

Inflammatory, Lipid, Thrombotic, and Genetic Markers of Coronary Heart Disease Risk in the Women's Health Initiative Trials of Hormone Therapy

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Background: Clinical trials of postmenopausal hormone therapy (HT) have shown increased risk of coronary heart disease (CHD) in the first few years after initiation of therapy and no overall benefit.

Methods: This nested case-control study evaluates a range of inflammatory, lipid, thrombotic, and genetic markers for their association with CHD in the 4 years after randomization and assesses whether any of these markers modified or mediated the initially increased risk associated with HT in postmenopausal women aged 50 to 79 years at baseline. Conjugated equine estrogens, 0.625 mg/d, or placebo was given to 10 739 hysterectomized women, and the same estrogen plus medroxyprogesterone acetate, 2.5 mg/d, was given to 16 608 women with an intact uterus.

Results: In multivariate-adjusted analyses of 359 cases and 820 controls in the combined trials, baseline levels of 12 of the 23 biomarkers studied were associated with CHD events: interleukin 6, matrix metalloproteinase 9, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, total cholesterol, triglycerides,

D-dimer, factor VIII, von Willebrand factor, leukocyte count, homocysteine, and fasting insulin. Biomarkers tended to be more strongly associated with CHD in the initial 2 years after randomization. The genetic polymorphism glycoprotein IIIa leu33pro was significantly associated with CHD. Baseline low-density lipoprotein cholesterol interacted significantly with HT so that women with higher levels were at higher risk for CHD when given HT ($P = .03$ for interaction). The levels of several biomarkers were changed by HT, but these changes did not seem to be associated with future CHD events.

Conclusions: Several thrombotic, inflammatory, and lipid biomarkers were associated with CHD events in postmenopausal women, but only low-density lipoprotein cholesterol modified the effect of HT. Further research is needed to identify the mechanisms by which HT increases the risk of CHD.

Trial Registration: clinicaltrials.gov Identifier: NCT00000611

Arch Intern Med. 2008;168(20):2245-2253

THE WOMEN'S HEALTH INITIATIVE (WHI) trials of postmenopausal hormone therapy (HT) tested whether the use of estrogen alone or combined with progestin would reduce the risk of coronary heart disease (CHD) in predominantly healthy postmenopausal women. The trial of conjugated equine estrogens plus medroxyprogesterone acetate in women with an intact uterus was stopped early because of an increase in cardiovascular events (CHD, stroke, and venous thromboembolism [VTE]) and in breast cancer.¹ The parallel trial of conjugated equine estrogens alone in hysterectomized women was also stopped early because of increased strokes and lack of

benefit for CHD.² After publication of these findings, current recommendations state that HT should not be started or continued for the prevention of CHD.³

In the conjugated equine estrogens plus medroxyprogesterone trial, the cumulative hazard ratio (HR) for CHD after a mean of 5.6 years of follow-up was 1.24, with a 95% confidence interval (CI) of 1.00 to 1.54; however, the initial risks were higher, with HRs in years 1 through 6 or more of 1.81, 1.34, 1.27, 1.25, 1.45, and 0.70, respectively ($P = .02$ for trend).⁴ Similar trends for higher initial risks were found in the Heart and Estrogen/progestin Replacement Study of conjugated equine estrogens plus medroxyprogesterone.⁵ In the WHI trial of conjugated equine estro-

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gens, there was no overall effect on CHD, with a cumulative HR of 0.95 (95% CI, 0.79-1.16) after 7.1 years. Risks of CHD were modestly elevated in the first 2 years, but the overall temporal trend was not significant, with HRs of 1.11, 1.20, 0.89, 0.79, 1.39, and 0.81 in years 1 through 6 or more, respectively ($P = .14$ for trend).⁶

To elucidate the mechanisms by which HT might initially increase the risk of CHD, the WHI investigators conducted a nested case-control study that included all centrally adjudicated cases of CHD that occurred in the first 4 years of the study. The possible effects of lipids, lipoproteins, and coagulation factors on trial results were pre-specified in the study protocol. Other laboratory markers were chosen on the basis of a priori knowledge from other studies of their relationship to CHD, with a focus on those affected by HT. In this article, we report on the association of markers of inflammation, lipid metabolism, thrombosis, and other markers and candidate genes with CHD and their potential interaction with HT effect on CHD.

