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Estrogen in the Prevention of Atherosclerosis

A Randomized, Double-Blind, Placebo-Controlled Trial

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Background: **Although observational studies suggest that estrogen replacement therapy (ERT) reduces cardiovascular morbidity and mortality in postmenopausal women, use of unopposed ERT for prevention of coronary heart disease in healthy postmenopausal women remains untested.**

Objective: **To determine the effects of unopposed ERT on the progression of subclinical atherosclerosis in healthy postmenopausal women without preexisting cardiovascular disease.**

Design: **Randomized, double-blind, placebo-controlled trial.**

Setting: **University-based clinic.**

Patients: **222 postmenopausal women 45 years of age or older without preexisting cardiovascular disease and with low-density lipoprotein cholesterol levels of 3.37 mmol/L or greater (≥130 mg/dL).**

Intervention: **Unopposed micronized 17β-estradiol (1 mg/d) or placebo. All women received dietary counseling. Women received lipid-lowering medication if their low-density lipoprotein cholesterol level exceeded 4.15 mmol/L (160 mg/dL).**

Measurements: **The rate of change in intima–media thickness of the right distal common carotid artery far wall in computer image processed B-mode ultrasonograms obtained at baseline and every 6 months during the 2-year trial.**

Results: **In a multivariable mixed-effects model, among women who had at least one follow-up measurement of carotid intima–media thickness ($n = 199$), the average rate of progression of subclinical atherosclerosis was lower in those taking unopposed estradiol than in those taking placebo (−0.0017 mm/y vs. 0.0036 mm/y); the placebo–estradiol difference between average progression rates was 0.0053 mm/y (95% CI, 0.0001 to 0.0105 mm/y) ($P = 0.046$). Among women who did not receive lipid-lowering medication ($n = 77$), the placebo–estradiol difference between average rates of progression was 0.0147 mm/y (CI, 0.0055 to 0.0240) ($P = 0.002$). Average rates of progression did not differ between estradiol and placebo recipients who took lipid-lowering medication ($n = 122$) ($P > 0.2$).**

Conclusions: **Overall, the average rate of progression of subclinical atherosclerosis was slower in healthy postmenopausal women taking unopposed ERT with 17β-estradiol than in women taking placebo. Reduction in the progression of subclinical atherosclerosis was seen in women who did not take lipid-lowering medication but not in those who took these medications.**

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Coronary heart disease is the leading cause of death in women, and mortality rates from this disease substantially and steadily increase after menopause (1–3). Population studies indicate that estrogen reduces the incidence of coronary heart disease in women. Bilateral oophorectomy before natural menopause increases the risk for coronary heart disease (4). This pattern of risk for coronary heart disease suggests that endogenous estrogens, including 17β-estradiol, play a cardioprotective role before menopause.

More than 40 observational studies have suggested that hormone replacement therapy (HRT) reduces cardiovascular morbidity and mortality in postmenopausal women (5, 6). Most of these studies were conducted in healthy postmenopausal women who used unopposed estrogen replacement therapy (ERT). Although observational studies are important, selection bias is a potential problem, especially when studying HRT, since healthier women tend to use hormones (7). Only randomized, controlled trials can ensure that patients are assigned to treatment in an unbiased manner and can establish the efficacy of HRT for reducing the progression of atherosclerosis and its clinical sequelae.

The effect of unopposed ERT on progression of atherosclerosis in healthy postmenopausal women without preexisting cardiovascular disease remains untested in randomized, controlled trials. We report the results of the Estrogen in the Prevention of Atherosclerosis Trial (EPAT), a randomized, double-blind, placebo-controlled trial designed to test whether unopposed micronized 17β-estradiol reduces progression of subclinical atherosclerosis in healthy postmenopausal women with-

out preexisting cardiovascular disease. Our primary hypothesis was that unopposed ERT significantly reduces the progression of subclinical atherosclerosis.

METHODS

Study Design

Potential participants were prescreened by telephone and seen at three screening visits 2 to 4 weeks apart to collect baseline data and to determine final study eligibility. Women were eligible if they were postmenopausal (serum estradiol level < 73.4 pmol/L [<20 pg/mL]), 45 years of age or older, and had a low-density lipoprotein (LDL) cholesterol level of 3.37 mmol/L or greater (≥130 mg/dL). Women were excluded if breast or gynecologic cancer had been diagnosed in the past 5 years or if these cancers were identified during screening; if they had previously used HRT for more than 10 years or had used HRT within 1 month of the first screening visit; if they had five or more hot flushes daily that interfered with daily activity and precluded randomization, diastolic blood pressure greater than 110 mm Hg, untreated thyroid disease, life-threatening disease with a survival prognosis of less than 5 years, total triglyceride level of 4.52 mmol/L or greater (≥400 mg/dL), high-density lipoprotein (HDL) cholesterol level less than 0.78 mmol/L (<30 mg/dL), or serum creatinine concentration greater than 221 μmol/L (>2.5 mg/dL); or if they were current smokers. All women, including those with diabetes mellitus, were included provided that their fasting blood glucose level was less than 11.1 mmol/L (<200 mg/dL). All participants gave written informed consent, and the study protocol was approved by the University of Southern California Institutional Review Board.

Packets of study medications were prepared in a blinded manner (to both the clinical staff and participants) before the start of the study. Computer-generated random numbers were used to assign participants to unopposed estradiol or placebo in one of eight strata, defined by LDL cholesterol level (<4.15 mmol/L [<160 mg/dL] or ≥4.15 mmol/L), previous duration of HRT use (<5 years or ≥5 years), and diabetes mellitus (yes or no). As a new participant was determined to be eligible for randomization, the next packet in sequence in the appropriate stratum was obtained and recorded. The Data Coordinating Center monitored adherence to sequential assignment of medication packets. The participants, gynecologists, clinical staff, and image analysts were blinded to treatment assignment. The data monitor and data analyst were blinded to treatment assignment until analyses were completed.

Participants were followed every month for the first 6 months and every other month thereafter for a total of 2 years. All participants received dietary counseling according to step II American Heart Association dietary recommendations: 200 mg of cholesterol or less per day, 25% of energy as total-fat calories, and 7% of energy as saturated-fat calories. Dietary intake was monitored at each clinic visit by using 3-day dietary booklets (Nutrition Scientific, South Pasadena, California). Participants received lipid-lowering medication (primarily hydroxymethylglutaryl coenzyme A reductase inhibitors) if their LDL cholesterol level exceeded 4.15 mmol/L (160 mg/dL). Vital signs; clinical events; adherence; and use of nonstudy medications, dietary supplements, and nutriceuticals were ascertained at each visit.

