Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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Vascular Effects of Early versus Late Postmenopausal Treatment with Estradiol

Supplementary Appendix

Table of Contents

I. ELITE Research Group Members 1
II. Supplementary Methods 2
III. Supplementary Results 7
IV. Supplementary Tables 7
V. References 16

I. ELITE Research Group Members

ELITE Research Group members are the following, with primary trial investigators designated by an asterisk: Study Chair: Howard N. Hodis, M.D.* Clinical Center Staff: Liny Zurbrugg, R.N. (clinic coordinator), Esther Bhimani, M.A., Martha Charlson, R.D., Irma Flores, M.A., Martha Huerta, Thelma LaBree, M.A., Sonia Lavender, M.A., Violetta McElreath, R.N., Janie Teran, Philip Zurbrugg. Ultrasound Image Acquisition and Processing Laboratory: Robert H. Selzer, M.S.* (director), Yanjie Li, M.D. (technical director), Mei Feng, M.D., Lora Whitfield-Maxwell, R.N., Ming Yan, M.D., Ph.D. Data Coordinating Center: Wendy J. Mack, Ph.D.* (director), Stanley P. Azen, Ph.D.,* Farzana Choudhury, M.S., Carlos Carballo, Laurie Dustin, M.S., Adrian Herbert, Naoko Kono, M.P.H., George Martinez, Olga Morales. Atherosclerosis Research Unit Core Lipid/Lipoprotein Laboratory: Juliana Hwang-Levine, PharmD* (director), Gail Izumi, C.L.S., Arletta Ramirez, CLS, Luci Rodriguez. Gynecology and Mammography: Donna Shoupe, M.D.,* Juan C. Felix, M.D., Pulin Sheth, M.D., Mary Yamashita, M.D. USC Reproductive Endocrinology Research Laboratory: Frank Z. Stanczyk, Ph.D. (director). USC Endocrinology Laboratory: Carole Spencer. Cognition and Mood: Victor W. Henderson, M.D., M.S.,* Carol A. McCleary, Ph.D., Janet A. St. John, M.P.H. Kaiser Permanente Medical Center Recruitment Site: Malcolm G. Munro, M.D. Cardiac Computed Tomography Core Center: Matthew J. Budoff, M.D. (director), Lily Honoris, M.D., Chris Dailing, Sivi Carson. Apolipoprotein E Genotyping: Hooman Allayee, Ph.D. Data Safety Monitoring Board: Leon Speroff, M.D. (chair), Robert H. Knopp, M.D.

(deceased), Richard H. Karas, M.D., Joan Hilton, Ph.D., Judy Hannah (ex-officio, National Institute on Aging).

II. Supplementary Methods

Exclusion criteria

ELITE exclusion criteria were the following:[1] indeterminate time-since-menopause; fasting plasma triglyceride level >500 mg/dl (5.65 mmol/L); diabetes mellitus or fasting serum glucose >140 mg/dl; serum creatinine >2.0 mg/dl (177 mmol/L); diastolic blood pressure >110 mmHg or systolic blood pressure >160 mmHg; untreated thyroid disease; liver disease; life-threatening disease with prognosis <5 years; history of deep vein thrombosis (DVT) or pulmonary embolism (PE); history of breast cancer; current postmenopausal hormone therapy within 1 month of screening.

Assessment of atherosclerosis progression

Subclinical atherosclerosis progression measured as carotid artery intima-media thickness (CIMT) change was the primary outcome. Carotid artery ultrasound image acquisition and CIMT measurements were conducted using standardized procedures and technology developed specifically for longitudinal atherosclerosis measurements (Patents 2005, 2006, 2011).[2-11] Using a linear array 7.5 MHz transducer attached to a Siemens Acuson CV70 (Mountain View, California) ultrasound imaging system, high-resolution B-mode ultrasound carotid artery images were acquired. Ultrasound images were simultaneously recorded along with a single lead electrocardiogram (ECG). The carotid artery was imaged transversely and then longitudinally with the jugular vein stacked above the carotid artery. Internal anatomical landmarks used for reproducing probe angulation were included in all images. Using a split-screen system designed for repeat image acquisition for longitudinal studies, the baseline image for each individual was used as an online guide for follow-up examinations. For each individual, depth of field, gain, input power, dynamic range, monitor intensity and all other instrumentation settings used at the baseline examination were maintained for all follow-up ultrasound image acquisition. This procedure establishes instrument setup standardization that encompasses the full dynamic

range of the ultrasound echo across all examinations within the same participant. Employing the foregoing standardized procedures results in reproducible imaging and processing of the same portion of the arterial wall at each examination necessary for accurately tracking atherosclerosis change.[2-9] Using automated computerized edge detection software, far wall CIMT was measured at sub-pixel resolution (Patents 2005, 2006, 2011).[8,9] Just proximal to the carotid artery bulb at the same point in the cardiac cycle standardized to the ECG signal, CIMT was determined as the average of 70 to 100 measurements between the intima-lumen and media-adventitia echo interfaces along a 1 cm length determined by an electronic ruler. This procedure standardizes the timing, location and distance over which CIMT is measured, ensuring comparability within and across participants.[8,9] This CIMT method of acquisition and measurement is correlated with the change in coronary artery disease assessed by serial quantitative coronary angiography[10] and is predictive of clinical cardiovascular events.[11] The coefficient of variation of repeated CIMT measurements is typically <3% and often approaches 1%.