METHODS

Details of the design, recruitment, randomization, data collection, intervention, and outcomes ascertainment procedures in the WHI HT trials, including CONSORT (Consolidated Standards of Reporting Trials) diagrams, have been published previously.^{1,2}

STUDY POPULATION AND INTERVENTIONS

The WHI hormone trials enrolled 27 347 postmenopausal women aged 50 to 79 years between September 1, 1993, and December 31, 1998, at 40 US clinical centers based on hysterectomy status: 16 608 without hysterectomy in a trial of conjugated equine estrogens plus medroxyprogesterone and 10 739 with hysterectomy in a trial of conjugated equine estrogens alone. At baseline, women completed screening and baseline questionnaires by interview and self-report, and a physical examination was performed. Blood specimens were collected at baseline and at the 1-year visit. The study was approved by the human subjects review committee at each participating institution, and all the participants provided written informed consent.

Participants were randomly assigned to take a single daily tablet containing placebo or active medication: women without hysterectomy took 0.626 mg of conjugated equine estrogens plus 2.5 mg of medroxyprogesterone acetate (Prempro), and women with hysterectomy took 0.625 mg of conjugated equine estrogens (Premarin). Study drugs and placebo were supplied by Wyeth-Ayerst (St Davids, Pennsylvania). The planned end date of the trials was March 31, 2005, for a total follow-up of 8.4 years; however, conjugated equine estrogens plus medroxyprogesterone trial medications were stopped on July 7, 2002, and conjugated equine estrogens were stopped on March 1, 2004, after mean follow-up periods of 5.6 and 7.1 years, respectively.^{4,6}

All centrally adjudicated cases of CHD, stroke, and VTE occurring during the first 4 years of follow-up were included in biomarker studies. Controls were matched on age, randomization date, hysterectomy status, and prevalent cardiovascular disease (CVD) at baseline. Matching on prevalent disease was specific to the case type so that cases of CHD were matched on prevalent myocardial infarction (MI), cases of stroke on prevalent stroke, and cases of VTE on prevalent VTE. All controls

for the 3 case types were used after excluding any with incident CHD, stroke, or VTE. The CHD biomarker study included 359 cases of CHD and 820 controls. Of the 359 participants with CHD, 11 also had a stroke, 9 had a VTE, and 1 had all 3 events. Analyses involving year 1 biomarker data involved 236 case patients who experienced their CHD event after the year 1 visit and 560 corresponding controls. The parallel case-control study for stroke has been published,⁷ and that for VTE is in preparation.

FOLLOW-UP AND OUTCOME ASCERTAINMENT

Clinical outcomes were identified by semiannual questionnaires and were classified by centrally trained local adjudicators after medical record review. All locally adjudicated cases of CHD were reviewed by central adjudicators (including J.E.R., S.H., and J.H.). Coronary heart disease included nonfatal and silent MI and, CHD death. Definite and probable nonfatal MI required overnight hospitalization and was defined according to an algorithm based on standardized criteria using cardiac pain, cardiac enzyme and troponin levels, and electrocardiographic findings and included MI occurring during surgery and aborted MI. Coronary heart disease death was defined as death consistent with an underlying cause of CHD plus 1 or more of the following: hospitalization for MI within 28 days before death, previous angina or MI, death due to a procedure related to CHD, or a death certificate consistent with an underlying cause of atherosclerotic CHD. Definite silent MI was diagnosed from baseline and years 3 and 6 electrocardiograms (Novacode 5.1 and 5.2).⁸