Carotid artery ultrasonography was performed at baseline (two visits 1 to 3 weeks apart) and every 6 months thereafter. The baseline intima–media thickness was the average of the two measurements. Pelvic examination (and uterine ultrasonography in participants with a uterus), Papanicolou smear, and mammography were done yearly in all participants. Uterine biopsy was performed if endometrial thickness was 5 mm or more. Adverse clinical symptoms and bleeding were assessed by the study gynecologist, who was blinded to treatment assignment.

The primary trial end point was the rate of change in intima–media thickness of the right distal common carotid artery far wall in computer image processed B-mode ultrasonograms (8–15). Power calculations indicated that a sample size of 200 (100 participants per treatment group) was needed to detect a treatment effect size (the standardized difference in progression rates between the two treatment groups) of 0.40 or greater with 80% power. Two hundred twenty-two participants (111 per treatment group) were recruited to accommodate the anticipated dropout rate.

Assessment of the Progression of Subclinical Atherosclerosis

High-resolution B-mode ultrasonograms of the right common carotid artery were obtained by using a

7.5-MHz linear-array transducer attached to a Toshiba SSH 140A ultrasonography system (Toshiba Corp., Tokyo, Japan). The ultrasonographers were blinded to treatment assignment. Participants were placed in a supine position with the head rotated to the left by using a 45-degree head block. The jugular vein and carotid artery were located in the transverse view, with the jugular vein stacked above the carotid artery according to modification of a procedure described by Beach and colleagues (16). The transducer was then rotated 90 degrees around the central line of the transverse image of the stacked jugular vein and carotid artery to obtain a longitudinal image while the stacked position of the vessels was maintained. All images contained anatomic landmarks for reproducing probe angulation, and a hard copy of each participant's baseline image was used as a guide for follow-up examinations. For each participant, the depth of field, gain, monitor intensity setting, and other instrumentation settings used at baseline examination were used at all follow-up examinations. These techniques significantly reduce measurement variability (14, 15). All images were recorded with the electrocardiogram tracing on super-VHS video tape.

An image analyst who was blinded to treatment assignment measured the intima–media thickness of the distal common carotid artery far wall with automated computerized edge detection using an in-house software package (Prosound, University of Southern California, Los Angeles, California), as described elsewhere (14, 15). Carotid intima–media thickness was the average of approximately 70 to 100 individual measurements between the intima–lumen and media–adventitia interfaces along a 1-cm length just distal to the carotid artery bulb. This method standardized the location and the distance over which intima–media thickness was measured and ensured that the same portion of the arterial wall was measured in each image and compared within and across all participants.

Laboratory Measurements

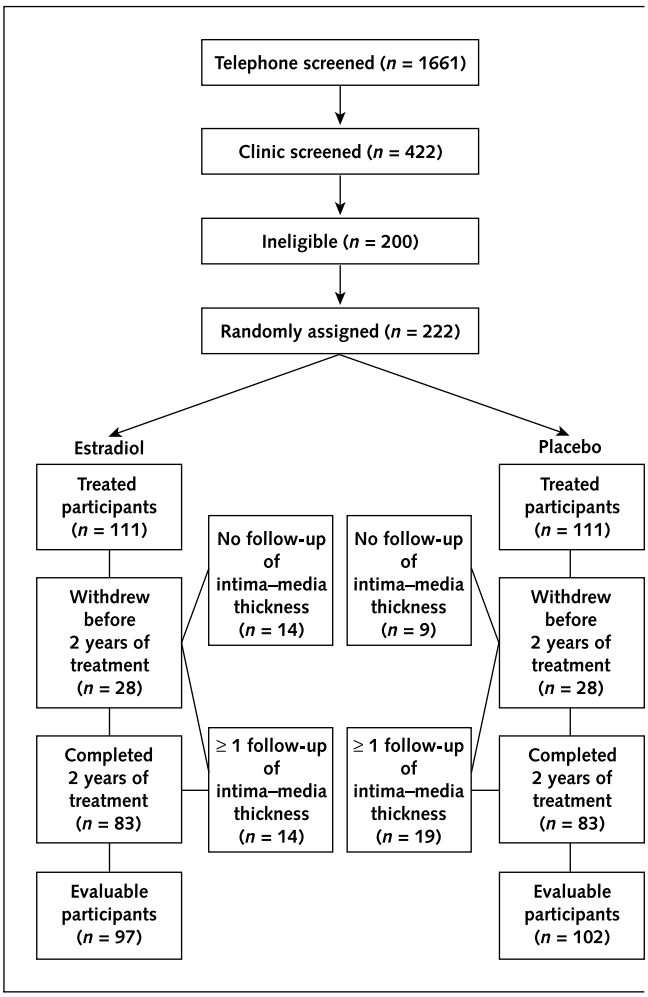
Participants fasted for 8 hours before sample collection. Total plasma cholesterol and triglyceride levels were measured by using an enzymatic method of the Standardization Program of the National Centers for Disease Control and Prevention. High-density lipoprotein cholesterol levels were measured after lipoproteins containing apolipoprotein B were precipitated in whole plasma by using heparin manganese chloride. Low-density lipoprotein cholesterol levels were estimated by using the Friedewald equation (17). Serum estradiol and fasting insulin levels were measured by using radioimmunoassay. Fasting serum glucose levels were measured by using the glucose oxidase technique on a Beckman Glucose II analyzer (Beckman Instruments, Brea, California). Hemoglobin A_{1c} levels were measured by using high-performance liquid chromatography (Bio-Rad Diamat, Bio-Rad Corp., Hercules, California).

Statistical Analysis

We compared demographic and baseline clinical and laboratory values between the estradiol and placebo groups by using a *t*-test for independent samples for means or a chi-square test for proportions. At each study visit, adherence to study treatment was determined by calculating the percentage pill adherence (number of pills consumed divided by the number that should have been consumed) and by measuring estradiol levels. The average percentage pill adherence (over the entire study and by 6-month study period) and average serum estradiol levels (over the entire study) were compared between treatment groups by using a *t*-test for independent samples.