Cardiac computed tomography

Coronary artery calcium (CAC) and cardiac computed tomography angiography (CCTA) for measurement of coronary artery stenosis were assessed with cardiac computed tomography (CT). In a single session, participants underwent non-contrast CAC followed by contrast CCTA scans using a cardiac GE 64 slice multi-detector computed tomography (64 MDCT) scanner. For non-contrast CT CAC scanning, prospective ECG gating at 70-80% of the R-R interval was performed according to standardized procedures.[12] The following CT imaging parameters were used: tube voltage = 120 killivolts (kV); tube current = 150 milliamperes (mA); gantry rotation speed = 0.35 seconds; slice thickness = 2.5 mm; rows = 64; range = 128-160 mm. A Field of View (FOV) of 25 cm included the heart from below the carina to below the diaphragm. During a 5 ml/sec intravenous iodinated contrast infusion, CCTA images were collected according to standardized procedures.[13] One minute prior to CCTA scanning, participants were given sublingual nitroglycerin to improve epicardial vasodilation. If required, a β-blocker was administered to maintain heart rate between 50-70 beats per minute. Prospective ECG gating at 70-80% of the R-R interval was performed with the following CT parameters: tube voltage = 100 kV for participants who weighed <85 kg and 120 kV for participants who weighed >85 kg,

tube current = 300-600 mA (based on body habitus), gantry rotation speed = 0.35 seconds, slice thickness = 0.5-0.625 mm, rows = 64, range = 128-160 mm.

The non-contrast CT images were used to calculate CAC with standard methods as previously described.[12] CAC was defined as a plaque with a density of >130 HU over a minimum of 3 contiguous pixels (area 1.02 mm^2). Lesion score was determined by multiplying lesion area by maximum HU density within this area.[14] By summing individual lesion scores from each of 4 anatomic sites (right coronary, left main, left anterior descending, left circumflex), a total CAC score was calculated.

For CCTA image analysis, thin CT sections (0.5-0.625 mm) were transferred to a workstation (GE Advantage Workstation 4.4, GE Healthcare, Milwaukee, Wisconsin). For each coronary artery segment, curved maximum intensity projection (MIP) was performed at the end diastolic frame or the frame with the least motion artifact. Areas of abnormalities were identified from the curved MIP and the points of minimum luminal diameter determined. Semi-automated software (GE Advantage Workstation 4.4, GE Healthcare, Milwaukee, Wisconsin) was used for multi-planar reformatting to generate cross-sectional images of coronary segments. This yields a vessel centerline using the full-width-half maximum standard method to delineate the contrast-filled vessel. At any cross-section along the vessel centerline, maximum and minimum diameters were automatically determined. Semi-automatic software was used to reconstruct a cross-sectional 5 mm MIP image and to outline the intimal surface providing cross-sectional vessel area. In cases where coronary segments were normal, the most proximal cross-sectional image was used for analysis. CT measurements were obtained from the most visible images such as axial source or multi-planar images of the long axis at each site of the coronary arteries. The modified 15-segment model of the American Heart Association[15] was used in the evaluation of all of the data sets: The right coronary artery defined to include segments 1-4; the left main artery and the left anterior descending artery to include segments 5-10; the left circumflex artery to include segments 11-15; and, if present, the intermediate artery was designated as segment 16. Side branches were used as anatomical landmarks to define coronary segments.

In all assessable coronary segments, the degree of coronary artery stenosis was assessed by using axial images, multi-planar reconstructions and curved MIPs to assess the degree of luminal narrowing. Segments with 1-25% diameter narrowing were defined as minimal stenosis, 26%-50% diameter narrowing was defined as mild stenosis, 51-75% diameter narrowing was defined as moderate stenosis and >75% diameter narrowing was defined as severe stenosis. Since spatial resolution of CCTA cannot achieve the precision of quantitative coronary angiography, percent diameter stenosis and not area stenosis was determined. Even if plaque was eccentric, the most narrowed diameter in each coronary segment was determined. A segment stenosis score was determined from the degree of stenosis in each coronary segment (0=no plaque, 1=1-25% stenosis, 2=26-50% stenosis, 3=51-75% stenosis, 4=>75% stenosis). A Total Stenosis Score (TSS) ranging from 0 to 60 was calculated by summing the extent scores of all 15 individual coronary segments.

