

Arterial Imaging Outcomes and Cardiovascular Risk Factors in Recently Menopausal Women

A Randomized Trial

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Background: Whether menopausal hormone therapy (MHT) protects against cardiovascular disease (CVD) remains unclear.

Objective: To assess atherosclerosis progression and CVD risk factors after MHT initiated in early menopause.

Design: Randomized, controlled trial. (ClinicalTrials.gov: NCT00154180)

Setting: Nine U.S. academic centers.

Participants: Healthy menopausal women aged 42 to 58 years between 6 and 36 months from last menses without prior CVD events who had a coronary artery calcium (CAC) score less than 50 Agatston units and had not received estrogen or lipid-lowering therapy for at least 90 days.

Intervention: Oral conjugated equine estrogens (o-CEE), 0.45 mg/d, or transdermal 17 β -estradiol (t-E₂), 50 mcg/d, each with 200 mg of oral progesterone for 12 days per month, or placebo for 48 months.

Measurements: Primary end point was annual change in carotid artery intima-media thickness (CIMT). Secondary end points included changes in markers of CVD risk.

Results: Of 727 randomly assigned women, 89.3% had at least 1 follow-up CIMT and 79.8% had CIMT at 48 months. Mean CIMT increases of 0.007 mm/y were similar across groups. The percentages of participants in whom CAC score increased did not differ significantly across groups. No changes in blood pressure were observed with o-CEE or t-E₂. Low- and high-density lipoprotein cholesterol levels improved and levels of C-reactive protein and sex hormone-binding globulin but not interleukin-6 increased with o-CEE. Insulin resistance decreased with t-E₂. Serious adverse events did not differ by treatment.

Limitation: Power to compare clinical events was insufficient.

Conclusion: Four years of early MHT did not affect progression of atherosclerosis despite improving some markers of CVD risk.

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Incidence of cardiovascular disease (CVD), the leading cause of death in women, increases after menopause (1). A low-risk, preventive intervention applicable to many women would be highly desirable. Observational studies have suggested that long-term menopausal hormone therapy (MHT) has favorable effects on several CVD risk factors (2–9) and varying effects on blood pressure (10–13) and reduces CVD incidence (2–17). However, data from randomized, controlled trials from the large Women's Health Initiative (WHI), which involved predominantly older women, did not find cardioprotective effects of MHT (18–21).

Further analyses of WHI data and new findings have suggested that the cardiovascular benefits of MHT may be limited to women who begin treatment at a younger age, closer to menopause, or both (19, 20, 22–31). We therefore conducted KEEPS (Kronos Early Estrogen Prevention Study), a randomized, controlled trial of MHT in women who were within 36 months of their last menses, to assess effects of early initiation of oral or transdermal MHT versus placebo on rates of progression of atherosclerosis. This progression was measured as changes in carotid artery intima-media thickness (CIMT) by ultrasonography and

coronary artery calcium (CAC) score, each of which quantifies atherosclerosis independently; predicts risk for CVD events; and responds to interventions (32–41), including estrogens (31, 42).

METHODS

Design Overview

KEEPS was a randomized, double-blind, placebo-controlled trial comparing daily oral or transdermal estrogen, both with cyclic progesterone treatment, with placebo. The primary and secondary outcomes were changes in CIMT (42) and CAC score (43, 44), respectively. Age and time since menopause at study entry were specified to be similar to those at usual clinical initiation of MHT. A

See also:

Summary for Patients. I-19

Web-Only
Supplement

Context

Although a randomized, controlled trial (RCT) of predominantly older women did not find cardioprotective effects of menopausal hormone therapy (MHT), observational studies suggest benefit when MHT is initiated in younger women or closer to the onset of menopause.

Contribution

This RCT of recently menopausal women found no effect of up to 4 years of MHT on the progression of carotid artery intima-media thickness or coronary artery calcium. Some blood markers of risk for cardiovascular disease did improve.

Implication

Four years of MHT does not protect against progression of atherosclerosis. Whether longer-term use alters cardiovascular events is uncertain.

—The Editors

separate study of cognitive and affective outcomes was done on a subset of KEEPS participants, the results of which will be reported separately.

Setting and Participants

The study was approved by the Western Institutional Review Board (Olympia, Washington) and local institutional review boards at each participating institution (Appendix 1, available at www.annals.org). Informed consent was obtained before screening and randomization procedures.

Participants were recruited through mass mailings, posters in hospitals and clinics, print and electronic media advertisements, and an Internet Web page. They were studied at 9 academic medical centers. Recruitment occurred between July 2005 and June 2008.

Full inclusion and exclusion criteria for KEEPS have been published elsewhere (45). In brief, women aged 42 to 58 years who were between 6 and 36 months from their last menses and had plasma follicle-stimulating hormone levels of 35 IU/L or greater, estradiol (E_2) levels less than 147 pmol/L, or both were eligible. Women with a history of clinical CVD, including myocardial infarction, angina, congestive heart failure, stroke, transient ischemic attack, or thromboembolic disease, were excluded. Benign results on Papanicolaou smear and normal results on mammography within 1 year before randomization were required. Former or current recipients of MHT (approximately 20% [Table 1]) were screened only after having discontinued therapy for at least 90 days.

All women meeting initial eligibility criteria had a complete blood count, a chemistry panel, and E_2 and follicle-stimulating hormone levels measured at the clinical laboratories at each study center. Lipids and TSH were measured at Kronos Science Laboratories (Phoenix, Ari-

zona). Women were screened for CAC, and those with scores of 50 Agatston units or greater were excluded. Eligible women had baseline CIMT measurements (42).

Subsequently, the participants had short follow-up visits every 90 days (in person or by telephone with medications mailed) to assess adverse events (AEs) and adherence (pill or patch counts). Longer in-person visits were done at 18 months and annually to measure end points. Study visits concluded in March 2012.

Randomization and Interventions

The KEEPS unblinded officer created the randomization schema using the Excel (Microsoft) random-number generator to create randomly sequenced blocks of 13, stratified by study center, in a ratio of 4:4:5 (oral conjugated equine estrogens [o-CEE]–transdermal 17β -estradiol [t- E_2]–placebo) to overcome a higher anticipated dropout rate in the placebo group. The order of randomization for each center was supplied to the KEEPS database programmer so that a study identification number specifying treatment could be assigned to each enrolled participant using the database's randomization function. The randomization key in the database was not accessible to study personnel, and the original Excel version was a locked file kept in the custody of the unblinded officer who was not a KEEPS investigator. It was shared only with the study pharmacist who provided blinded packets of study drugs for each participant.

The active study treatments were o-CEE (Premarin, Pfizer Pharmaceuticals), 0.45 mg/d, or t- E_2 (Climara, Bayer HealthCare), 50 mcg/d (patch replaced weekly). The use of lower-dose estrogen than in the WHI and prior trials was meant to reduce the risk for AEs while providing sufficient estrogen to prevent bone loss and relieve vasomotor symptoms. Women receiving either active estrogen also received progesterone capsules (Prometrium, Abbott), 200 mg/d, on days 1 to 12 of each month.

Women assigned to active o-CEE received placebo patches and those assigned to active t- E_2 received placebo tablets. Placebo participants received placebo tablets, patches, and capsules.

Outcomes and Follow-up**Demographic Characteristics and Anthropometric and Safety Measures**

Participants reported their ethnicity; income and education levels; history of smoking; use of lipid-lowering or antihypertensive medications, hormones, oral contraceptives, and bisphosphonates; amount and intensity of exercise; caffeine and alcohol consumption; and pregnancy history. Height and weight were measured using clinical scales and a stadiometer. Blood pressure and pulse rates were measured twice after 10 minutes of rest in the sitting position and averaged. Waist circumference was measured at the top of the iliac crest by tape measure. Measurements were repeated at annual visits, as were a Papanicolaou smear and mammography.