The preplanned intention-to-treat analysis of the primary study end point, progression of subclinical atherosclerosis (defined as the rate of change in common carotid artery intima–media thickness), was done for all evaluable randomly assigned participants (those who had carotid artery ultrasonography at baseline and at one or more follow-up visits). No missing intima–media thickness values were imputed. To test the hypothesis of treatment differences in the average rates of change in intima–media thickness, we used a linear, multivariate mixed-effects model (random coefficients corresponding to participants) that included all available determinations of intima–media thickness (18). In this situation, intima–media thickness at a given follow-up time was regressed on the follow-up time while adjusting for the randomization stratification factors (baseline LDL cholesterol level, duration of previous hormone therapy, and diabetes mellitus) and for the variables found to be

Figure 1. **Profile of the Estrogen in the Prevention of Atherosclerosis Trial.**



different across treatment groups at baseline. The regression coefficient associated with follow-up time represents the average rate of change in intima–media thickness. All variance and covariance terms were estimated, and no assumption of homogeneity of variances between treatment groups was made. Differences between treatment groups in the average rates of change in intima–media thickness were tested for significance by including a treatment × follow-up time interaction term in the mixed-effects model.

Restricted maximum likelihood procedures were used to estimate and test hypotheses about the parameters. To evaluate the goodness-of-fit of the preplanned model, we performed a calibration analysis using a chi-square goodness-of-fit test to compare the final predicted intima–media thickness with the final observed intima–media thickness for each participant. The null hypothesis was that the model fits the data (as opposed to not fitting the data). Evaluation of higher-order terms (for example, quadratic) for time in the model did not significantly improve the goodness of fit.

All statistical analyses were done by using SAS software (SAS, Inc., Cary, North Carolina). The PROC MIXED procedure was used to estimate the multivariable mixed-effects model.

Because the effect of lipid-lowering therapy on reducing the progression of carotid intima–media thickness is well established (8–10, 19, 20), we performed a planned subgroup analysis of the benefit of unopposed ERT on subclinical atherosclerosis in participants receiving and not receiving lipid-lowering therapy after randomization. We used similar statistical methods as in the full cohort to assess the subgroups.

Results of analyses of changes in lipid, glucose, insulin, and glycosylated hemoglobin values are presented for evaluable participants. We used a mixed-effects, random coefficients model that included all available follow-up determinations to compare laboratory variables between treatment groups.

Adverse events were reported for the entire cohort. Major cardiovascular events that were monitored included myocardial infarction (fatal and nonfatal), cerebrovascular accident, transient ischemic attack, peripheral vascular disease, deep venous thrombosis, pulmonary embolus, and arterial revascularization procedures. Other clinical events reported were death and cancer. The proportions of participants experiencing events were compared between treatment groups by using a Fisher exact test.

Role of the Funding Source

This was an investigator initiated and conducted trial. The authors were solely responsible for the design, conduct, data collection, data management, statistical analyses, and data interpretation. The funding source played no role in these functions. Data were not reported to the funding source, and the funding source had no role in deciding whether or where the study would be submitted for publication.

Table 1. **Baseline Carotid Artery Intima–Media Thickness and Demographic and Clinical Characteristics***

Variable	Evaluable Placebo Recipients (n = 102)	Evaluable Estradiol Recipients (n = 97)	Nonevaluable Participants from Both Groups (n = 23)
Carotid artery intima–media thickness, *mm*	0.776 ± 0.149	0.752 ± 0.111	0.743 ± 0.142
Age, *y*	62.1 ± 7.1	60.9 ± 6.7	58.0 ± 6.6†
Ethnicity, *n (%)*			
White	63 (62)	55 (57)	8 (35)
Black	10 (10)	12 (12)	3 (13)
Hispanic	19 (18)	21 (22)	7 (30)
Asian	10 (10)	8 (8)	5 (22)
Other	0 (0)	1 (1)	0 (0)
Marital status, *n (%)*			
Single	6 (6)	9 (9)	2 (9)
Married	48 (47)	43 (44)	8 (35)
Divorced or widowed	48 (47)	45 (47)	13 (56)
Education, *n (%)*			
<12 y	16 (16)	14 (14)	6 (26)
≥12 y	86 (84)	83 (86)	17 (74)
Smoking history, *n (%)*			
Former smoker	48 (47)	55 (57)	15 (65)
Nonsmoker	54 (53)	42 (43)	8 (35)
Pulse rate, *beats/min*	64.4 ± 5.7	65.4 ± 5.8	65.4 ± 5.7
Blood pressure, *mm Hg*			
Systolic	128.6 ± 14.0	127.8 ± 14.6	128.3 ± 14.2
Diastolic	77.2 ± 7.2	78.1 ± 8.0	79.1 ± 7.8
Body weight, *kg*	75.0 ± 15.4	74.3 ± 16.1	79.0 ± 24.5
Waist-to-hip ratio	0.8 ± 0.06	0.8 ± 0.06	0.8 ± 0.07
Body mass index, kg/m^2	29.0 ± 5.3	28.7 ± 5.5	30.5 ± 7.4
Diabetes mellitus, *n (%)*	4 (4)	2 (2)	4 (17)‡
Hysterectomy, *n (%)*	33 (32)	43 (44)	13 (57)
Oophorectomy, *n (%)*	19 (19)	31 (32)§	6 (26)

* Evaluable participants were women who had baseline and at least one follow-up measurement of carotid artery intima–media thickness. Nonevaluable participants were women with no follow-up carotid artery intima–media thickness measurements. Data with the plus/minus sign are the mean ± SD.
† Evaluable participants were significantly older than nonevaluable participants ($P = 0.02$).
‡ A significantly higher proportion of nonevaluable participants than evaluable participants had diabetes mellitus ($P = 0.01$).
§ A significantly higher proportion of evaluable estradiol recipients than evaluable placebo recipients had complete or partial oophorectomy ($P = 0.03$).

RESULTS

Baseline Characteristics

Figure 1 shows the trial profile. Of the 1661 potential participants who were prescreened by telephone, 422 were invited for on-site screening visits and 222 were randomly assigned (111 participants to each treatment group). The mean participant age was 62.2 years (range, 46 to 80 years); 42% belonged to an ethnic minority group.

Of the 222 randomly assigned participants, 166 (83 per group) completed the 2-year study. Fifty-six participants (28 per group) withdrew: Eighteen participants dropped out for personal reasons, 1 died, 13 were lost to follow-up or moved, 10 wanted HRT, and 14 did not want HRT. Of the 56 persons who dropped out, 33 (14 in the estradiol group and 19 in the placebo group) were evaluable for the primary trial end point because they had at least one follow-up measurement of carotid intima–media thickness. Thus, 199 participants (97 in the estradiol group and 102 in the placebo group) were evaluable (**Figure 1**).

Tables 1 and **2** summarize the baseline characteristics of the 199 evaluable participants, stratified by treatment assignment. Except for rates of oophorectomy, the groups did not differ significantly at baseline in carotid intima–media thickness, demographic or clinical characteristics (**Table 1**), or laboratory values (**Table 2**). Compared with placebo recipients, proportionately more estradiol recipients had complete or partial oophorectomy ($P = 0.03$). Thus, subsequent statistical analyses of the rate of change in intima–media thickness were conducted with and without oophorectomy as a covariate.