In all affected coronary segments, plaque quantification was determined by manually tracing area of the plaque in each CT image slice. The area of each coronary plaque visualized in a minimum of 2 adjacent slices (reconstructed slice thickness 0.6 mm) was determined in all affected slices and total plaque per coronary segment was summed. A semi-quantitative plaque score was determined for each participant.[16] Each plaque was multiplied by 1 for small plaque (defined as < 1 mm in diameter perpendicular to the artery), 2 for medium plaque (defined as 1-2 mm in diameter perpendicular to the artery) and 3 for large plaque (defined as >2 mm in diameter perpendicular to the artery). The number of evaluable coronary segments with individual plaque scores was summed to determine a Total Plaque Score (TPS; maximum plaque score = 45 [score of 3 for all 15 segments]).

Follow-up

At each clinic visit, study product compliance, non-study medications, nutritional supplement intake, clinical adverse events and vital signs were ascertained. Participants completed flushing, vaginal bleeding and cramping, breast pain and 3-day dietary (Nutrition Scientific) diaries prior to each clinic visit. At each 6-month visit, fasting (8-12 hours) blood samples were obtained for sex hormone,[17] lipid[2] and hemoglobin A1c[2] levels and questionnaires covering medical history, smoking, alcohol intake and physical activity were administered and waist:hip circumferences

measured. Safety laboratories were obtained annually along with a 12-lead electrocardiogram. Annually and as indicated, mammography and gynecological examinations including Pap smear, transvaginal uterine ultrasound and endometrial biopsy (if indicated) were performed. All baseline examinations, questionnaires and laboratory determinations were conducted prior to randomization.

Statistics

Generalized linear models were used, specifying the CAC and CCTA measures as dependent variables. The primary independent variables of interest were indicator variables for treatment group and postmenopause stratum. A treatment-by-menopause stratum interaction tested whether the treatment group differences differed by time-since-menopause. The 2 randomization stratification variables (type of menopause and dichotomous baseline CIMT) were included as covariates. To account for the fact that the end-of-study visit differed across participants, indicator variables for the study visit at which the CAC and CCTA measures were obtained were included as covariates. Treatment group comparisons were performed for participants who completed the CAC and/or CCTA end points no more than 6 months after the final clinic visit and were at least 80% adherent by pill count.

Safety analysis and evaluation of adverse events were performed on all randomized participants using exact methods, comparing event proportions among the four study groups [early postmenopause-estradiol treated; early postmenopause-placebo treated; late postmenopause-estradiol treated; late postmenopause-placebo treated]) (Table S8). The following major clinical events were evaluated and compared: 1) cardiovascular events, including fatal/nonfatal myocardial infarction (MI), silent MI and sudden death, hospitalization for unstable angina and coronary revascularization procedures (coronary artery bypass grafting and percutaneous transluminal coronary angioplasty); 2) stroke; 3) venous thromboembolism (DVT and PE); 4) cancer (breast, uterine, ovarian, gastrointestinal, lung); 5) bone fractures; and, 6) all-cause mortality and noncoronary mortality.

III. Supplementary Results

Within postmenopause stratum, baseline characteristics did not differ by completion of CT outcomes, except participants in the early postmenopause group who did not have CT outcomes were less educated (61.5% vs. 78.4% college graduates) and had more previous hormone therapy (59.6% vs. 45.5%) than participants who did have CT outcomes, while late postmenopause participants without CT outcomes had greater hypertensive medication use (34.2% vs. 24.8%) than participants who did have CT outcomes.

IV. Supplementary Tables

Table S1. Baseline demographic, clinical, laboratory and ultrasound characteristics of all randomized participants

Table S2. Mean carotid artery intima-media thickness progression per protocol adherent analysis

Table S3. Mean carotid artery intima-media thickness progression – no hysterectomy at baseline

Table S4. Mean carotid artery intima-media thickness progression stratified by lipid-lowering and/or hypertensive therapy at baseline – any vs. none

Table S5. Mean carotid artery intima-media thickness progression – imputed CIMT data for n=47 participants missing CIMT follow-up

Table S6. Metabolic, clinical and estradiol levels during the trial

Table S7. Metabolic, clinical and estradiol levels during the trial in women without hysterectomy at baseline

Table S8. Serious adverse events

Table S1
Baseline Demographic, Clinical, Laboratory and Ultrasound Characteristics of all Randomized Participants (n=643)*

