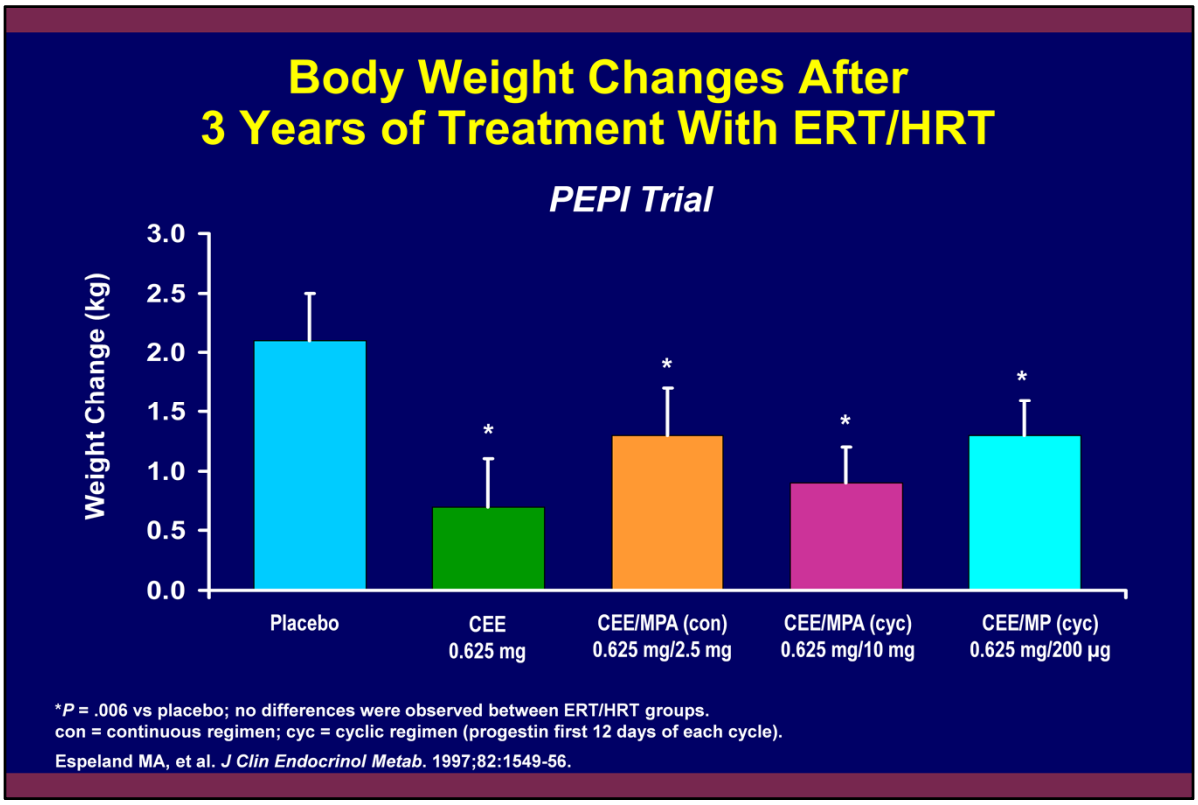
[1]Douchi T, Yamamoto S, Nakamura S, et al. The effect of menopause on regional and total body lean mass. *Maturitas*. 1998; 29:247-252.

[2]Dionne IJ, Kinaman KA, Poehlman ET. Sarcopenia and muscle function during menopause and hormone-replacement therapy. *J Nutr Health Aging*. 2000;4:156-161.

[3]Ley CJ, Lees B, Stevenson JC. Sex- and menopause-associated changes in body-fat distribution. *Am J Clin Nutr*. 1992;55:950-954.

[4]Sørensen MB, Rosenfalck AM, Højgaard L, Ottesen B. Obesity and sarcopenia after menopause are reversed by sex hormone replacement therapy. *Obes Res*. 2001;9:622-626.

[5]Jensen J, Christiansen C, Rodbro P. Oestrogen-progestogen replacement therapy changes body composition in early post-menopausal women. *Maturitas*. 1986;8:209-216.