Table 1 also summarizes baseline characteristics of the 23 nonevaluable participants (those with no follow-up measurement of intima–media thickness). Compared with evaluable participants (both treatment groups combined), nonevaluable participants were younger ($P = 0.02$) and had a higher rate of diabetes mellitus

Table 2. **Laboratory Values in Evaluable Participants, by Treatment Group***

Variable	All Evaluable Participants			Evaluable Participants Receiving No Lipid-Lowering Medications		
	Placebo Recipients ($n = 102$)	Estradiol Recipients ($n = 97$)	*P* Value	Placebo Recipients ($n = 35$)	Estradiol Recipients ($n = 42$)	*P* Value
Total cholesterol level						
Baseline value, *mmol/L*†	6.42 ± 0.86 (102)	6.50 ± 0.82 (97)	>0.2‡	5.74 ± 0.61 (35)	5.93 ± 0.51 (42)	0.13
Change, %	−6.3 ± 0.9 (102)	−7.7 ± 0.9 (97)	>0.2§	0.4 ± 1.0 (35)	−3.3 ± 0.9 (42)	0.004
Triglyceride level						
Baseline value, *mmol/L*‖	1.81 ± 0.92 (102)	1.78 ± 0.73 (97)	>0.2	1.58 ± 1.05 (35)	1.68 ± 0.68 (42)	>0.2
Change, %	−2.9 ± 2.0 (102)	5.0 ± 2.1 (97)	0.006	−0.8 ± 3.6 (35)	5.6 ± 3.3 (42)	>0.2
HDL cholesterol level						
Baseline value, *mmol/L*¶	1.40 ± 0.31 (102)	1.38 ± 0.31 (97)	>0.2	1.43 ± 0.27 (35)	1.40 ± 0.32 (42)	>0.2
Change, %	8.0 ± 1.0 (102)	14.0 ± 1.0 (97)	<0.001	7.9 ± 1.6 (35)	12.6 ± 1.4 (42)	0.03
LDL cholesterol level						
Baseline value, *mmol/L***	4.20 ± 0.69 (102)	4.30 ± 0.75 (97)	>0.2	3.61 ± 0.41 (35)	3.75 ± 0.37 (42)	0.12
Change, %	−10.4 ± 1.2 (102)	−16.0 ± 1.2 (97)	0.001	−1.1 ± 1.4 (35)	−10.5 ± 1.2 (42)	0.001
Fasting glucose level						
Baseline value, *mmol/L*††	5.03 ± 0.71 (88)	4.85 ± 0.59 (90)	0.06	4.93 ± 0.65 (30)	4.74 ± 0.49 (36)	0.18
Change, %	2.1 ± 0.9 (88)	−0.03 ± 0.9 (90)	0.09	0.5 ± 1.5 (30)	0.4 ± 1.4 (36)	>0.2
Insulin level						
Baseline value, *pmol/L*	59.0 ± 42.6 (90)	55.5 ± 29.0 (90)	>0.2	53.9 ± 36.8 (32)	54.1 ± 25.6 (36)	>0.2
Change, %	−3.7 ± 2.9 (90)	−14.0 ± 2.9 (90)	0.01	−2.3 ± 4.9 (32)	−17.3 ± 4.6 (36)	0.03
Hemoglobin A_{1c} level						
Baseline value, %	5.10 ± 0.42 (89)	4.99 ± 0.67 (90)	0.17	5.11 ± 0.4 (32)	4.87 ± 0.33 (36)	0.008
Change, %	1.6 ± 0.6 (89)	−0.8 ± 0.6 (90)	0.007	0.1 ± 0.9 (32)	−0.5 ± 0.9 (36)	>0.2

* Participants with baseline and at least one follow-up measurement of carotid artery intima–media thickness. Baseline data are presented as the mean ± SD; change data are presented as the mean ± SE of percentage change from baseline computed as 100 (during trial value − baseline value)/baseline value. Numbers in parentheses are the number of participants for whom values were available. HDL = high-density lipoprotein; LDL = low-density lipoprotein.

† To convert total cholesterol values to mg/dL, divide by 0.02586.

‡ Student *t*-test.

§ Mixed-effects random coefficient (random coefficients corresponding to participants) model. The dependent variable is change (%) in laboratory measurement, the independent variable is treatment group, and covariates are randomization stratification factors (baseline LDL cholesterol level, duration of previous hormone therapy, and diabetes mellitus).

‖ To convert triglyceride values to mg/dL, divide by 0.01129.

¶ To convert HDL cholesterol values to mg/dL, divide by 0.02586.

** To convert LDL cholesterol values to mg/dL, divide by 0.02586.

†† To convert fasting glucose values to mg/dL, divide by 0.05551.

($P = 0.01$). Evaluable and nonevaluable participants did not differ for any laboratory variable (data not shown).

Adherence

During the trial, mean pill adherence was 95% in the estradiol group and 92% in the placebo group ($P = 0.08$). Pill adherence in the estradiol and placebo groups was maintained throughout the trial: 96.2% versus 92.1% at 6 months, 94.6% versus 94.7% at 12 months, 95.2% versus 94.8% at 18 months, and 94.8% versus 94.6% at 24 months, respectively. An appropriate increase in mean serum estradiol level was observed in the estradiol group, from 49.5 pmol/L (13.5 pg/mL) at baseline to 218.7 pmol/L (59.6 pg/mL) during follow-up ($P < 0.001$). Serum estradiol levels did not change in the placebo group (49.2 pmol/L [13.4 pg/mL] at baseline and 52.1 pmol/L [14.2 pg/mL] during follow-up ($P > 0.2$). Serum estradiol levels differed significantly between treatment groups during follow-up ($P < 0.001$).