Table 1. Baseline Characteristics of Participants, by Treatment Group

Variable	Placebo (n = 275)	o-CEE (n = 230)	t-E ₂ (n = 222)*	All (n = 727)
Demographic characteristics				
Mean age (SD), y	52.5 (2.5)	52.8 (2.6)	52.7 (2.6)	52.7 (2.6)
Mean BMI (SD), kg/m ²	26.4 (4.3)	26.0 (4.3)	26.0 (4.4)	26.2 (4.3)
Race, n (%)				
White	211 (77)	177 (77)	169 (76)	557 (77)
African American	23 (8)	17 (7)	14 (6)	54 (7)
Asian or Hispanic	27 (10)	25 (11)	22 (10)	74 (10)
Other	14 (5)	11 (5)	17 (8)	42 (6)
Education, n (%)				
High school diploma, GED, or less	28 (10)	16 (7)	14 (6)	58 (8)
More than high school	46 (17)	47 (20)	39 (18)	132 (18)
College degree or higher	195 (71)	166 (72)	166 (75)	527 (72)
Unknown	6 (2)	1 (0)	3 (1)	10 (1)
Annual family income, n (%)				
<\$60 000	48 (17)	44 (19)	47 (21)	139 (19)
\$60 000–\$100 000	40 (15)	32 (14)	29 (13)	101 (14)
>\$100 000	52 (19)	36 (16)	34 (15)	122 (17)
Unknown	135 (49)	118 (51)	112 (50)	365 (50)
Marital status, n (%)†				
Married or partnered	189 (69)	158 (69)	137 (63)	484 (67)
Never married	32 (12)	22 (10)	30 (14)	84 (12)
Divorced, separated, or widowed	53 (19)	48 (21)	52 (24)	153 (21)
Participants with term pregnancies, n (%)‡				
0	51 (19)	50 (22)	57 (26)	158 (22)
1–2	122 (45)	111 (49)	99 (45)	332 (47)
≥3	96 (36)	66 (29)	62 (28)	224 (31)
Hormone replacement status, n (%)				
Never	223 (81)	171 (74)	181 (82)	575 (79)
Past/current	52 (19)	59 (26)	41 (18)	152 (21)
Smoking status, n (%)				
Never	214 (78)	181 (79)	176 (79)	571 (79)
Former	42 (15)	35 (15)	29 (13)	106 (15)
Current	19 (7)	14 (6)	17 (8)	50 (7)
Cardiovascular risk factors				
Mean systolic BP (SD), mm Hg	119.8 (14.4)	119.0 (14.8)	117.4 (15.6)	118.8 (14.9)
Mean diastolic BP (SD), mm Hg	75.4 (9.5)	75.3 (8.3)	74.1 (9.7)	75.0 (9.2)
Mean total cholesterol level (SD)				
mmol/L	5.4 (0.9)	5.4 (0.8)	5.4 (0.9)	5.4 (0.9)
mg/dL	207.4 (33.9)	207.7 (31.6)	209.3 (35.6)	208.1 (33.7)
Mean LDL cholesterol level (SD)				
mmol/L	2.9 (0.7)	2.9 (0.7)	2.9 (0.8)	2.9 (0.7)
mg/dL	110.9 (26.6)	110.8 (27.8)	111.0 (29.2)	110.9 (27.8)
Mean HDL cholesterol level (SD)				
mmol/L	1.8 (0.4)	1.9 (0.4)	1.9 (0.4)	1.9 (0.4)
mg/dL	70.3 (13.7)	72.9 (14.5)	73.2 (15.6)	72.0 (14.6)
Mean triglyceride level (SD)				
mmol/L	1.0 (0.7)	0.9 (0.6)	0.9 (0.6)	0.9 (0.6)
mg/dL	91.6 (60.3)	83.8 (55.9)	84.6 (49.7)	87.0 (55.9)
Mean non-HDL cholesterol level (SD)				
mmol/L	3.5 (0.8)	3.5 (0.7)	3.5 (0.8)	3.5 (0.8)
mg/dL	137.0 (29.7)	134.7 (28.6)	136.1 (32.6)	136.0 (30.2)
Insulin-related				
Mean fasting insulin level (SD), pmol/L	42.3 (43.7)	35.4 (30.5)	48.6 (91.7)	42.4 (60.4)
Mean fasting glucose level (SD)				
mmol/L	44.2 (5.4)	44.0 (4.8)	44.4 (6.3)	44.2 (5.5)
mg/dL	79.7 (9.8)	79.2 (8.7)	80.0 (11.4)	79.6 (10.0)
Mean HOMA-IR score (SD), unit	1.24 (1.35)	1.02 (0.94)	1.55 (3.77)	1.27 (2.32)
Other				
Mean CRP level (SD), nmol/L	22.19 (36.28)	17.14 (23.62)	21.52 (34.19)	20.38 (32.19)
Mean IL-6 level (SD), pg/mL	3.81 (9.49)	4.28 (14.6)	3.79 (13.7)	3.95 (12.6)
Mean SHBG level (SD), nmol/L	59.6 (29.2)	62.0 (28.5)	62.3 (29.7)	61.2 (29.1)

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Table 1—Continued

Variable	Placebo (n = 275)	o-CEE (n = 230)	t-E ₂ (n = 222)*	All (n = 727)
Vascular imaging				
Mean CIMT (SD), mm	0.720 (0.089)	0.726 (0.089)	0.718 (0.092)	0.721 (0.090)
CAC score, n (%)				
0 Agatston units	240 (87.0)	203 (88.0)	192 (86.0)	635 (87.0)
1–5 Agatston units	20 (7.3)	12 (5.2)	15 (6.8)	47 (6.5)
>5 Agatston units	15 (5.2)	15 (6.7)	15 (6.8)	45 (6.2)

BMI = body mass index; BP = blood pressure; CAC = coronary artery calcium; CIMT = carotid artery intima-media thickness; CRP = C-reactive protein; HDL = high-density lipoprotein; HOMA-IR = Homeostasis Model Assessment of Insulin Resistance; IL-6 = interleukin-6; LDL = low-density lipoprotein; o-CEE = oral conjugated equine estrogens; SHBG = sex hormone-binding globulin; t-E₂ = transdermal 17 β -estradiol.

* Patch.

† Total n = 721.

‡ Total n = 714.

Assessment of Symptoms

Menopausal symptoms (dyspareunia, hot flashes, night sweats, insomnia, palpitations, depression, vaginal dryness, mood swings, and irritability) were assessed at baseline and at each annual visit using a Likert scale (none, mild, moderate, or severe). Only vasomotor symptoms are reported here.

CIMT

Change in CIMT was the predesignated primary end point. Technicians trained at the University of Southern California Carotid Artery Intima-Media Thickness Core Imaging and Reading Center obtained CIMT scans with high-resolution ultrasonography equipment using standardized methods for reproducing transducer angulation and cardiac gating (patents obtained in 2005, 2006, and 2011) (33, 42, 46). The intima-media thickness of the far wall of the distal common carotid artery was determined as the average of 70 to 100 standardized measurements between the intima-lumen and media-adventitia interfaces by automated computerized edge detection with a software package developed in-house.

Readers blinded to treatment allocation did all measurements of carotid wall thickness at the CIMT reading center. Two baseline measurements of CIMT were done at separate visits (generally 3 days to 6 weeks apart), and the results were averaged to provide an estimate of baseline CIMT. The mean coefficient of variation between baseline scans was 0.6% (SD, 0.7 [range, 0.0% to 7.7%]). Subsequently, 1 CIMT scan was obtained annually.

CAC

A single chest CT was done at baseline and study end. Images of the coronary arteries were obtained by high-speed axial tomography using standard methods (44). An experienced reader blinded to study group quantified the CAC score at the CAC center using the Agatston scoring method (47).

Biochemical End Points

At baseline and 12, 36, and 48 months, fasting levels of serum total and high- and low-density lipoprotein cholesterol and triglycerides were measured at Kronos Science Laboratories. Interleukin-6, high-sensitivity C-reactive protein, sex hormone-binding globulin, glucose, and insulin levels were also measured at the laboratory. Estrone (E₁) and E₂ levels were measured by highly sensitive radiometric assays in the Reproductive Endocrine Research Laboratory at the University of Southern California Keck School of Medicine (Los Angeles, California) (48). Levels of E₁ and E₂ at baseline and 12 months were assessed on a random sample of approximately 60% of participants (excluding those who did not complete follow-up and those without samples at these times); 36- and 48-month assays were done on a random subsample of 99 (33 per treatment group) participants with baseline and 12-month values who completed the study receiving study medications.

All assays were done as a batch at the end of the study. The **Supplement** (available at www.annals.org) includes details of methods and statistics on the assay quality. The Homeostasis Model Assessment of Insulin Resistance was calculated from fasting glucose and insulin levels (49).

AEs

Serious adverse events (SAEs) were reported immediately, and AEs were solicited and recorded every 3 months at study calls or visits. The **Supplement** describes these procedures.

Statistical Analysis

Data were entered at study centers into online forms in Perl, and all data were transferred to the Kronos Coordinating Center into a structured query language database and then uploaded for analysis and converted to SAS data sets at the data coordinating center at the University of California, San Francisco. Data from core centers (for example, the CAC score, CIMT, and laboratory measurements) were uploaded from Excel into SAS (SAS Institute).

Before initiation, study power was calculated as 92% to detect a difference of 0.008 mm/y in the rate of CIMT progression between a treatment group and the placebo group by using a repeated-measures linear mixed-effects model and assuming a cross-sectional SD of 0.15 mm (42), correlation between measurements of 0.5 (50), rate of loss to follow-up of 4% per year, and a 2-sided significance level of 0.05. The actual correlations within participants were much higher than projected, and the resulting estimated variances were lower. For CAC score, assuming an overall rate of progression of 18%, we could have detected a reduction in progression of approximately 50% with a power of 90%.