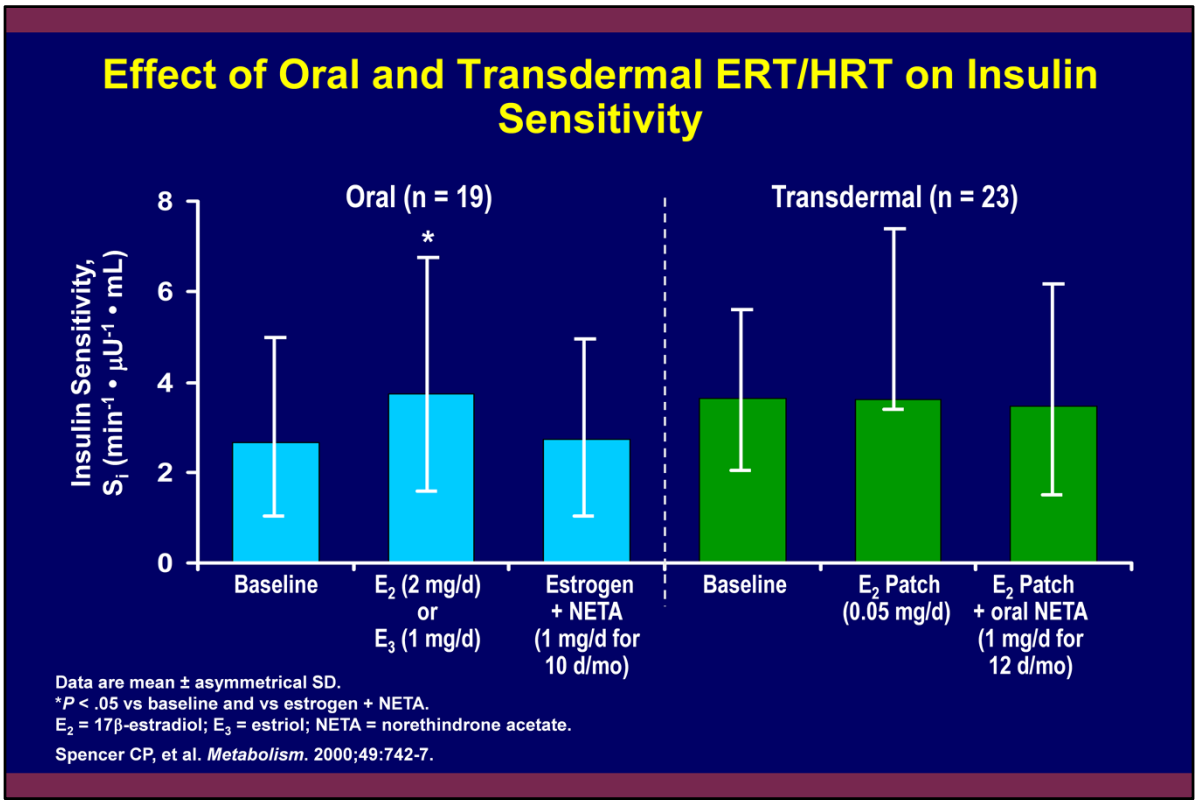
In addition to lean versus fat comparisons (as seen in the previous slide), an effect of ERT/HRT on total body weight changes has been examined.

The Postmenopausal Estrogen/Progestin Interventions (PEPI) trial was a 3-year, placebo-controlled, randomized clinical trial of 875 women that evaluated potential cardiovascular risk factor modification by each of 4 hormone regimens (shown above): CEE 0.625 mg alone (with placebo), CEE with continuous MPA, CEE with cyclical MPA, or CEE with cyclical MP.

The PEPI trial also evaluated the effects of CEE alone or combined with a progestin (MPA or MP) on total body weight.