Analysis of Subclinical Atherosclerosis

Evaluable participants (97 in the estradiol group and 102 in the placebo group) had a mean (±SD) of 5.6 ± 0.8 and 5.5 ± 0.9 measurements of carotid intima–media thickness, respectively. The proportion of missing data on intima–media thickness (intermediate or final) for all evaluable participants was 8% at 6 months, 11% at 12 months, 14% at 18 months, and 7% at 24 months. These data were missing because participants missed visits, not for technical reasons. The groups did not differ in the proportion of missing intima–media thickness measurements ($P > 0.2$). No trend in

Table 2—**Continued**

Evaluable Participants Receiving Lipid-Lowering Medications		
Placebo Recipients (*n* = 67)	**Estradiol Recipients (*n* = 55)**	***P* Value**
6.77 ± 0.75 (67)	6.94 ± 0.74 (55)	>0.2
−9.7 ± 1.0 (67)	−10.9 ± 1.1 (55)	>0.2
1.92 ± 0.82 (67)	1.86 ± 0.77 (55)	>0.2
−4.0 ± 2.4 (67)	4.6 ± 2.6 (55)	0.02
1.39 ± 0.32 (67)	1.37 ± 0.32 (55)	>0.2
8.1 ± 1.2 (67)	14.9 ± 1.3 (55)	<0.001
4.50 ± 0.60 (67)	4.72 ± 0.70 (55)	0.06
−15.1 ± 1.4 (67)	−20.0 ± 1.6 (55)	0.02
5.08 ± 0.75 (58)	4.92 ± 0.64 (54)	>0.2
2.9 ± 1.1 (58)	−0.3 ± 1.1 (54)	0.04
61.8 ± 45.6 (58)	56.4 ± 31.2 (54)	>0.2
−4.3 ± 3.6 (58)	−11.9 ± 3.8 (54)	0.15
5.09 ± 0.43 (57)	5.06 ± 0.81 (54)	>0.2
2.4 ± 0.8 (57)	−0.9 ± 0.8 (54)	0.004

the pattern of missing intima–media thickness measurements was observed across visits.

One hundred twenty-two of the 199 evaluable participants (61%) received lipid-lowering medication after randomization; the proportions were similar in the estradiol and placebo groups (57% and 66%, respectively; $P = 0.19$). Baseline carotid intima–media thickness was similar in the estradiol and placebo groups for women receiving lipid-lowering medication (0.766 ± 0.110 mm vs. 0.764 ± 0.128 mm; $P > 0.2$) and for those not receiving lipid-lowering medication (0.734 ± 0.111 mm vs. 0.799 ± 0.182 mm; $P = 0.07$).

The top panel of **Figure 2** shows the time course of changes in common carotid artery intima–media thickness that was predicted by the mixed-effects model (goodness-of-fit P value > 0.2). In the placebo group, subclinical atherosclerosis progressed by 0.0036 mm/y. In contrast, the estradiol group experienced regression of subclinical atherosclerosis (negative average rate of change in intima–media thickness) at a rate of −0.0017 mm/y. The difference in the average rates of progression between the two treatment groups was 0.0053 mm/y (95% CI, 0.0001 to 0.0105 mm/y) ($P = 0.046$, and $P = 0.045$ after adjustment for oophorectomy status).

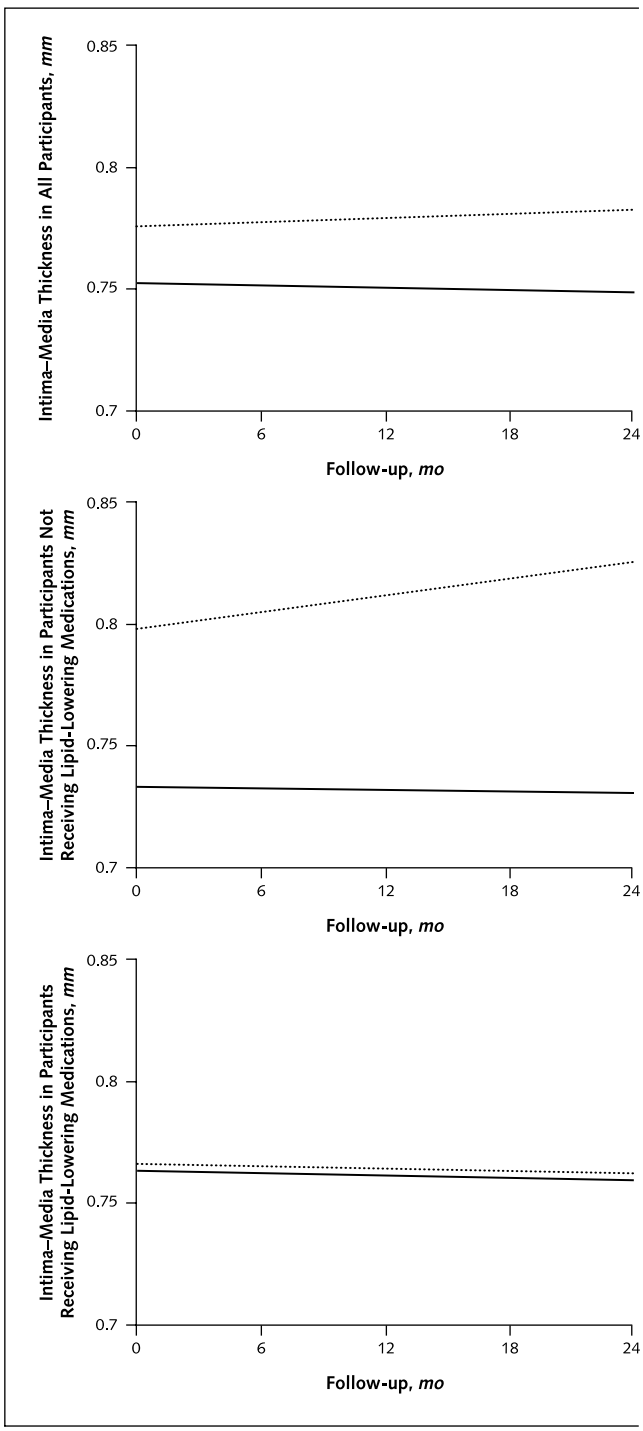
Subgroup analyses were warranted because our data indicated an interaction between lipid-lowering medication and treatment by time ($P = 0.007$). The middle panel of **Figure 2** shows the time course of changes in common carotid artery intima–media thickness in women who did not receive lipid-lowering medication. Among these women, carotid artery intima–media thickness changed 0.0134 mm/y in placebo recipients and −0.0013 mm/y in estradiol recipients (difference, 0.0147 mm/y [CI, 0.0055 to 0.0240 mm/y]; $P = 0.002$ with and without adjustment for oophorectomy status). This more pronounced difference between treatment groups resulted mainly from the greater rate of progression in the placebo group.

The bottom panel of **Figure 2** shows the time course of changes in common carotid artery intima–media thickness in women who received lipid-lowering medication. In this subgroup, rates of progression of atherosclerosis in placebo and estradiol recipients did not statistically differ (−0.0016 mm/y vs. −0.0019 mm/y; difference, 0.0003 mm/y [CI, −0.0056 to 0.0061 mm/y]; $P > 0.2$).