Variable	Postmenopausal < 6 years (n=271)		Postmenopausal ≥ 10 years (n=372)	
	Placebo (n=134)	Estradiol (n=137)	Placebo (n=186)	Estradiol (n=186)
Median time from menopause, years*	3.7(1.9,5.0)	3.5(1.9,5.2)	14.0(11.4,18.1)	14.9(11.5,19.0)
Age at enrollment, years	55.4(52.5,57.7)	55.4(53.2,57.9)	63.0(59.9,66.9)	64.5(60.5,68.8)
Race or ethnicity				
White, non-Hispanic	77 (57.5%)	97 (70.8%)	132 (71.0%)	134 (72.0%)
Black, non-Hispanic	16 (11.9%)	8 (5.8%)	16 (8.6%)	20 (10.8%)
Hispanic	23 (17.2%)	18 (13.1%)	26 (14.0%)	23 (12.4%)
Asian	18 (13.4%)	14 (10.2%)	12 (6.5%)	9 (4.8%)
Education				
Less than high school	0 (0%)	1 (0.7%)	2 (1.1%)	0 (0%)
High school or some college	42 (31.3%)	33 (24.1%)	75 (40.3%)	62 (33.3%)
College graduate	92 (68.7%)	103 (75.2%)	109 (58.6%)	124 (66.7%)
Smoking history				
Current	6 (4.5%)	5 (3.6%)	4 (2.2%)	7 (3.8%)
Former	48 (35.8%)	41 (29.9%)	76 (40.9%)	71 (38.2%)
Never smoked	80 (59.7%)	91 (66.4%)	106 (57.0%)	108 (58.1%)
Anti-hypertension medications	28 (20.9%)	22 (16.1%)	50 (26.9%)	57 (30.6%)
Cholesterol lowering medications	21 (15.7%)	19 (13.9%)	41 (22.0%)	47 (25.3%)
Type of menopause				
Surgical	3 (2.2%)	6 (4.4%)	33 (17.7%)	27 (14.5%)
Natural	131 (97.8%)	131 (95.6%)	153 (82.3%)	159 (85.5%)
Carotid artery intima-media thickness, mm	0.73(0.68,0.80)	0.73(0.68,0.79)	0.76(0.71,0.85)	0.76(0.71,0.83)
Body mass index, kg/m^2	26.1(23.2,29.9)	26.3(23.4,30.5)	26.6(23.1,29.6)	27.2(23.3,31.3)
Pulse rate, beats/min	65.0(62.0,68.7)	65.3(62.7,68.0)	65.3(62.0,69.3)	66.0(62.7,70.0)
Blood pressure, mmHg				
Systolic	115(106,125)	117(108,123)	116(110,125)	121(112,127)
Diastolic	77(70,81)	76(71,80)	73(69,78)	75(70,79)
Cholesterol, mg/dl	223(198,246)	226(207,246)	222(205,243)	219(198,244)
Triglycerides, mg/dl	90(74,129)	95(65,120)	93(70,127)	92(68,134)
HDL-cholesterol, mg/dl	63(51,75)	63(54,76)	66(55,80)	63(52,77)
LDL-cholesterol, mg/dl	134(115,160)	139(119,161)	133(115,155)	132(113,156)
Glucose, mg/dl	94(88,101)	95(90,101)	93(88,98)	94(87,102)
Hemoglobin A1c, %	5.6(5.3,5.8)	5.5(5.3,5.8)	5.7(5.4,5.9)	5.7(5.3,5.9)
Total estradiol, pg/ml	<10(<10,12)	<10(<10,12)	<10(<10,13)	<10(<10,12)
Previous hormone use, n(%)	68 (50.7%)	70 (51.1%)	158 (84.9%)	163 (87.6%)
Current hormone use n(%) requiring 1 month washout†	8 (6.0%)	13 (9.5%)	28 (15.1%)	22 (11.8%)

***** Median (interquartile range) for continuous variables; n (%) for categorical variables. Treatment group comparisons conducted within postmenopause strata, using Wilcoxon rank sum for continuous variables, chi-square for categorical variables. All $p>0.05$ except: 1) Age in ≥10 year stratum ($p<0.05$); and, 2) Systolic blood pressure in ≥10 year stratum ($p<0.05$).

† Women using hormone therapy stopped use ≥1 month prior to screening.

Table S2
Mean Carotid Artery Intima-Media Thickness (CIMT) Progression (mm/yr) per Protocol Adherent Analysis (n=499)

Postmenopause Stratum	CIMT Rate (95% CI)* Placebo (n=249)	CIMT Rate (95% CI)* Estradiol (n=250)	P-value treatment within menopause stratum	P-value for menopause interaction
				0.006
< 6 years (n=102,118)	0.0076 (0.0056, 0.0096)	0.0042 (0.0023, 0.0060)	0.022	
≥ 10 years (n=147,132)	0.0086 (0.0069, 0.0102)	0.0102 (0.0085, 0.0120)	0.14	

* Mixed effects model, adjusted for randomization stratification factors: Baseline carotid artery intima-media thickness (<0.75 mm, ≥0.75 mm) and hysterectomy status (yes, no). Analysis includes 499 participants with carotid artery intima-media thickness follow-up who were at least 80% compliant by pill count throughout trial follow-up.