Data were analyzed on the basis of original treatment assignment (intention to treat). Comparisons of baseline characteristics across the 3 groups were done by analysis of variance or the chi-square or Fisher exact test, as appropriate. Available data were used without imputation for missing values in the primary analysis.

For CIMT, we used a linear mixed-effects model for repeated measurements to compare CIMT progression over time among treatment groups. We modeled the covariance structure among repeated measurements by participant by using an unstructured variance-covariance matrix and fitted the model using restricted maximum likelihood. Intercept and follow-up (years) were modeled as fixed effects. The interaction term of treatment by follow-up (years) was used to test for treatment differences in average CIMT progression rates.

Our primary focus of inference was the annual changes from the model. We compared results between each of the treated groups versus the placebo group separately and determined significance levels without adjustment for 2 comparisons. We analyzed changes in CVD risk factors (for example, blood pressure and lipid levels) and hormone levels by using the same model as for CIMT. We implemented these analyses using PROC MIXED in SAS, version 9.2.

For CAC, we analyzed change from baseline as a binary outcome as agreed on a priori after discussion with the director of the CAC reading center. This value was defined as any increase from 0 Agatston units at baseline or, if the CAC score was greater than 0 units at baseline, an increase of 5 units or greater. We compared each treatment group with the placebo group with respect to this binary outcome using Poisson models with robust SEs (51). We used a similar model for hot flush symptoms. We implemented these analyses in Stata, version 13.1 (StataCorp), using the poisson and margins commands. In the model for hot flushes, the SEs accounted for clustering by participant. We compared the adverse events in the 2 treated groups with those in the placebo group by using 2-tailed Fisher exact tests.

To allow for any clinical center effect, we performed a sensitivity analysis for CIMT in which site was added as a fixed effect. We also did analyses to assess the potential bias

in the CIMT and CAC outcomes due to missing data not being missing at random. The **Supplement** describes these methods and results.

Role of the Funding Source

The Aurora Foundation provided funding for KEEPS through a grant to the Kronos Longevity Research Institute. Bayer HealthCare and Abbott Pharmaceuticals donated study drugs. Pfizer Pharmaceuticals provided a small grant for post hoc assessment of unexpected bleeding. The funding sources had no input into the design or conduct of the study or the writing, review, or approval of this manuscript.

RESULTS

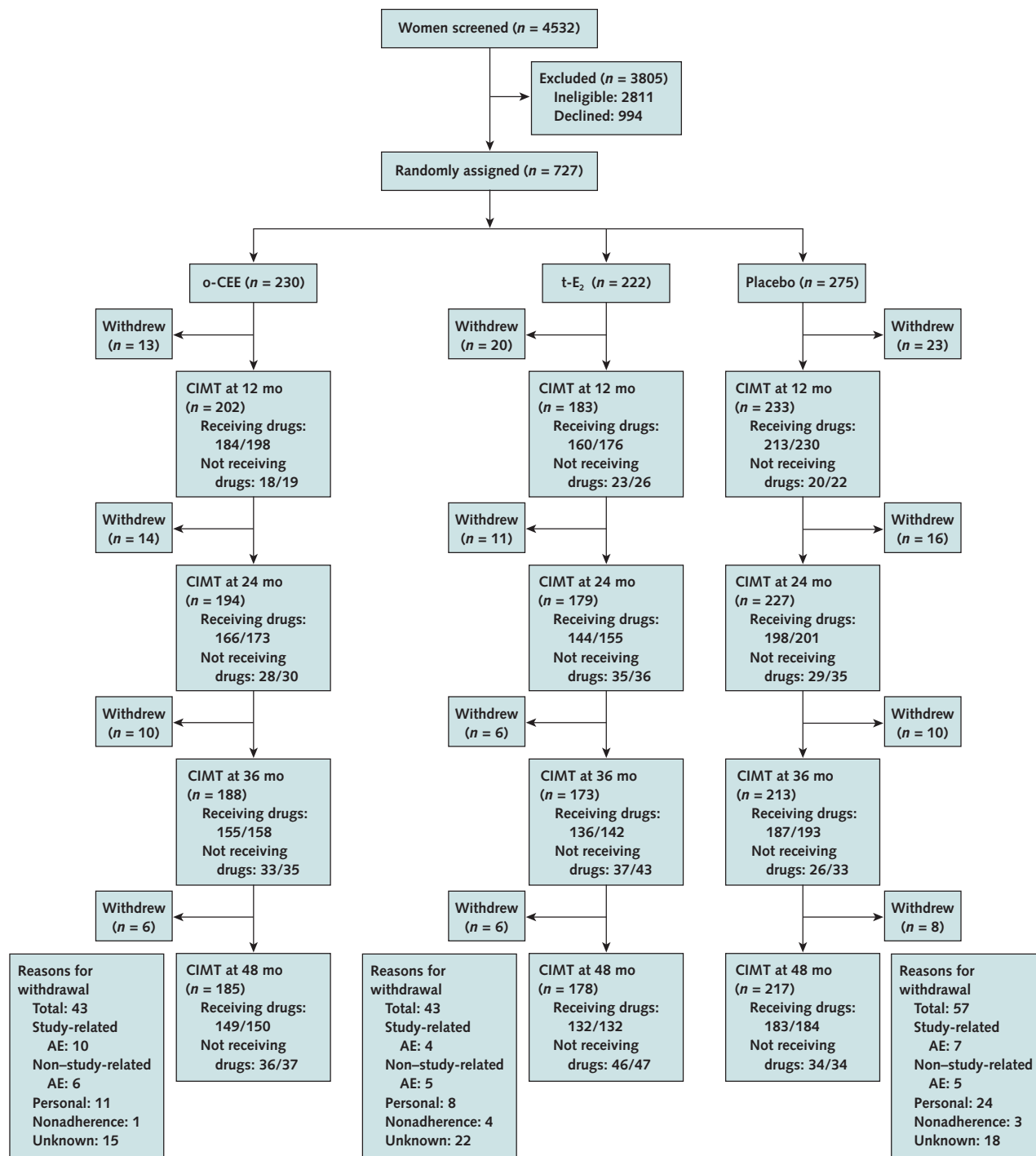
Participation and Adherence

Of 4532 women contacted, 727 were randomly assigned: 230 (31.6%) to o-CEE, 222 (30.5%) to t-E₂, and 275 (37.8%) to placebo (**Figure 1**). Of women who discontinued study medications, approximately one half continued to be followed. As shown by the number of CIMT measurements compared with the number of participants assigned to a particular intervention who continued to receive study drugs (on study medications) and those in whom study drugs had been discontinued (off study medications), some participants missed CIMT measurement at each follow-up visit. At 48 months, CIMT was available for 580 women (79.8% of randomly assigned participants), of whom 464 (63.8% of randomly assigned participants) were still receiving study medications (**Figure 1**).

Among participants who completed the trial while continuing to receive study medications, based on data from follow-up visits that participants attended adherence in participants continuing to receive these medications averaged 94% to 95% for tablets, patches, and capsules, with no differences by study group. Mean duration of treatment was 37.4 months (SD, 16.6) for o-CEE, 34.6 months (SD, 18.3) for t-E₂, and 37.6 months (SD, 17.3) for placebo. The proportions of participants who completed the study who did and did not receive medications and those who did not complete the study were similar in the 3 groups.

In the o-CEE, t-E₂, and placebo groups, 16, 9, and 12 women, respectively, withdrew after AEs. Approximately one half of these events in the t-E₂ and placebo groups but nearly two thirds of those in the o-CEE group were classified as possibly or probably study-related (**Figure 1**). Serious AEs leading to study withdrawal included 6 cases of breast cancer (3 in the o-CEE group, 2 in the t-E₂ group, and 1 in the placebo group) among 8 diagnosed cases, 1 transient ischemic attack in the o-CEE group, 1 suspected stroke (later determined not to be) in the t-E₂ group, and 2 cases of venous thrombotic disease (1 in the t-E₂ group and 1 in the placebo group). Other commonly cited AEs included breast tenderness, vaginal bleeding, migraine headaches, and dermatitis, but no event had a clear pattern of distribution among the treatment groups.

Figure 1. Study flow diagram.



The number of remaining active participants receiving and not receiving drugs is shown as a denominator, and the number of CIMT scans (primary end point) obtained from those participants is shown as a numerator (i.e., "Receiving drugs: 184/198" means that, of 198 available participants receiving study medications, CIMT was measured in 184). Personal reasons for withdrawal include logistical problems, family concerns, fear of cancer, and relocation. AEs include serious and nonserious events. AE = adverse event; CIMT = carotid artery intima-media thickness; o-CEE = oral conjugated equine estrogens; t-E₂ = transdermal 17 β -estradiol.