Among placebo recipients, those who received lipid-lowering medication had less progression of atherosclerosis than did those who did not receive such medication. The difference in the average progression rates was 0.0150 mm/y (CI, 0.0069 to 0.0230 mm/y) ($P < 0.001$). In contrast, in a subgroup analysis among estradiol recipients stratified by lipid-lowering medication, progression of subclinical atherosclerosis did not statistically differ between those women who did and did not receive lipid-lowering medication; the difference in the average progression rates was 0.0008 mm/y (CI, −0.0057 to 0.0072) ($P > 0.2$). A further subgroup analysis demonstrated that progression of subclinical atherosclerosis did not differ between placebo recipients who took lipid-lowering medication and estradiol recipients who did not receive lipid-lowering medication (difference in progression rates, −0.0005 mm/y [CI, −0.0064 to 0.0053 mm/y)]; $P > 0.2$).

To confirm our modeling procedures, we reran the statistical analysis after controlling for oophorectomy and other risk factors (hysterectomy status and smoking history) that, although nonsignificant at baseline, may be confounders. These analyses were conducted with and without two-way and three-way interactions in the model. Parameter estimates and results of hypothesis tests for the major variables (treatment; use of lipid-

Figure 2. **Time course of progression of common carotid artery intima–media thickness in the estradiol (*solid lines*) and placebo (*dotted lines*) groups.**



Top. Stratified by treatment group. The mean (±SD) baseline intima–media thickness in all 199 evaluable participants (102 placebo recipients and 97 estradiol recipients) was 0.776 ± 0.149 mm compared with 0.752 ± 0.111 mm ($P > 0.2$). The mean rate of change in intima–media thickness in the placebo and estradiol groups was 0.0036 mm/y versus −0.0017 mm/y ($P = 0.046$). **Middle. Participants not taking lipid-lowering medications.** The mean (±SD) baseline intima–media thickness in 77 evaluable participants (35 placebo recipients and 42 estradiol recipients) not taking lipid-lowering medications was 0.799 ± 0.182 mm compared with 0.734 ± 0.111 mm ($P = 0.07$). The mean rate of change in intima–media thickness in the placebo and estradiol groups was 0.0134 mm/y versus −0.0013 mm/y ($P = 0.002$). **Bottom. Participants taking lipid-lowering medications.** The mean (±SD) baseline intima–media thickness in 122 evaluable participants (67 placebo recipients and 55 estradiol recipients) taking lipid-lowering medications was 0.764 ± 0.128 mm compared with 0.766 ± 0.110 mm ($P > 0.2$). The mean rate of change in intima–media thickness in the placebo and estradiol groups was −0.0016 mm/y versus −0.0019 mm/y ($P > 0.2$).

lowering medication; and the stratification variables LDL cholesterol level, HRT use, and diabetes mellitus status) did not change (**Appendix Table**).

Changes in Lipid, Nonlipid, and Clinical Variables

Table 2 shows results of mixed-effects modeling of follow-up lipid levels in the 199 evaluable participants. Overall, the percentage increase in HDL cholesterol ($P < 0.001$) and triglyceride ($P = 0.006$) levels was greater in the estradiol group than the placebo group. In addition, the estradiol group had a greater percentage decrease in LDL cholesterol level ($P = 0.001$). Regardless of lipid-lowering medication status, the increase in HDL cholesterol levels and decrease in LDL cholesterol levels were greater in the estradiol group than the placebo group ($P < 0.05$ for all comparisons). Among women taking lipid-lowering medication, the increase in triglyceride level was greater in estradiol recipients than in placebo recipients ($P = 0.02$). Among women not taking lipid-lowering medication, the decrease in total cholesterol level was greater in estradiol recipients ($P = 0.004$).

Overall, estradiol had a positive effect on carbohydrate metabolism compared with placebo, as shown by greater percentage decreases in fasting glucose ($P = 0.09$), insulin ($P = 0.01$), and hemoglobin A_{1c} levels ($P = 0.007$). Body weight, waist-to-hip ratio, body mass index, pulse rate, and systolic and diastolic blood pressures did not change significantly from baseline to follow-up and did not differ significantly between treatment groups (data not shown).

Adverse Gynecologic and Clinical Events

Of the total cohort of 222 participants, uterine biopsy was performed in 5 placebo recipients and 30 es-

tradiol recipients who had endometrial thickness of 5 mm or more. No uterine-related adverse events, such as cancer, complex hyperplasia, or atypia, were found in either treatment group. Five estradiol recipients had simple hyperplasia without atypia. One placebo recipient developed breast cancer. No other cancers, deep venous thrombosis, or pulmonary embolus events were reported during the trial.

As per protocol, 30 of 222 (13.5%) participants with endometrial thickness of 5 mm or greater on ultrasonography, with endometrial hyperplasia on biopsy, or advised by the study gynecologist, received medroxyprogesterone acetate, 10 mg/d, for 14 days at least once. In the placebo group, two women received medroxyprogesterone acetate once; one woman received it twice. In the estradiol group, 14 women received medroxyprogesterone acetate once, 10 women received it 2 to 5 times, and 3 women received it 6 to 14 times. Carotid intima–media thickness and clinical and biochemical variables were measured at least 4 weeks after the last dose of medroxyprogesterone acetate to avoid confounding effects.

Nine cardiovascular events occurred in 7 participants (3 of 111 [3%] estradiol recipients and 4 of 111 [4%] placebo recipients; $P > 0.2$). Among placebo recipients, one femoral angioplasty and one percutaneous transluminal coronary angioplasty was done; among estradiol recipients, 1 had a cerebrovascular accident and 1 had a transient ischemic attack. One estradiol recipient and 1 placebo recipient had a subendocardial myocardial infarction, resulting in coronary artery bypass grafting in the estradiol recipient and percutaneous transluminal coronary angioplasty in the placebo recipient. One placebo recipient died of myocardial infarction.

DISCUSSION

Summary of Findings

Overall, subclinical atherosclerosis progressed more slowly in healthy postmenopausal women who randomly received unopposed ERT with 17β-estradiol than in women who received placebo ($P = 0.046$). On stratification, the difference in atherosclerosis progression was greatest in women who did not receive lipid-lowering medication ($P = 0.002$) and no difference was observed in women who received lipid-lowering medication ($P > 0.2$). The significant interaction between lipid-lowering medication and treatment ($P = 0.007$) supports the validity of these findings. Placebo recipients who took lipid-lowering medication had less progression of subclinical atherosclerosis than did placebo recipients who did not receive such medication ($P < 0.001$). Finally, the reduction in progression of subclinical atherosclerosis was similar in placebo recipients who took lipid-lowering medication and estradiol recipients who did not receive such medication ($P > 0.2$). Although only 5 participants (3% incidence) had endometrial hyperplasia without atypia (all of whom received estradiol), women should not take unopposed estradiol if they have a uterus because of the risk for uterine cancer. Although ERT improved markers of carbohydrate metabolism, the treatment benefits that we observed should not be generalized to postmenopausal women with diabetes, since only 6 evaluable participants had diabetes mellitus.