Table S3
Mean Carotid Artery Intima-Media Thickness (CIMT) Progression (mm/yr)
No Hysterectomy at Baseline (n=487)*

Postmenopause Stratum	CIMT Rate (95% CI)† Placebo (n=246)	CIMT Rate (95% CI)† Estradiol (n=241)	P-value treatment within menopause stratum	P-value for menopause interaction
				0.009
< 6 years (n=120,117)	0.0081 (0.0062, 0.0099)	0.0044 (0.0025, 0.0062)	0.007	
≥ 10 years (n=126,124)	0.0082 (0.0064, 0.0099)	0.0093 (0.0075, 0.0111)	0.35	

* P-value for differential treatment effects by hysterectomy status, p=0.19.

† Mixed effects model, adjusted for randomization stratification factor, baseline carotid artery intima-media thickness (<0.75 mm, ≥0.75 mm).

Table S4
Mean Carotid Artery Intima-Media Thickness (CIMT) Progression (mm/yr)
Stratified by Lipid-Lowering and/or Hypertensive Therapy at Baseline – Any vs. None*

	No Lipid-Lowering or Anti-Hypertensive Therapy at Baseline (n=383)			
Postmenopause Stratum	**CIMT Rate (95% CI)†** **Placebo (n=191)**	**Estradiol (n=192)**	**P-value treatment within menopause stratum**	**P-value for menopause interaction**
< 6 years (n=83,95)	0.0081 (0.0058, 0.0103)	0.0040 (0.0019, 0.0061)	0.01	0.03
≥ 10 years (n=108,97)	0.0097 (0.0077, 0.0116)	0.0102 (0.0081, 0.0122)	0.70	

	Lipid-Lowering or Anti-Hypertensive Therapy at Baseline (n=213)			
Postmenopause Stratum	**CIMT Rate (95% CI)†** **Placebo (n=108)**	**Estradiol (n=105)**	**P-value treatment within menopause stratum**	**P-value for menopause interaction**
< 6 years (n=40,30)	0.0073 (0.0042, 0.0104)	0.0055 (0.0019, 0.0090)	0.47	0.16
≥ 10 years (n=68,75)	0.0075 (0.0051, 0.0099)	0.0097 (0.0075, 0.0120)	0.16	

* P-value for differential treatment effects by lipid-lowering/anti-hypertension medication, p=0.90.

† Mixed effects model, adjusted for randomization stratification factors: Baseline carotid artery intima-media thickness (<0.75 mm, ≥0.75 mm) and hysterectomy status (yes, no).

Table S5
Mean Carotid Artery Intima-Media Thickness (CIMT) Progression (mm/yr)
Imputed CIMT Data for n=47 Participants Missing CIMT Follow-up (n=643)*

Postmenopause Stratum	CIMT Rate (95% CI)† Placebo (n=320)	CIMT Rate (95% CI)† Estradiol (n=323)	P-value treatment within menopause stratum	P-value for menopause interaction
				0.011
< 6 years (n=134,137)	0.0071 (0.0054, 0.0089)	0.0041 (0.0024, 0.0057)	0.016	
≥ 10 years (n=186,186)	0.0083 (0.0069, 0.0098)	0.0093 (0.0079, 0.0108)	0.32	

***** CIMT follow-up data imputed for n=47 participants who had only baseline CIMT. Follow-up CIMT imputed for follow-up time = 30 months (CIMT values imputed at 6 month, 12 month, 18 month, 24 month, 30 month visits). This was the median follow-up time of 83 participants who had some CIMT follow-up but not complete follow-up. For each of the 47 participants, CIMT at each visit was imputed as a normal random variable with mean = baseline CIMT (simulating no change in CIMT over follow-up) and variance = model residual from analysis of full data (Table 2).

† Mixed effects model, adjusted for randomization stratification factors: Baseline carotid artery intima-media thickness (<0.75 mm, ≥0.75 mm) and hysterectomy status (yes, no).