Table 2. Changes in CIMT, by Treatment*

Treatment	Baseline		Year 4		Annual Slope		
	Participants, n	Mean CIMT (95% CI), mm	Participants, n	Mean CIMT (95% CI), mm	Estimated Change in CIMT† (95% CI), mm/y	Mean Difference From Placebo (95% CI), mm/y	P Value
Placebo	275	0.7213 (0.7106 to 0.7319)	217	0.7503 (0.7388 to 0.7619)	0.0072 (0.0058 to 0.0086)	—	—
o-CEE	230	0.7268 (0.7152 to 0.7384)	185	0.7591 (0.7465 to 0.7717)	0.0080 (0.0065 to 0.0095)	0.0008 (−0.0012 to 0.0029)	0.43
t-E ₂	222	0.7176 (0.7058 to 0.7294)	178	0.7488 (0.7359 to 0.7616)	0.0077 (0.0061 to 0.0092)	0.0005 (−0.0016 to 0.0026)	0.64

CIMT = carotid artery intima-media thickness; o-CEE = oral conjugated equine estrogens; t-E₂ = transdermal 17 β -estradiol.

* Means and 95% CIs for baseline, year 4, and annual slope are derived from linear mixed-effects models for repeated measures (see Methods). Annual slopes are estimated from models and calculated using all values available at baseline and follow-up years 1, 2, 3, and 4.

† Change between baseline and year 4 as annual slope.

Participant Baseline Characteristics

Participants had a mean age of 52.7 years (range, 42 to 58 years) and were an average of 1.4 years after menopause (range, 0.5 to 3.0 years). Of participants reporting, 72% had college degrees or higher and 62% had annual incomes greater than \$60 000 (Table 1). More than 90% of participants reported having never smoked or having stopped smoking at least 12 months earlier, and 79% had no prior use of MHT. None of the baseline characteristics differed significantly among treatment groups except for the high-density lipoprotein cholesterol level, which was lower in the placebo group.

Nonstudy Medication Use

Women were excluded at screening if they reported taking lipid-lowering drugs (statins, fibrates, or high-dose niacin). During the study, some women started antihypertensive ($n = 116$ [16.0%]) or lipid-lowering ($n = 53$ [7.3%]) therapy. Only a few ($n = 39$ [5.4%]) started non-study prescription MHT. Proportions did not differ significantly by treatment group.

Vascular Imaging

Measurements of CIMT were available for at least 1 follow-up visit for 649 participants (89.3%). During the 4-year follow-up, CIMT increased similarly in all 3 groups at a mean rate of 0.0076 mm/y (Table 2 and Figure 2). The difference in rates of change between the o-CEE and placebo groups and the t-E₂ and placebo groups was 0.0008 mm/y (95% CI, −0.0012 to 0.0029 mm/y; $P = 0.43$) and 0.0005 mm/y (CI, −0.0016 to 0.0026 mm/y; $P = 0.64$), respectively. Adjustment for clinical site did not affect the overall results. We also assessed the potential bias if missing data were not missing at random and concluded that results were robust to various patterns of missing data (Supplement).

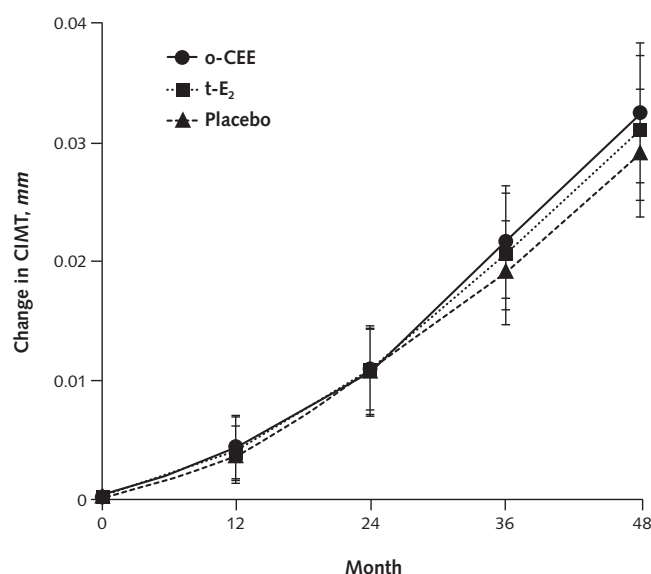
At baseline and 48 months, CAC scores were available for 570 participants (78.4% of those randomly assigned). The CAC score increased in 17.4% of the o-CEE group, 18.9% of the t-E₂ group, and 21.0% of the placebo group, with no significant differences (risk difference for o-CEE vs. placebo group and t-E₂ vs. placebo group, −3.6 percentage points [CI, −11.4 to 4.1 percentage points] and −2.1 percentage points [−10.0 to 5.7 percentage points],

respectively) (Table 3). Results for CAC were also robust to any plausible bias induced by missing data (the Supplement includes sensitivity results).

Serum Estrogen Levels

In the placebo group, neither E₂ nor E₁ levels changed significantly from baseline to 12 months or thereafter (Appendix Figure, available at www.annals.org). In the t-E₂ group compared with placebo, the mean increase in E₂ levels was 138.8 pmol/L (CI, 116.0 to 161.1 pmol/L); that in E₁ levels was smaller (52.0 pmol/L [CI, 21.1 to 85.0 pmol/L]). By contrast, in the o-CEE group compared with placebo, the change in mean E₂ levels (30.1 pmol/L [CI, 7.3 to 53.2 pmol/L]) was low, but E₁ levels increased by 167.9 pmol/L (CI, 135.7 to 199.7 pmol/L). In the subset

Figure 2. Effects of treatment on CIMT.



The change in CIMT (primary end point) from baseline to 12, 24, 36, and 48 mo after randomization by treatment group is shown. Bars represent 95% CIs. All values are derived from the linear mixed-effects model for repeated measurements (see Methods section). CIMT = carotid artery intima-media thickness; o-CEE = oral conjugated equine estrogens; t-E₂ = transdermal 17 β -estradiol.

Table 3. Changes in CAC Score, by Treatment

Treatment	Participants With CAC Change*, n	Risk Difference vs. Placebo (95% CI), percentage points†	P Value
Placebo	217 (21.0)	–	
o-CEE	181 (17.4)	–3.6 (–11.4 to 4.1)	0.36
t-E ₂	172 (18.9)	–2.1 (–10.0 to 5.7)	0.59

CAC = coronary artery calcium; o-CEE = oral conjugated equine estrogens; t-E₂ = transdermal 17 β -estradiol.

* Preset criteria for change in CAC score was an increase in CAC score >0 Agatston units if the baseline CAC score was 0 Agatston units or an increase in CAC score \geq 5 Agatston units if the baseline CAC score was >0 Agatston units. Results from generalized estimating equation model (see Methods section).

† Hormone group minus the placebo group.

of 99 women who continued study medications at 36 and 48 months, the mean levels of both hormones were similar to those of the larger sample shown at baseline and 12 months (Appendix Figure).

Cardiovascular Risk Factors, Including Inflammatory Measures

Table 4 shows the estimated overall changes from baseline in CVD risk factors, averaged across the 4 follow-up visits. Changes in some CVD risk factors were greater with MHT than placebo but many were not (Table 4 and Appendix Figure). Levels of low-density lipoprotein cholesterol decreased with o-CEE, whereas levels of high-density lipoprotein cholesterol, C-reactive protein, and SHBG increased. Levels of total and non-high-density lipoprotein cholesterol and insulin and the Homeostasis Model Assessment of Insulin Resistance score decreased with t-E₂. Variables lacking significant differences in changes from baseline between active treatment and placebo groups included blood pressure and interleukin-6 levels (Table 4).

Menopausal Symptoms

Menopausal symptoms were reported at baseline, 6 months, and annual visits thereafter. At baseline, 85.7% of participants reported hot flashes (43.7% moderate or severe). At 6 months, 28.3% of women in the placebo group reported moderate or severe hot flashes, significantly more than the 4.2% and 7.4% in the o-CEE and t-E₂ groups, respectively ($P < 0.001$). Women receiving placebo continued to report more vasomotor symptoms than those receiving estrogen through the 48-month visit (Appendix Table 1, available at www.annals.org, and Supplement), but differences between the MHT and placebo groups attenuated during the study and were no longer significant for the t-E₂ group compared with the placebo group at 48 months.

AEs

More than 48% of women reported at least 1 AE (49.1%, 47.3%, and 47.6% in the o-CEE, t-E₂, and placebo groups, respectively). The most common AEs were classified as skin and hair changes, including rashes

(15.0%); musculoskeletal, including fractures (13.8%); central nervous system, including headaches (10.9%); and genitourinary and reproductive (9.9%). No class of AE or specific AE was significantly more common in estrogen recipients versus placebo recipients except for vaginal bleeding, which was significantly more common in the pooled treated groups than the placebo group ($P < 0.001$) (Appendix Table 2, available at www.annals.org).