Thickening of the intima–media of the arterial wall is the earliest detectable anatomic change in the development and progression of atherosclerosis. High-resolution B-mode ultrasonography is used to noninvasively quantitate carotid intima–media thickness as a measure of subclinical atherosclerosis (21). Carotid intima–media thickness is a marker of generalized atherosclerosis and is predictive of clinical cardiovascular events (11, 12, 22–25).

Comparison of EPAT with Other Studies

Our results are consistent with those of more than 40 observational studies indicating that postmenopausal women who use ERT have lower rates of coronary heart disease than do postmenopausal women who do not use ERT (5, 6). Cross-sectional studies using angiographic end points show less coronary atherosclerosis in ERT users than in nonusers (26–29). Results of population-based studies of subclinical atherosclerosis are less consistent; at least three studies have shown thinner carotid intima–media in ERT users than in nonusers (30–32), but one study did not find this association (33). As in other epidemiologic studies, ERT use in these population-based studies was associated with a more favorable cardiovascular risk factor profile, health-related behaviors, and socioeconomic and demographic factors; these findings underscore the importance of the results from EPAT, as a randomized, controlled clinical trial. The lipid and carbohydrate metabolic effects of unopposed 17β-estradiol are also consistent with those observed in

previous studies and may be an important mechanism in slowing the progression of atherosclerosis (34).

Of particular relevance to EPAT was a subgroup analysis of 186 postmenopausal women who randomly received placebo or lovastatin in the Asymptomatic Carotid Artery Progression Study (35). Of the 186 women, 63 reported current use of ERT at least on one visit, and more than half of the users reported current use at more than 90% of their visits over the 3-year study. Women who self-selected ERT tended to have a healthier cardiovascular risk factor profile than those who did not use ERT. The effects on the progression of subclinical atherosclerosis were similar to those that we observed: Estrogen replacement therapy reduced progression of atherosclerosis in women not receiving lipid-lowering therapy but had no additional effect on progression in women receiving lovastatin. In addition, data from the Asymptomatic Carotid Artery Progression Study indicated a reduction in the progression of carotid intima–media thickness in a subgroup of women who randomly received lovastatin plus warfarin versus placebo (36), a finding that supports our nonrandomized results in women taking lipid-lowering medication.

Although observational data (predominantly in asymptomatic women) suggest that combined estrogen–progestogen hormone therapy is associated with a reduced incidence of coronary heart disease (37–39), daily continuous combined conjugated equine estrogen–medroxyprogesterone acetate therapy for 4.1 years did not reduce the rate of nonfatal myocardial infarction and coronary heart disease death in the Heart and Estrogen/progestin Replacement Study (HERS), a randomized trial involving 2763 postmenopausal women with established coronary heart disease (40). Despite the overall null results of HERS, the investigators reported a significant early increase in risk for cardiovascular and thromboembolic events and a late time trend, with fewer nonfatal myocardial infarctions in the HRT group than in the placebo group (40). In a substudy of HERS, rates of change in carotid intima–media thickness did not differ between 455 participants randomly assigned to therapy with continuous combined conjugated equine estrogen–medroxyprogesterone acetate versus placebo (41). Although the null results of HERS are not consistent with current observational data, nonhuman primate studies have demonstrated that continuous combined estrogen–medroxyprogesterone acetate therapy negates the beneficial effect of unopposed estrogen therapy on coronary and carotid atherosclerosis (42, 43).

The results of HERS were confirmed and expanded by the Estrogen Replacement and Atherosclerosis (ERA) trial, a randomized, controlled trial of 309 postmenopausal women treated for 3.2 years with a serial coronary angiographic end point (44). In ERA, therapy with neither conjugated equine estrogen–medroxyprogesterone acetate nor unopposed conjugated equine estrogen slowed the progression of coronary artery atherosclerosis compared with placebo (44). As in HERS, women in ERA had established coronary artery disease.

Reasons for Divergent Outcomes among Trials

Many potential reasons explain the divergent outcomes between EPAT and HERS and ERA. The three studies not only had different end points (intima–media thickness vs. events and coronary angiography) but also had at least three other important differences. First, EPAT was conducted in healthy postmenopausal women without symptoms of cardiovascular disease (primary prevention), whereas HERS and ERA were conducted in women with preexisting coronary heart disease (secondary prevention). Second, 17β-estradiol was used in EPAT, whereas conjugated equine estrogen was used in HERS and ERA. Conjugated equine estrogen, a mixture of many steroidal compounds derived from the urine of pregnant horses, and 17β-estradiol, the endogenous human hormone, differ greatly. Randomized, controlled trials are needed to determine whether other estrogen compounds have the same beneficial effect on subclinical atherosclerosis as does unopposed 17β-estradiol. Finally, participants in EPAT were younger than participants in HERS and ERA, and the time from menopause to randomization was approximately 10 years shorter in EPAT. Early intervention in the progression of atherosclerosis, especially at the start of menopause, may be the key to successful prevention of cardiovascular disease with HRT. Because nonevaluable participants were younger than evaluable participants, the treatment benefit in EPAT might have been greater if these participants had completed the trial.

Use of proven atherosclerosis- and event-reducing therapies in addition to the study therapy is inevitable in cardiovascular end point studies of secondary prevention. In HERS and ERA, more than 50% of participants

were taking other therapies proven to reduce cardiovascular events, such as aspirin, β-blockers, angiotensin-converting enzyme inhibitors, and lipid-lowering medication, alone or in combination. In those studies, the cardiovascular effects of HRT may have been masked by or added no benefit over the effects of the other event-reducing therapies. Comparison of EPAT participants who received lipid-lowering medication and ERT with participants who received ERT but no lipid-lowering medication confirms this idea. Subanalyses of other nonrandomized treatment regimens have not yet been reported in HERS or ERA.

Although observational data indicate that HRT is associated with a lower incidence of coronary heart disease in postmenopausal women, most of these data are associations with unopposed ERT in women without symptomatic coronary heart disease (5, 6). The observational arterial imaging studies that support an HRT-related reduction of atherosclerosis have studied unopposed ERT (26–32).