Table S6
Metabolic, Clinical and Estradiol Levels During the Trial (n=596)*

Variable, Postmenopause Stratum	N_1/N_2†	Placebo N = 299	Estradiol N = 297	Multiplicity adjusted P-value
Body mass index, kg/m^2	586 / 13310	27.1 (26.9 – 27.2)	27.0 (26.8 – 27.2)	0.72
<6	247 / 5701	27.1 (26.9 – 27.4)	27.1 (26.8 – 27.4)	
≥10	339 / 7609	27.0 (26.8 – 27.2)	26.9 (26.7 – 27.1)	
Systolic blood pressure, mm Hg	586 / 13336	115.3 (114.3 – 116.2)	114.7 (113.7 – 115.7)	0.72
<6	247 / 5728	114.5 (113.0 – 116.0)	113.9 (112.4 – 115.3)	
≥10	339 / 7608	116.0 (114.9 – 117.1)	115.5 (114.4 – 116.7)	
Diastolic blood pressure, mm Hg	586 / 13336	73.8 (73.2 – 74.4)	73.2 (72.6 – 73.8)	0.33
<6	247 / 5728	74.1 (73.2 – 75.0)	73.4 (72.4 – 74.3)	
≥10	339 / 7608	73.5 (72.8 – 74.2)	73.0 (72.3 – 73.8)	
Total cholesterol, mg/dL	561 / 4781	215.4 (212.5 – 218.3)	212.2 (209.5 – 214.9)	0.23
<6	239 / 2103	216.6 (212.2 – 221.0)	212.5 (208.8 – 216.1)	
≥10	322 / 2678	214.2 (210.9 – 217.4)	211.9 (208.5 – 215.3)	
Total triglycerides, mg/dL‡	561 / 4783	92.5 (89.9 – 95.1)	96.8 (94.2 – 99.5)	0.025
<6	239 / 2103	95.3 (91.4 – 99.3)	99.5 (95.7 – 103.8)	
≥10	322 / 2680	89.7 (87.3 – 92.3)	94.2 (91.2 – 97.5)	
High-density lipoprotein cholesterol, mg/dL‡	561 / 4783	68.1 (67.3 – 69.0)	70.8 (69.8 – 71.8)	<.0001
<6	239 / 2103	68.2 (66.8 – 69.7)	70.5 (69.0 – 71.8)	
≥10	322 / 2678	68.1 (67.0 – 69.0)	71.1 (70.0 – 72.3)	
Low-density lipoprotein cholesterol, mg/dL	561 / 4783	124.8 (122.2 – 127.5)	118.1 (115.5 – 120.8)	0.0002
<6	239 / 2103	125.9 (122.0 – 129.8)	118.2 (114.6 – 121.8)	
≥10	322 / 2678	123.8 (120.7 – 126.9)	118.1 (114.9 – 121.3)	
Glucose, mg/dL	535 / 2387	91.4 (90.6 – 92.3)	90.3 (89.4 – 91.1)	0.11
<6	227 / 1045	91.2 (89.9 – 92.4)	89.9 (88.7 – 91.2)	
≥10	308 / 1342	91.7 (90.7 – 92.7)	90.6 (89.6 – 91.6)	
Hemoglobin A1c, %	559 / 4850	5.83 (5.80 – 5.86)	5.73 (5.70 – 5.76)	<.0001
<6	240 / 2125	5.84 (5.80 – 5.89)	5.73 (5.69 – 5.77)	
≥10	319 / 2725	5.81 (5.78 – 5.85)	5.73 (5.69 – 5.77)	
Total estradiol, pg/ml‡	564 / 4984	12.6 (12.0 – 13.2)	41.7 (38.7 – 44.9)	<.0001
<6	241 / 2186	12.8 (12.0 – 13.7)	44.1 (39.6 – 49.1)	
≥10	323 / 2798	12.4 (11.9 – 13.0)	39.4 (36.2 – 42.9)	

***** Treatment groups compared using generalized estimating equations with identity link function and exchangeable correlation structure. Tabled numbers are least square means (95% confidence interval), adjusted for randomization strata and baseline levels of the characteristic. All p-values for treatment-by-postmenopause stratum interaction ≥0.23.

† N_1 = number of subjects; N_2 = number of observations.

‡ Log transformed for analysis; results shown are back transformed.

Table S7
Metabolic, Clinical and Estradiol Levels During the Trial in Women without Hysterectomy at Baseline (n=487)*