More women in the estrogen groups than in the placebo group had SAEs (9.7% vs. 6.5%), but this difference was not statistically significant ($P = 0.135$) and no particular class or specific type of SAE seemed responsible for this trend. Moreover, the number of participants having potentially hormone-related cardiovascular or neoplastic SAEs was too small ($n = 19$ [2.6%]) to evaluate their statistical significance. These events did not seem unevenly distributed by study group (Appendix Table 2). The sole myocardial infarction occurred in a newly randomly assigned participant before her first dose of the study drug, whereas the 1 death was due to cancer of uncertain origin that had spread throughout the pelvis and uterine wall.

DISCUSSION

In this double-blind, placebo-controlled, randomized 4-year study in recently menopausal women who generally had low risk for CVD, 2 low-dose MHT regimens favorably altered certain CVD risk factors (lipid levels with o-CEE and insulin resistance with t-E₂). Despite these favorable results, the effect of MHT on atherosclerosis progression by arterial imaging was neutral. Neither MHT regimen altered blood pressure, but both relieved vasomotor symptoms.

Prior studies have shown favorable effects of estrogen on CIMT and CAC score. For CIMT, EPAT (Estrogen in the Prevention of Atherosclerosis) (42) showed a regression of CIMT in the MHT group and progression in the placebo group, with a mean difference of 0.0147 mm/y ($P = 0.046$) across groups in those not receiving lipid-lowering medications and 0.0053 mm/y in the whole study. Other studies of CIMT have shown similar magnitudes of change (51, 52).

Several factors may explain our finding of no apparent effect of MHT on CIMT. We selected participants with low risk for CVD and low atherosclerosis burden at entry by excluding candidates with a history of CVD or a CAC score of 50 Agatston units or greater. Because baseline CVD risk and extent of atherosclerosis are excellent predictors of progression, KEOPS selection criteria may have minimized the potential to observe effects of treatment during the study. Prior MHT intervention trials showing reduced progression or regression of CIMT (42, 53–55) have generally enrolled participants with high baseline CIMT, high risk for CVD, or both—the opposite of the selection strategy in KEOPS.

Table 4. Overall Changes in Cardiac Risk Factors, by Treatment*

	Placebo	o-CEE		t-E ₂ †	
	Mean Value (95% CI)	Mean Value (95% CI)	Difference From Placebo (95% CI)	Mean Value (95% CI)	Difference From Placebo (95% CI)
Vital signs					
Systolic BP, mm Hg	−0.56 (−1.73 to 0.61)	−0.60 (−1.87 to 0.67)	−0.04 (−1.77 to 1.68)	−1.26 (−2.56 to 0.05)	−0.70 (−2.45 to 1.06)
Diastolic BP, mm Hg	−1.41 (−2.24 to −0.59)	−0.65 (−1.55 to 0.24)	0.76 (−0.46 to 1.98)	−1.71 (−2.63 to −0.78)	−0.29 (−1.53 to 0.95)
Lipids					
Total cholesterol level					
mmol/L	0.07 (−0.01 to 0.14)	0.02 (−0.06 to 0.10)	−0.05 (−0.15 to 0.06)	−0.08 (−0.16 to 0.01)	−0.14 (−0.25 to −0.03)
mg/dL	2.62 (−0.22 to 5.45)	0.73 (−2.34 to 3.79)	−1.89 (−6.07 to 2.29)	−2.90 (−6.06 to 0.25)	−5.52 (−9.76 to −1.28)
LDL cholesterol level					
mmol/L	0.01 (−0.06 to 0.07)	−0.13 (−0.20 to −0.06)	−0.13 (−0.23 to −0.04)	−0.07 (−0.15 to −0.003)	−0.08 (−0.18 to 0.02)
mg/dL	0.24 (−2.23 to 2.70)	−4.86 (−7.53 to −2.20)	−5.10 (−8.73 to −1.47)	−2.87 (−5.61 to −0.13)	−3.10 (−6.79 to 0.58)
HDL cholesterol level					
mmol/L	0.01 (−0.01 to 0.04)	0.08 (0.05 to 0.11)	0.07 (0.03 to 0.11)	−0.03 (−0.06 to −0.003)	−0.04 (−0.08 to −0.005)
mg/dL	0.45 (−0.55 to 1.45)	3.20 (2.12 to 4.28)	2.75 (1.27 to 4.22)	−1.24 (−2.35 to −0.13)	−1.69 (−3.19 to −0.19)
Triglyceride level					
mmol/L	−0.02 (−0.07 to 0.02)	0.15 (0.10 to 0.20)	0.17 (0.10 to 0.24)	−0.0007 (−0.05 to 0.05)	0.02 (−0.05 to 0.09)
mg/dL	−2.03 (−6.06 to 1.99)	13.11 (8.77 to 17.45)	15.14 (9.22 to 21.06)	−0.06 (−4.52 to 4.40)	1.97 (−4.04 to 7.99)
Non-HDL cholesterol level					
mmol/L	0.06 (−0.001 to 0.12)	−0.07 (−0.13 to 0.002)	−0.13 (−0.22 to −0.03)	−0.05 (−0.12 to 0.02)	−0.11 (−0.20 to −0.02)
mg/dL	2.39 (−0.04 to 4.81)	−2.54 (−5.16 to 0.09)	−4.92 (−8.50 to −1.35)	−1.85 (−4.54 to 0.85)	−4.23 (−7.86 to −0.61)
Insulin-related					
Fasting insulin level, pmol/L	−2.92 (−7.08 to 1.32)	−7.71 (−12.22 to −3.20)	−4.79 (−10.97 to 1.39)	−9.72 (−14.38 to −5.07)	−6.81 (−13.06 to −0.56)
Fasting blood glucose level					
mmol/L	0.09 (0.05 to 0.14)	0.05 (0.008 to 0.10)	−0.03 (−0.10 to 0.03)	0.02 (−0.03 to 0.07)	−0.07 (−0.14 to −0.007)
mg/dL	1.66 (0.85 to 2.46)	1.01 (0.15 to 1.88)	−0.64 (−1.83 to 0.54)	0.33 (−0.56 to 1.22)	−1.33 (−2.53 to −0.13)
HOMA-IR score, unit	−0.09 (−0.22 to 0.04)	−0.29 (−0.43 to −0.15)	−0.19 (−0.38 to −0.003)	−0.33 (−0.47 to −0.19)	−0.23 (−0.43 to −0.04)
Other					
CRP level, nmol/L	5.05 (1.33 to 8.76)	15.52 (11.42 to 19.52)	10.48 (4.95 to 15.91)	5.14 (0.95 to 9.24)	0.09 (−5.52 to 5.62)
IL-6 level, pg/mL	−0.11 (−1.25 to 1.02)	−0.09 (−1.31 to 1.13)	0.02 (−1.65 to 1.69)	−0.49 (−1.75 to 0.78)	−0.37 (−2.07 to 1.33)
SHBG level, nmol/L	−1.55 (−4.09 to 0.99)	37.16 (34.43 to 39.89)	38.71 (34.98 to 42.44)	2.82 (0.01 to 5.64)	4.38 (0.58 to 8.17)

BP = blood pressure; CRP = C-reactive protein; HDL = high-density lipoprotein; HOMA-IR = Homeostasis Model Assessment of Insulin Resistance; IL-6 = interleukin-6; LDL = low-density lipoprotein; o-CEE = oral conjugated equine estrogens; SHBG = sex hormone-binding globulin; t-E₂ = transdermal 17β-estradiol.

* Baseline to mean postrandomization value. Reported mean changes are estimates of the overall changes from baseline, averaged across the 4 follow-up visits. These values were estimated from linear mixed-effects models for repeated measures (see Methods section).

† Patch.

In a WHI follow-up study (31), CAC progression was significantly reduced in estrogen-treated women. In our study, the CAC score increased in only 3.0% and 1.5% fewer women receiving o-CEE and t-E₂, respectively, compared with placebo. When only women with a measurable baseline CAC score (≥ 1 Agatston unit) were considered, CAC progressed in 19% in the o-CEE group, 22% in the t-E₂ group, and 26% in the placebo group with respective differences of 7 and 4 percentage points in this subgroup. However, KEEPS had power to detect with confidence only on the order of a 50% difference. Also, KEEPS' 4-year treatment duration (vs. 7-year follow-up for the WHI CAC study) was probably not sufficient to fully evaluate potential effects.

Finally, KEEPS used lower estrogen doses than prior studies in which a protective effect was observed. The arterial wall is dose-responsive to estrogen. Increasing the dose of oral E₂ from 1 mg/d to 2 to 4 mg/d in young estrogen-deficient women progressively decreased the

CIMT (56). However, mean E₁ and E₂ levels in the o-CEE and t-E₂ groups were in the range seen in cycling follicular-phase women, and our observation of significant symptom relief suggests that doses used were clinically relevant. Endothelial function also may respond better to higher estrogen doses (57, 58).