Unopposed ERT inhibited development of atherosclerosis in several animal models (45–48). It is conceivable that HRT in general, and unopposed ERT specifically, may be most effective as primary prevention, when atherosclerosis is in its early stages, rather than as secondary prevention, when atherosclerosis is in a later, established phase. In other words, ERT may be effective in the prevention rather than the treatment of atherosclerosis. This notion is consistent with the possible proinflammatory effects of estrogen. Estrogen increases levels of C-reactive protein (49–52), a marker of underlying inflammation that is associated with cardiovascular events in women (53). Estrogen may reduce early preintrusive atherosclerosis in healthy women but not cardiovascular events if it promotes arterial thrombosis or atherosclerotic plaque destabilization in women with established cardiovascular disease. This scenario appears to be consistent with the early events reported in HERS (40).

It is unclear whether sequential therapy with medroxyprogesterone acetate or other progestational agents would result in a different clinical outcome than that seen with continuous combined therapy in HERS and ERA. These and other questions concerning the role of HRT in the prevention and treatment of atherosclerotic disease in postmenopausal women need further study. As the initial randomized, controlled trials of atherosclerosis end points in this important area of women's health, EPAT, HERS, and ERA indicate new directions for further investigation.

Conclusions

Overall, we found that healthy postmenopausal women without preexisting cardiovascular disease who randomly received unopposed ERT with 17β-estradiol had less progression of subclinical atherosclerosis than did those who received placebo. In subgroup analyses, this result was seen only in women not receiving lipid-lowering medication. The reduction of the progression of subclinical atherosclerosis associated with ERT was of the same magnitude as that in women who received lipid-lowering medication. Furthermore, lipid-lowering medication appeared not to add benefit to unopposed ERT in reducing the progression of subclinical atherosclerosis. Although the use of lipid-lowering medication was not randomized in EPAT, these results are important because they suggest that unopposed ERT can serve as an alternative to lipid-lowering medication as preventive therapy to slow the progression of subclinical atherosclerosis in postmenopausal women who cannot or do not wish to use lipid-lowering medication.

Since most women who enter menopause are asymptomatic for cardiovascular disease and 95% of women who develop cardiovascular disease do so after menopause, our results suggest that administration of unopposed 17β-estradiol to women entering menopause may slow the progression of atherosclerosis and its clinical sequelae (11, 12, 22–25). Two ongoing large clinical trials in predominantly healthy women, the Women's Health Initiative (27 000 participants) and the Women's International Study of Long Duration Oestrogen for Menopause (36 000 participants), will provide important information on conjugated equine estrogen for primary prevention of coronary heart disease. If confirmed in other studies, the cardiovascular benefit of ERT may outweigh any risks associated with this preventive therapy (54). Additional randomized clinical trials are needed to elucidate the role of unopposed and opposed ERT in primary and secondary prevention of cardiovascular disease in postmenopausal women. As the results of EPAT, HERS, and ERA indicate, the questions remaining in this important area of investigation are complicated by the variety of HRT regimens, deliv-

Appendix Table. **Parameter Estimates and 95% Confidence Intervals for All Models***

Parameter Effect	Estimate (95% CI)	
	Preplanned Model†	Adjusted for Risk Factors
Intercept	0.73780 (0.70560 to 0.77000)	0.74020 (0.63446 to 0.84594)
Receipt of placebo	0.02143 (−0.01479 to 0.05765)	0.03108 (−0.00555 to 0.06771)
Years on study	−0.00168 (−0.00542 to 0.00206)	−0.00168 (−0.00542 to 0.00206)
Lipid-lowering treatment on study	–	–
Stratification variables		
LDL cholesterol level ≥ 3.37 mmol/L (≥130 mg/dL)	0.02691 (−0.00939 to 0.06321)	0.02291 (−0.01321 to 0.05903)
Past HRT use	−0.00194 (−0.04753 to 0.04365)	−0.01722 (−0.06448 to 0.03004)
Diabetes mellitus	0.09316 (−0.01348 to 0.19980)	0.09657 (−0.00888 to 0.20202)
Two-way interactions		
Years × receipt of placebo	0.00532 (0.00012 to 0.01051)	0.00534 (0.00014 to 0.01054)
Lipid-lowering treatment × receipt of placebo	–	–
Years × lipid-lowering treatment	–	–
Three-way interaction: years × lipid-lowering treatment × receipt of placebo	–	–
Covariates		
Oophorectomy	–	0.04889 (−0.00997 to 0.10775)
Hysterectomy	–	−0.01265 (−0.0663 to 0.04100)
Former smoker	–	0.01081 (−0.0250 to 0.04670)

* Restricted maximum likelihood procedures were used to estimate and test the parameters. HRT = hormone replacement therapy; LDL = low-density lipoprotein.
† Treatment differences in the preplanned model were as follows: all evaluable participants, $P = 0.046$; those not taking lipid-lowering medications, $P = 0.002$; those taking lipid-lowering medications, $P > 0.2$.
‡ Treatment differences in full model 2 were as follows: all evaluable participants, $P = 0.044$; those not taking lipid-lowering medications, $P = 0.0018$; those taking lipid-lowering medications, $P > 0.2$.

ery routes, and dosages; confounding treatments; and varying groups of women at risk.

APPENDIX: THE EPAT RESEARCH GROUP

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Appendix Table—**Continued**

Estimate (95% CI)	
Full Model 1	**Full Model 2‡**
0.72840 (0.68702 to 0.76978)	0.73100 (0.62010 to 0.84190)
0.06494 (0.00687 to 0.12301)	0.07364 (0.01553 to 0.13175)
−0.00120 (−0.00677 to 0.00437)	−0.00120 (−0.00677 to 0.00437)
0.00493 (−0.0530 to 0.06289)	0.00846 (−0.04943 to 0.06636)
0.03986 (−0.00395 to 0.08367)	0.03373 (−0.01008 to 0.07754)
−0.00125 (−0.04711 to 0.04461)	−0.01549 (−0.06310 to 0.03212)
0.09953 (−0.00704 to 0.20610)	0.10190 (−0.00361 to 0.20741)
0.01477 (0.00659 to 0.02296)	0.01478 (0.00660 to 0.02297)
−0.06725 (−0.14177 to 0.00727)	−0.06763 (−0.14256 to 0.00730)
−0.00072 (−0.00803 to 0.00659)	−0.00072 (−0.00803 to 0.00659)
−0.01428 (−0.02470 to −0.00386)	−0.01428 (−0.02470 to −0.00386)
–	0.04828 (−0.01081 to 0.10737)
–	−0.01105 (−0.06477 to −0.04267)
–	0.00462 (−0.03172 to 0.04096)

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