Variable, Postmenopause Stratum	N_1/N_2†	Placebo N = 246	Estradiol N = 241	Multiplicity adjusted P-value
Body mass index, kg/m^2	480 / 10979	26.9 (26.7 – 27.0)	27.0 (26.8 – 27.1)	0.59
<6	236 / 5468	27.1 (26.8 – 27.3)	26.9 (26.6 – 27.2)	
≥10	244 / 5511	26.9 (26.6 – 27.1)	26.8 (26.6 – 27.0)	
Systolic blood pressure, mm Hg	480 / 11005	114.7 (113.8 – 115.6)	113.8 (112.9 – 114.8)	0.59
<6	236 / 5495	113.6 (112.3 – 114.9)	113.0 (111.8 – 114.3)	
≥10	244 / 5510	115.7 (114.5 – 116.9)	114.6 (113.2 – 116.1)	
Diastolic blood pressure, mm Hg	480 / 11005	73.6 (73.0 – 74.1)	72.6 (72.0 – 73.3)	0.19
<6	236 / 5495	73.7 (72.9 – 74.5)	72.8 (71.9 – 73.6)	
≥10	244 / 5510	73.4 (72.6 – 74.2)	72.5 (71.6 – 73.4)	
Total cholesterol, mg/dL	458 / 3951	214.9 (212.2 – 217.7)	211.8 (209.6 – 214.1)	0.37
<6	228 / 2012	216.3 (212.3 – 220.2)	211.6 (208.7 – 214.5)	
≥10	230 / 1939	213.6 (209.8 – 217.5)	212.0 (208.5 – 215.6)	
Total triglycerides, mg/dL‡	458 / 3951	90.4 (88.4 – 92.5)	93.5 (91.1 – 95.8)	0.30
<6	228 / 2012	92.6 (89.4 – 95.9)	96.3 (92.9 – 99.9)	
≥10	230 / 1939	88.4 (85.9 – 91.0)	90.7 (87.4 – 94.0)	
High-density lipoprotein cholesterol, mg/dL‡	458 / 3951	67.3 (66.5 – 68.2)	69.7 (68.8 – 70.6)	0.001
<6	228 / 2012	67.3 (66.1 – 68.6)	69.3 (68.1 – 70.6)	
≥10	230 / 1939	67.3 (66.1 – 68.5)	70.1 (68.8 – 71.4)	
Low-density lipoprotein cholesterol, mg/dL	458 / 3951	125.7 (123.2 – 128.2)	119.6 (117.4 – 121.8)	0.002
<6	228 / 2012	127.0 (123.6 – 130.4)	119.4 (116.3 – 122.4)	
≥10	230 / 1939	124.4 (120.8 – 128.0)	119.7 (116.5 – 123.0)	
Glucose, mg/dL	437 / 1961	91.3 (90.5 – 92.0)	90.6 (89.7 – 91.4)	0.59
<6	218 / 999	91.2 (90.1 – 92.3)	90.1 (88.7 – 91.3)	
≥10	218 / 962	91.4 (90.4 – 92.3)	91.1 (89.9 – 92.2)	
Hemoglobin A1C, %	456 / 3995	5.82 (5.79 – 5.85)	5.72 (5.69 – 5.75)	<.0001
<6	229 / 2033	5.84 (5.80 – 5.88)	5.71 (5.68 – 5.75)	
≥10	227 / 1962	5.80 (5.76 – 5.84)	5.73 (5.68 – 5.77)	
Total estradiol, pg/ml‡	461 / 4111	12.0 (11.6 – 12.3)	37.6 (35.3 – 40.1)	<.0001
<6	230 / 2093	11.8 (11.4 – 12.3)	39.9 (36.4 – 43.7)	
≥10	231 / 2018	12.1 (11.6 – 12.6)	35.5 (32.5 – 38.8)	

***** Treatment groups compared using generalized estimating equations with identity link function and exchangeable correlation structure. Tabled numbers are least squares mean (95% confidence interval), adjusted for randomization strata and baseline levels of the characteristic. All p-values for treatment-by-postmenopause stratum interactions >0.06.

† N_1 = number of subjects; N_2 = number of observations.

‡ Log transformed for analysis; results shown are back transformed.

Table S8
Serious Adverse Events (n=643)*

Adverse Event (n)	Postmenopausal < 6 years (n=271)		Postmenopausal ≥ 10 years (n=372)	
	Placebo (134)	Estradiol (137)	Placebo (186)	Estradiol (186)
Deaths (2)	Pancreatic cancer (1)	None	None	Glioblastoma (1)
Cancer (34)	Breast (3) Gastric (1) Malignant peritoneal neoplasm (1) Pancreatic (1)	Breast (3) Colorectal (1) Uterine (1)	Breast (5) Colorectal (2) Uterine (2) Ovarian epithelial (1) B-cell lymphoma (1)	Breast (7) Colorectal (2) Uterine (1) Glioblastoma (1) Mycosis fungoides (1)
Cardiovascular (14)	Myocardial infarction (1) Transient ischemic attack (1)	None	Myocardial infarction (2) Transient ischemic attack (1) Deep vein thrombosis (2)	Myocardial infarction (1) Transient ischemic attack (1) Deep vein thrombosis (1) Pulmonary embolus (2) Unstable angina (2)
Other (38)	6†	5‡	14§	13¶

* Within each postmenopause stratum, the number of serious adverse events did not differ significantly between treatment groups (early postmenopause stratum P=0.62; late postmenopause stratum P=0.58).

† Other serious adverse events were fracture (3 events), non-cardiac chest pain, pneumonia and cellulitis.

‡ Other serious adverse events were non-cardiac chest pain, syncope, psychotic disorder, systemic lupus erythematosus and drug hypersensitivity.