An important strength of our study is the high precision of the CIMT results, including narrow 95% CIs for differences in rates of progression that exclude those of 0.002 mm/y or more. This finding suggests that MHT may not clinically meaningfully reduce CIMT progression. Of note, these results are specific to the KEEPS population and the duration, types, and doses of MHT used in this study and should not be extrapolated beyond them.

Another strength is that our study directly compared oral versus transdermal routes of estrogen dosing in MHT. To our knowledge, KEEPS is the largest and longest trial comparing these 2 treatment methods with each other and with placebo. Although the effects of oral and transdermal

treatments on the results of atherosclerosis imaging and menopausal symptoms over 4 years were similar, the effects on the measured CVD risk factors were not. Changes in plasma lipid levels were generally more favorable with o-CEE, whereas glycemic effects seemed better with t-E₂. Further studies are needed to pursue differences and similarities between these routes of estrogen administration and their relevance to CVD pathogenesis.

Our study has limitations. Duration and size were insufficient to examine the implications of clinical CVD or other AEs. Our power for the CAC score end point was also limited. Because the primary focus was on atherosclerosis and traditional CVD risk factors, our study did not investigate other CVD risk factors, such as oxidation, inflammation, and thrombosis. KEEPS studied primarily well-educated white women, who are not fully representative of the general postmenopausal population of the United States. Finally, KEEPS examined relatively healthy women and may not be generalizable to women at greater cardiovascular risk due to smoking, obesity, type 2 diabetes, uncontrolled hypertension, dyslipidemia, or a history of CVD events.

The North American Menopause Society and other groups support the use of MHT for relief of menopausal symptoms (59) in women at low risk for known complications of this therapy. Results of KEEPS are consistent with this recommendation and provide novel information that, in recently menopausal women at low cardiovascular risk, 4 years of MHT neither increases nor decreases atherosclerosis progression as measured by CIMT or CAC score. To the extent that these imaging methods predict CVD events, our findings suggest that MHT neither is a risk nor is protective in the population studied.

In summary, in recently postmenopausal women with low CVD risk, 4 years of MHT with low-dose oral or transdermal estrogen, with cyclic oral progesterone, reduced menopausal vasomotor symptoms without negatively affecting blood pressure. Although some markers for CVD improved, MHT neither improved nor worsened atherosclerosis progression. The long-term effects of early initiation of MHT on risk for CVD events are uncertain.

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Note: Drs. Harman and Black had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Reproducible Research Statement: *Study protocol and statistical code:* Available at www.KEEPStudy.org. *Data set:* Qualified investigators may request selected data sets for specific projects approved by the KEEPS Continuation Committee by completing the application available at www.KEEPStudy.org.

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References

1. Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB, et al; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Executive summary: heart disease and stroke statistics—2012 update: a report from the American Heart Association. *Circulation*. 2012;125:188-97. [PMID: 22215894] doi:10.1161/CIR.0b013e3182456d46
2. Writing Group for the PEPI Trial. Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women. The Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial. *JAMA*. 1995;273:199-208. [PMID: 7807658]
3. Herrington DM, Werbel BL, Riley WA, Pusser BE, Morgan TM. Individual and combined effects of estrogen/progestin therapy and lovastatin on lipids and

flow-mediated vasodilation in postmenopausal women with coronary artery disease. *J Am Coll Cardiol*. 1999;33:2030-7. [PMID: 10362210]

4. Futterman LG, Lemberg L. Lp(a) lipoprotein—an independent risk factor for coronary heart disease after menopause. *Am J Crit Care*. 2001;10:63-7. [PMID: 11153187]

5. Störk S, von Schacky C, Angerer P. The effect of 17 β -estradiol on endothelial and inflammatory markers in postmenopausal women: a randomized, controlled trial. *Atherosclerosis*. 2002;165:301-7. [PMID: 12417281]

6. Guzik-Salobir B, Keber I, Seljeflot I, Arnesen H, Vrabec L. Combined hormone replacement therapy improves endothelial function in healthy postmenopausal women. *J Intern Med*. 2001;250:508-15. [PMID: 11902819]

7. Wakatsuki A, Okatani Y, Ikenoue N, Fukaya T. Effect of medroxyprogesterone acetate on endothelium-dependent vasodilation in postmenopausal women receiving estrogen. *Circulation*. 2001;104:1773-8. [PMID: 11591613]

8. Kawecka-Jaszcz K, Czarnecka D, Olszanecka A, Rajzer M, Jankowski P. The effect of hormone replacement therapy on arterial blood pressure and vascular compliance in postmenopausal women with arterial hypertension. *J Hum Hypertens*. 2002;16:509-16. [PMID: 12080436]

9. Moreau KL, Donato AJ, Seals DR, DeSouza CA, Tanaka H. Regular exercise, hormone replacement therapy and the age-related decline in carotid arterial compliance in healthy women. *Cardiovasc Res*. 2003;57:861-8. [PMID: 12618248]

10. Harvey PJ, Wing LM, Savage J, Molloy D. The effects of different types and doses of oestrogen replacement therapy on clinic and ambulatory blood pressure and the renin-angiotensin system in normotensive postmenopausal women. *J Hypertens*. 1999;17:405-11. [PMID: 10100079]

11. Scuteri A, Bos AJ, Brant LJ, Talbot L, Lakatta EG, Fleg JL. Hormone replacement therapy and longitudinal changes in blood pressure in postmenopausal women. *Ann Intern Med*. 2001;135:229-38. [PMID: 11511137]

12. Steiner AZ, Hodis HN, Lobo RA, Shoupe D, Xiang M, Mack WJ. Postmenopausal oral estrogen therapy and blood pressure in normotensive and hypertensive subjects: the Estrogen in the Prevention of Atherosclerosis Trial. *Menopause*. 2005;12:728-33. [PMID: 16278616]

13. Ichikawa J, Sumino H, Ichikawa S, Ozaki M. Different effects of transdermal and oral hormone replacement therapy on the renin-angiotensin system, plasma bradykinin level, and blood pressure of normotensive postmenopausal women. *Am J Hypertens*. 2006;19:744-9. [PMID: 16814131]

14. Bush TL, Cowan LD, Barrett-Connor E, Criqui MH, Karon JM, Wallace RB, et al. Estrogen use and all-cause mortality. Preliminary results from the Lipid Research Clinics Program Follow-Up Study. *JAMA*. 1983;249:903-6. [PMID: 6823043]

15. Stampfer MJ, Colditz GA, Willett WC, Manson JE, Rosner B, Speizer FE, et al. Postmenopausal estrogen therapy and cardiovascular disease. Ten-year follow-up from the Nurses' Health Study. *N Engl J Med*. 1991;325:756-62. [PMID: 1870648]

16. Ettinger B, Friedman GD, Bush T, Quesenberry CP Jr. Reduced mortality associated with long-term postmenopausal estrogen therapy. *Obstet Gynecol*. 1996;87:6-12. [PMID: 8532268]

17. Grodstein F, Manson JE, Colditz GA, Willett WC, Speizer FE, Stampfer MJ. A prospective, observational study of postmenopausal hormone therapy and primary prevention of cardiovascular disease. *Ann Intern Med*. 2000;133:933-41. [PMID: 11119394]

18. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, et al; 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;288:321-33. [PMID: 12117397]

19. Manson JE, Hsia J, Johnson KC, Rossouw JE, Assaf AR, Lasser NL, et al; Women's Health Initiative Investigators. Estrogen plus progestin and the risk of coronary heart disease. *N Engl J Med*. 2003;349:523-34. [PMID: 12904517]

20. Anderson GL, Limacher M, Assaf AR, Bassford T, Beresford SA, Black H, et al; Women's Health Initiative Steering Committee. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial. *JAMA*. 2004;291:1701-12. [PMID: 15082697]

21. Hsia J, Criqui MH, Rodabough RJ, Langer RD, Resnick HE, Phillips LS, et al; Women's Health Initiative Investigators. Estrogen plus progestin and the risk of peripheral arterial disease: the Women's Health Initiative. *Circulation*. 2004;109:620-6. [PMID: 14769684]

22. Grodstein F, Clarkson TB, Manson JE. Understanding the divergent data on postmenopausal hormone therapy. *N Engl J Med*. 2003;348:645-50. [PMID: 12584376]

23. Manson JE, Chlebowski RT, Stefanick ML, Aragaki AK, Rossouw JE, Prentice RL, et al. Menopausal hormone therapy and health outcomes during the intervention and extended poststopping phases of the Women's Health Initiative randomized trials. *JAMA*. 2013;310:1353-68. [PMID: 24084921] doi: 10.1001/jama.2013.278040

24. Mendelsohn ME, Karas RH. Molecular and cellular basis of cardiovascular gender differences. *Science*. 2005;308:1583-7. [PMID: 15947175]

25. Naftolin F, Taylor HS, Karas R, Brinton E, Newman I, Clarkson TB, et al; Women's Health Initiative. The Women's Health Initiative could not have detected cardioprotective effects of starting hormone therapy during the menopausal transition. *Fertil Steril*. 2004;81:1498-501. [PMID: 15193467]

26. Clarkson TB. Estrogen effects on arteries vary with stage of reproductive life and extent of subclinical atherosclerosis progression. *Menopause*. 2007;14:373-84. [PMID: 17438515]

27. Brinton EA, Hodis HN, Merriam GR, Harman SM, Naftolin F. Can menopausal hormone therapy prevent coronary heart disease? *Trends Endocrinol Metab*. 2008;19:206-12. [PMID: 18450469] doi:10.1016/j.tem.2008.03.002

28. Harman SM, Brinton EA. Biphasic effects of hormone treatment on risk of cardiovascular disease: Resolving the paradox in postmenopausal women. *Menopausal Med*. 2009;17:S5-8.