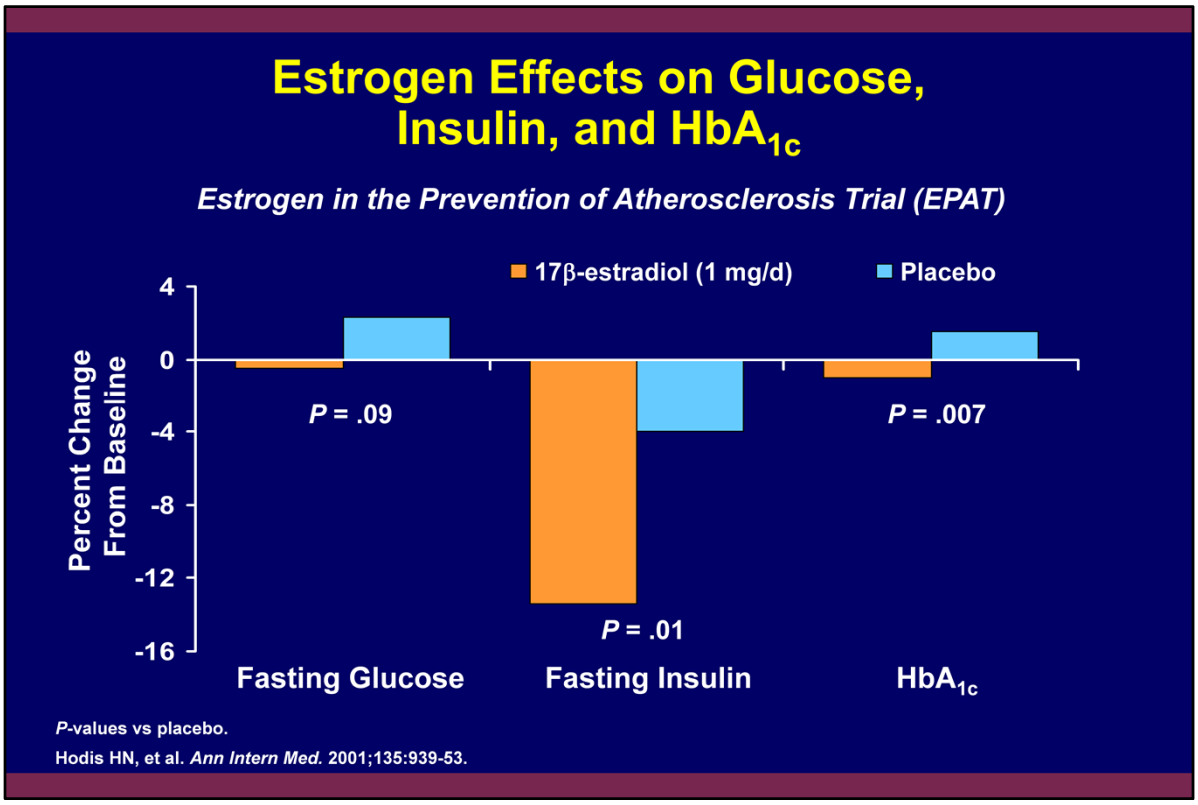
For all treatment regimens, women randomly assigned to active treatment gained significantly less weight than women randomized to placebo (*P* =.006). This amounted to approximately one less kilogram gained over the 3-year period compared with placebo.

Espeland MA, Stefanick ML, Kritz-Silverstein D, et al. Effect of postmenopausal hormone therapy on body weight and waist and hip girths. *J Clin Endocrinol Metab*. 1997;82:1549-1556.



There are many modes of delivery for ERT/HRT regimens. This randomized trial with 42 healthy postmenopausal women (mean age, 56.3 years) compared the effects of oral versus transdermal ERT/HRT on insulin and glucose metabolism. Nineteen women received oral 17b-estradiol (E_2)/estriol (E_3) with or without cyclical norethindrone acetate (NETA for 10 days/month). Twenty-three women received transdermal E_2 with or without oral NETA (for 12 days/month). S_i was evaluated by a glucose tolerance test where blood glucose was measured over a 3-hour period after an IV bolus challenge. S_i was measured at baseline and at 46 weeks with E_2 alone or at 48 weeks after combined E_2/NETA therapy. Oral E_2 or E_3 was associated with a significant increase in S_i compared with baseline or E_2/NETA values ($P < .05$). In contrast, transdermal E_2 with or without NETA did not affect S_i. Additional data from this study (not shown) demonstrated that insulin levels did not predict S_i as only oral E_2/NETA or transdermal E_2 alone were associated with significant reductions in fasting insulin levels. The study further demonstrated that all treatment groups had significant reductions in fasting glucose compared with within-group baselines.

Spencer CP, Godsland IF, Cooper AJ, Ross D, Whitehead MI, Stevenson JC. Effects of oral and transdermal 17β-estradiol with cyclical oral norethindrone acetate on insulin sensitivity, secretion, and elimination in postmenopausal women. *Metabolism*. 2000;49:742-747.



The Estrogen in the Prevention of Atherosclerosis Trial (EPAT) was a randomized, double-blind, placebo-controlled study examining the effect of ERT on the progression of carotid artery wall thickening; healthy postmenopausal women (n = 222) of mean age 61 years participated in the trial.

Participants were without preexisting CVD and with LDL-cholesterol levels of ≥130 mg/dL. The effect of unopposed micronized 17β-estradiol at 1 mg/d for 2 years was compared with placebo. At the end of the 2 years, there were 199 evaluable participants.

Thirty-two percent of the evaluable placebo recipients and 44% of the evaluable estradiol recipients had undergone hysterectomies.

All patients received dietary counseling. Lipid-lowering drugs were given only if the LDL-cholesterol exceeded 160 mg/dL.

Estradiol treatment reduced fasting glucose, fasting insulin, and HbA_{1c} in the evaluable subjects. These effects were significantly different from placebo for fasting insulin and HbA_{1c}.

Overall, estradiol had a positive effect on carbohydrate metabolism compared with that of placebo.

Hodis HN, Mack WJ, Lobo RA, et al. Estrogen in the prevention of atherosclerosis: a randomized, double-blind, placebo-controlled trial. *Ann Intern Med*. 2001;135:939-953.