§ Other serious adverse events were fracture (3 events), lobar pneumonia/pleuropericarditis, cellulitis, syncope, suicidal ideation, amnesia, atrial fibrillation, upper gastrointestinal hemorrhage, ulcerative colitis, colon adenoma, polycythemia vera and spinal laminectomy.

¶ Other serious adverse events were fracture, non-cardiac chest pain, pneumonia, dizziness, vertigo, atrial fibrillation, lower gastrointestinal hemorrhage, ischemic colitis, ileitis, gastroesophageal reflux disease, aplastic anemia, pancreatitis and pelvic abscess.

V. References

1. Hodis HN, Mack WJ, Shoupe D, et al. Methods and baseline cardiovascular data from the Early versus Late Intervention Trial with Estradiol testing the menopausal hormone timing hypothesis. Menopause 2015;22:391-401.

2. Hodis HN, Mack WJ, Lobo RA, Shoupe D, Sevanian A, Mahrer PR, et al. Estrogen in the prevention of atherosclerosis: a randomized, double-blind, placebo-controlled trial. Ann Intern Med 2001;135:939-53.

3. Blankenhorn DH, Hodis HN. Duff Memorial Lecture: arterial imaging and atherosclerosis reversal. Arterioscler Thromb 1994;14:177-92.

4. Hodis HN, Mack WJ, Kono N, Azen SP, Shoupe D, Hwang-Levine J, et al. Isoflavone soy protein supplementation and atherosclerosis progression in healthy postmenopausal women: a randomized controlled trial. Stroke 2011;42:3168-75.

5. Hodis HN, Mack WJ, LaBree L, Mahrer PR, Sevanian A, Liu CR, et al. Alpha tocopherol supplementation in healthy individuals reduces low-density lipoprotein oxidation but not atherosclerosis: the Vitamin E Atherosclerosis Prevention Study (VEAPS). Circulation 2002;106:1453-9.

6. Hodis HN, Mack WJ, Dustin L, Mahrer PR, Azen SP, Detrano R, et al. High-dose B-vitamin supplementation and progression of subclinical atherosclerosis: a randomized controlled trial. Stroke 2009;40:730-6.

7. Hodis HN, Mack WJ, LaBree L, Selzer RH, Liu CR, Liu CH, et al. Reduction in carotid arterial wall thickness using lovastatin and dietary therapy: a randomized, controlled clinical trial. Ann Intern Med 1996;124:548-56.

8. 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.

9. Selzer RH, Mack WJ, Lee PL, Kwong-Fu H, Hodis HN. Improved common carotid elasticity and intima-media thickness measurement from computer analysis of sequential ultrasound frames. Atherosclerosis 2001;154:185-93.

10. Mack WJ, LaBree L, Liu CL, Liu CH, Selzer RH, Hodis HN. Correlations between measures of atherosclerosis change using carotid ultrasonography and coronary angiography. Atherosclerosis 2000;150:371-9.

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

12. Carr JJ, Nelson JC, Wong ND, McNitt-Gray M, Arad Y, Jacobs DR, Jr., et al. Calcified coronary artery plaque measurement with cardiac CT in population-based studies: standardized protocol of Multi-Ethnic Study of Atherosclerosis (MESA) and Coronary Artery Risk Development in Young Adults (CARDIA) study. Radiology 2005;234:35-43.

13. Budoff MJ, Dowe D, Jollis JG, Gitter M, Sutherland J, Halamert E, et al. Diagnostic performance of 64-multidetector row coronary computed tomographic angiography for evaluation of coronary artery stenosis in individuals without known coronary artery disease: results from the prospective multicenter ACCURACY (Assessment by Coronary Computed Tomographic Angiography of Individuals Undergoing Invasive Coronary Angiography) Trial. J Am Coll Cardiol 2008;52:1724-32.

14. Agatston AS, Janowitz WR, Hilder FJ, Xusmer NR, Viamonte M, Jr., Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol 1990;15:827-32.

15. Austen WG, Edwards JE, Frye RL, Gensini GG, Gott VL, Griffith LS, et al. A reporting system on patients evaluated for coronary artery disease. Report of the Ad Hoc Committee for Grading of Coronary Artery Disease, Council on Cardiovascular Surgery, American Heart Association. Circulation 1975;51:5-40.

16. Rasouli ML, Shavelle DM, French WJ, McKay CR, Budoff MJ. Assessment of coronary plaque morphology by contrast-enhanced computed tomographic angiography: comparison with intravascular ultrasound. Coron Artery Dis 2006;17:359-64.

17. Karim R, Hodis HN, Stanczyk FZ, Lobo RA, Mack WJ. Relationship between serum levels of sex hormones and progression of subclinical atherosclerosis in postmenopausal women. J Clin Endocrinol Metab 2008;93:131-8.