29. Hsia J, Langer RD, Manson JE, Kuller L, Johnson KC, Hendrix SL, et al; Women's Health Initiative Investigators. Conjugated equine estrogens and coronary heart disease: the Women's Health Initiative. *Arch Intern Med*. 2006;166:357-65. [PMID: 16476878]

30. Rossouw JE, Prentice RL, Manson JE, Wu L, Barad D, Barnabei VM, et al. Postmenopausal hormone therapy and risk of cardiovascular disease by age and years since menopause. *JAMA*. 2007;297:1465-77. [PMID: 17405972]

31. Manson JE, Allison MA, Rossouw JE, Carr JJ, Langer RD, Hsia J, et al; WHI and WHI-CACS Investigators. Estrogen therapy and coronary-artery calcification. *N Engl J Med*. 2007;356:2591-602. [PMID: 17582069]

32. Arad Y, Spadaro LA, Goodman K, Lledo-Perez A, Sherman S, Lerner G, et al. Predictive value of electron beam computed tomography of the coronary arteries. 19-month follow-up of 1173 asymptomatic subjects. *Circulation*. 1996;93:1951-3. [PMID: 8640967]

33. Hodis HN, Mack WJ, LaBree L, Selzer RH, Liu CR, Liu CH, et al. The role of carotid arterial intima-media thickness in predicting clinical coronary events. *Ann Intern Med*. 1998;128:262-9. [PMID: 9471928]

34. Kennedy J, Shavelle R, Wang S, Budoff M, Detrano RC. Coronary calcium and standard risk factors in symptomatic patients referred for coronary angiography. *Am Heart J*. 1998;135:696-702. [PMID: 9539488]

35. Greenland P, Smith SC Jr, Grundy SM. Improving coronary heart disease risk assessment in asymptomatic people: role of traditional risk factors and non-invasive cardiovascular tests. *Circulation*. 2001;104:1863-7. [PMID: 11591627]

36. Raggi P, Cooil B, Shaw LJ, Aboulhson J, Takasu J, Budoff M, et al. Progression of coronary calcium on serial electron beam tomographic scanning is greater in patients with future myocardial infarction. *Am J Cardiol*. 2003;92:827-9. [PMID: 14516885]

37. Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzer M. Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. *Circulation*. 2007;115:459-67. [PMID: 17242284]

38. Folsom AR, Kronmal RA, Detrano RC, O'Leary DH, Bild DE, Bluemke DA, et al. Coronary artery calcification compared with carotid intima-media thickness in the prediction of cardiovascular disease incidence: the Multi-Ethnic Study of Atherosclerosis (MESA). *Arch Intern Med*. 2008;168:1333-9. [PMID: 18574091] doi:10.1001/archinte.168.12.1333

39. Crouse JR 3rd, Grobbee DE, O'Leary DH, Bots ML, Evans GW, Palmer MK, et al; METEOR Study Group. Carotid intima-media thickness in low-risk individuals with asymptomatic atherosclerosis: baseline data from the METEOR study. *Curr Med Res Opin*. 2007;23:641-8. [PMID: 17355745]

40. Raggi P, Callister TQ, Shaw LJ. Progression of coronary artery calcium and risk of first myocardial infarction in patients receiving cholesterol-lowering therapy. *Arterioscler Thromb Vasc Biol*. 2004;24:1272-7. [PMID: 15059806]

41. Houslay ES, Cowell SJ, Prescott RJ, Reid J, Burton J, Northridge DB, et al; Scottish Aortic Stenosis and Lipid Lowering Therapy, Impact on Regression trial Investigators. Progressive coronary calcification despite intensive lipid-lowering treatment: a randomised controlled trial. *Heart*. 2006;92:1207-12. [PMID: 16449511]

42. Hodis HN, Mack WJ, Lobo RA, Shoupe D, Sevanian A, Mahrer PR, et al; Estrogen in the Prevention of Atherosclerosis Trial Research Group. Estrogen in the prevention of atherosclerosis. A randomized, double-blind, placebo-controlled trial. *Ann Intern Med.* 2001;135:939-53. [PMID: 11730394]
43. Rumberger JA, Schwartz RS, Simons DB, Sheedy PF 3rd, Edwards WD, Fitzpatrick LA. Relation of coronary calcium determined by electron beam computed tomography and lumen narrowing determined by autopsy. *Am J Cardiol.* 1994;73:1169-73. [PMID: 8203333]
44. Nasir K, Raggi P, Rumberger JA, Braunstein JB, Post WS, Budoff MJ, et al. Coronary artery calcium volume scores on electron beam tomography in 12,936 asymptomatic adults. *Am J Cardiol.* 2004;93:1146-9. [PMID: 15110208]
45. Harman SM, Brinton EA, Cedars M, Lobo R, Manson JE, Merriam GR, et al. KEEPS: the Kronos Early Estrogen Prevention Study [Editorial]. *Climacteric.* 2005;8:3-12. [PMID: 15804727]
46. Selzer RH, Hodis HN, Kwong-Fu H, Mack WJ, Lee PL, Liu CR, et al. Evaluation of computerized edge tracking for quantifying intima-media thickness of the common carotid artery from B-mode ultrasound images. *Atherosclerosis.* 1994;111:1-11. [PMID: 7840805]
47. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M Jr, Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol.* 1990;15:827-32. [PMID: 2407762]
48. Stanczyk FZ, Jurow J, Hsing AW. Limitations of direct immunoassays for measuring circulating estradiol levels in postmenopausal women and men in epidemiologic studies. *Cancer Epidemiol Biomarkers Prev.* 2010;19:903-6. [PMID: 20332268] doi:10.1158/1055-9965.EPI-10-0081
49. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and β -cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* 1985;28:412-9. [PMID: 3899825]
50. MacMahon S, Sharpe N, Gamble G, Hart H, Scott J, Simes J, et al. Effects of lowering average of below-average cholesterol levels on the progression of carotid atherosclerosis: results of the LIPID Atherosclerosis Substudy. LIPID Trial Research Group. *Circulation.* 1998;97:1784-90. [PMID: 9603532]
51. Zou G. A modified poisson regression approach to prospective studies with binary data. *Am J Epidemiol.* 2004;159:702-6. [PMID: 15033648]
52. Mercuri M, Bond MG, Sirtori CR, Veglia F, Crepaldi G, Feruglio FS, et al. Pravastatin reduces carotid intima-media thickness progression in an asymptomatic hypercholesterolemic mediterranean population: the Carotid Atherosclerosis Italian Ultrasound Study. *Am J Med.* 1996;101:627-34. [PMID: 9003110]
53. ARBITER-2: evidence that targeting HDL cholesterol slows atherosclerosis. *Cardiovasc J S Afr.* 2005;16:61. [PMID: 15778778]
54. Espeland MA, Applegate W, Furberg CD, Lefkowitz D, Rice I, Hunninghake D. Estrogen replacement therapy and progression of intimal-medial thickness in the carotid arteries of postmenopausal women. ACAPS Investigators. Asymptomatic Carotid Atherosclerosis Progression Study. *Am J Epidemiol.* 1995;142:1011-9. [PMID: 7485045]
55. Liebson PR. ARBITER-6 HALTS and lipid concentrations and heart failure incidence: Framingham Heart Study. *Prev Cardiol.* 2010;13:141-4. [PMID: 20626670] doi:10.1111/j.1751-7141.2010.00069.x
56. Ostberg JE, Storry C, Donald AE, Attar MJ, Halcox JP, Conway GS. A dose-response study of hormone replacement in young hypogonadal women: effects on intima media thickness and metabolism. *Clin Endocrinol (Oxf).* 2007;66:557-64. [PMID: 17371475]
57. Lieberman EH, Gerhard MD, Uehata A, Walsh BW, Selwyn AP, Ganz P, et al. Estrogen improves endothelium-dependent, flow-mediated vasodilation in postmenopausal women. *Ann Intern Med.* 1994;121:936-41. [PMID: 7978718]
58. Vehkavaara S, Hakala-Ala-Pietilä T, Virkamäki A, Bergholm R, Ehnholm C, Hovatta O, et al. Differential effects of oral and transdermal estrogen replacement therapy on endothelial function in postmenopausal women. *Circulation.* 2000;102:2687-93. [PMID: 11094033]
59. North American Menopause Society. Estrogen and progestogen use in peri- and postmenopausal women: March 2007 position statement of The North American Menopause Society. *Menopause.* 2007;14:168-82. [PMID: 17259911]

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APPENDIX 1: INSTITUTIONAL REVIEW BOARD NUMBERS FOR KEEPS INSTITUTIONS

The central KEEPS and Phoenix KEEPS (institutional review board [IRB] protocol by the Western IRB): Study Number: 1058663 and Western IRB Protocol Number: 20040792KEEPS (main study and cognitive substudy) 10-02980 and MDBHAS 11-05383

Brigham and Women's Hospital (Partners): 2004-P-002144 BWH

Mayo Clinic: 2241-04

Columbia University: AAAA-8062

Yale University: 0409027022

University of Utah: 13257

Einstein/Montefiore: 04-08-213

University of Wisconsin: H-2005-0059

University of California, San Francisco: KEEPS (main study and cognitive substudy) 10-02980

University of Washington: 26702

Veterans Affairs Puget Sound Health Care System: 01048

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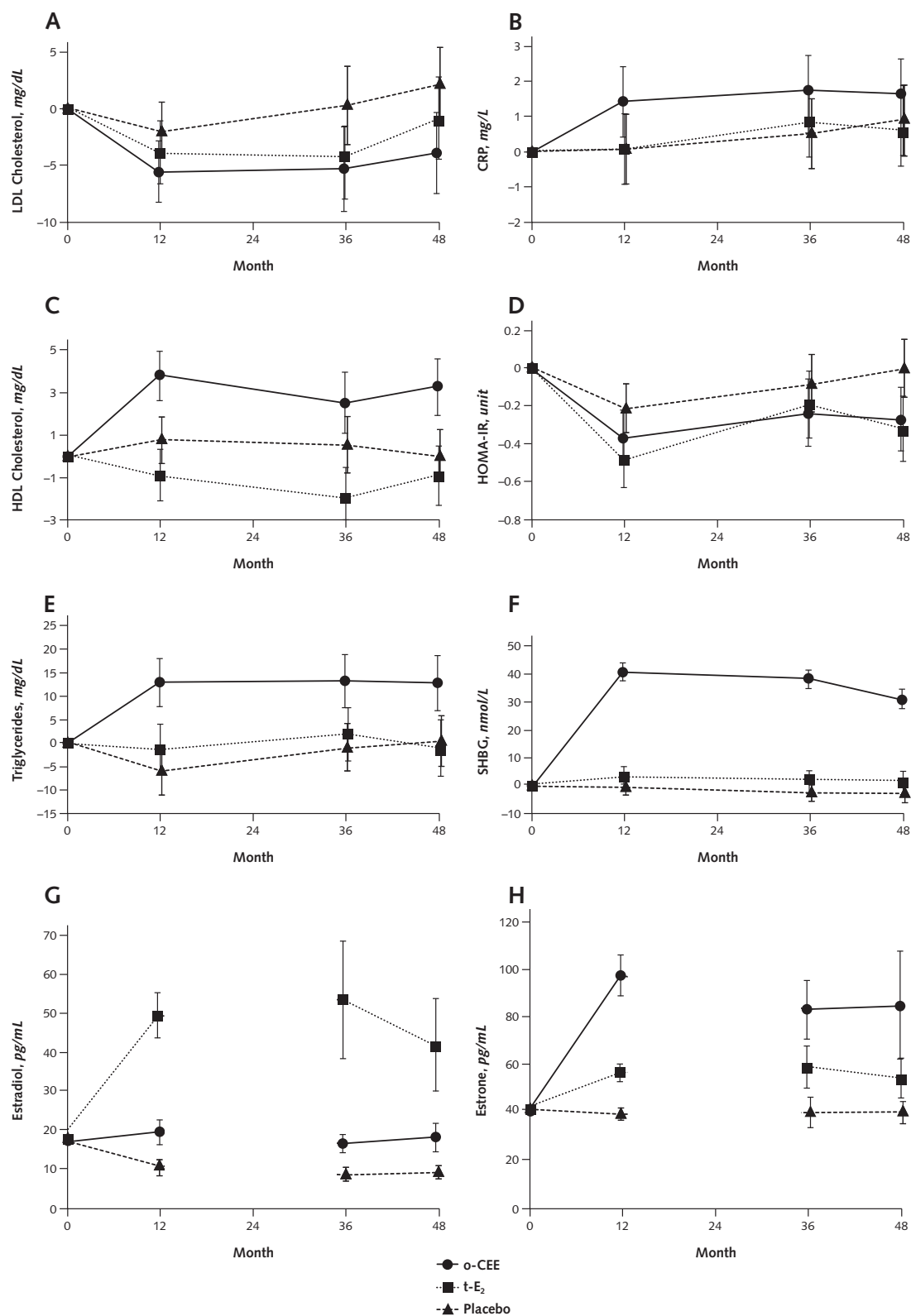
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Appendix Figure. Changes in laboratory values, by treatment and duration.



Continued on following page

CRP = C-reactive protein; HDL = high-density lipoprotein; HOMA-IR = Homeostasis Model Assessment of Insulin Resistance; LDL = low-density lipoprotein; o-CEE = oral conjugated equine estrogens; SHBG = sex hormone-binding globulin; t-E₂ = transdermal 17β-estradiol. A to F. Estimated means with bars representing 95% CIs are shown for changes from baseline in measured variables. Figures are included only for variables for which at least 1 of the treatment groups differed significantly from placebo (Table 3). All values are derived from repeated measures models. Numbers for each time point vary somewhat across measurements. G and H. Plotted values are estimates based on the linear mixed-effects model fit to all observed data (see Methods section). Bars represent 95% CIs around estimated means (all values are derived from repeated measures model). Levels at baseline and 12 mo were assessed on a random sample of approximately 60% of participants (excluding those who did not complete follow-up and those without samples at these times). The line discontinuity serves to indicate that 36- and 48-mo assays were done on a random subsample of 99 participants with baseline and 12-mo values who completed the study while receiving study medications. Means for the latter subset of 99 participants at baseline and 12 mo are similar to those shown for the larger samples.

Appendix Table 1. Percentage of Women Reporting Moderate to Severe Vasomotor Symptoms (Hot Flashes), by Study Visit and Treatment Group

Treatment	Baseline	6 mo	12 mo	24 mo	36 mo	48 mo
Participants who completed the symptom scale, n						
o-CEE	230	191	198	179	175	173
t-E ₂	222	189	187	168	170	170
Placebo	275	226	225	215	210	211
Participants who reported symptoms, %						
o-CEE	43.5	4.2	4.0	6.7	5.7	7.5
t-E ₂	41.4	7.4	9.6	10.1	10.0	12.9
Placebo	45.8	28.3	20.9	19.1	20.0	16.6
P value for difference from placebo using repeated measures models						
o-CEE	0.59	<0.001	<0.001	<0.001	<0.001	0.003
t-E ₂	0.32	<0.001	0.001	0.012	0.005	0.31

o-CEE = oral conjugated equine estrogens; t-E₂ = transdermal 17β-estradiol.

Appendix Table 2. Number of Women With AEs, by Type and Treatment Group

Event	o-CEE	t-E ₂	Placebo
Women with overall AEs, n			
Any AEs	113	105	131
SAEs*	24	20	18
Deaths	1	0	0
Women with AEs of special interest requiring emergency treatment, n†			
Cardiovascular disease-related			
Myocardial infarction	0	1	0
Venous thrombotic disease	0	1	1
Stroke	0	0	0
Neoplasia/hyperplasia			
Breast cancer	3	3	2
Endometrial cancer	2	1	0
Endometrial hyperplasia	2	1	1
Vaginal bleeding‡	78	92	25

AE = adverse event; o-CEE = oral conjugated equine estrogens; SAE = severe adverse event; t-E₂ = transdermal 17β-estradiol.

* Within categories, women were counted only once (≥1 event). They could have had multiple events across categories. For SAEs, by counting total events rather than participants with at least 1 event, numbers by treatment were 29 for the o-CEE group, 26 for the t-E₂ group, and 18 for the placebo group due to women reporting multiple SAEs.

† AEs of special interest were conditions reported as increased with menopausal hormone therapy in previous studies.

‡ Bleeding outside expected time.