GENETIC AND BIOMARKER ANALYSIS

Blood samples were collected from all the participants at baseline and at 1 year and were stored at -70°C . Analyses were run in single batches including cases and controls and 10% blind duplicates within 8 years of collection. Lipid profiles (analyzed in EDTA plasma with high-density lipoprotein [HDL] precipitation by heparin manganese [Dade Behring, Deerfield, Illinois]), interleukin 6 (IL-6), ultrasensitive enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, Minnesota), E-selectin, matrix metalloproteinase 9 (MMP-9), homocysteine, and lipoprotein(a) were measured at Medical Research Laboratories (Highland Heights, Kentucky). C-reactive protein (CRP) (N High-Sensitivity CRP; Dade Behring), fibrinogen (clot rate assay) (Diagnostica Stago, Parsippany, New Jersey), factor VIII activity (clotting time on mixing with factor VIII-deficient plasma using STA Deficient VIII; Diagnostica Stago), von Willebrand factor (vWF) activity and fibrin D-dimer (immunoturbidimetric assays) (Liatest von Willebrand factor and Liatest D-Di; Diagnostica Stago), plasminogen activator inhibitor 1 antigen and plasmin-antiplasmin complex (by in-house immunoassay), prothrombin fragment 1.2 (enzyme-linked immunosorbent assay, Dade Behring), and thrombin-activatable fibrinolysis inhibitor (immunoassay with antibodies from Affinity Biologicals, Ancaster, Ontario, Canada) were measured at the Laboratory for Clinical Biochemistry Research, University of Vermont. Complete blood cell counts were performed in the clinics' local laboratories. Genetic polymorphisms were assayed at Wake Forest University, Winston-Salem, North Carolina (estrogen receptor β -A1730G [rs4986938], GPIIb-IIIa-Thr145Met [rs6065], and glycoprotein IIIa [GpIIIa] leu33pro [rs5918]) and at Leiden University, Leiden, the Netherlands (factor V Leiden, prothrombin 20210, thermolabile variant of methylenetetrahydrofolate reductase, and plasminogen activator inhibitor 1 4G/5G).

STATISTICAL METHODS

All baseline marker values were log transformed owing to skewed distributions and for consistency; differences from baseline to year 1 were analyzed using the original scale. Logistic regression models were controlled for age and trial, body mass index, waist-hip ratio, smoking, alcohol consumption, physical activity, history of diabetes mellitus, history of high blood cholesterol concentration, prevalent CVD (other than MI), left ventricular hypertrophy on electrocardiography, systolic blood pressure, and use of antihypertensive medications, aspirin, or statins at baseline. In preliminary analyses, we fitted a model for each biomarker and polymorphism, including a term for interaction with trial assignment. Trial assignment was significant in 1 of 31 instances (1 or 2 would be expected by chance), suggesting that it was appropriate to combine the trials for subsequent analyses to increase statistical power.

We assessed the appropriateness of using biomarkers log linearly in generalized additive models using CHD as the response, correcting for risk factors. Because linearity was rejected for CRP, IL-6, factor VIII, and leukocyte count, we used quadratic models for these biomarkers. Although we used markers linearly or quadratically to assess significance (the more powerful analysis), we do not show the coefficients in the logistic regression model but rather the more easily interpreted odds ratios (ORs) per standard deviation increase. Thus, there is no 1-to-1 correspondence between $P < .05$ for models and CIs for ORs not containing 1. For the interaction of change in biomarker levels at 1 year, we show the ORs by tertiles of change, but the P values are computed from logistic coefficients for change as a continuous variable. We also examined whether changes in individual biomarker levels were an intermediate outcome in the pathway of hormone effects on CHD by comparing regression models with and without terms for biomarker change covariates. Outliers were identified by visual inspection of histograms and scatterplots; 1 result for factor VIII, 7 for hematocrit, and 2 for vWF were deemed to be outliers and were excluded from the analyses.

We tested for nominal significance at $P < .05$ without adjustment for multiple testing. In the adjusted models, we performed 31 tests for significance of the relationship of baseline biomarkers with CHD risk, of which 13 were significant (1 or 2 expected by chance) and, in analyses stratified by years since randomization, 20 of 62 tests were significant (3 expected); 31 tests of interaction of baseline levels with treatment assignment on CHD risk, of which 1 was significant (1 or 2 expected) and, stratified by years since randomization, 4 of 62 tests were significant (3 expected); and 23 tests of interaction of change in biomarker levels at 1 year with treatment assignment on CHD risk, of which none was significant (1 expected). Statistical analyses were performed using SAS statistical software (version 9; SAS Institute Inc, Cary, North Carolina).

RESULTS

BASELINE DATA

Baseline characteristics are shown by case-control status (**Table 1**). Baseline characteristics associated with CHD were used for adjusting subsequent multiple logistic regression models. Median baseline biomarker levels, notably CRP, tended to be higher in the conjugated equine estrogens placebo group than in the conjugated equine estrogens plus medroxyprogesterone placebo group, in keeping with the higher baseline risks of CHD in the trial of conjugated equine estrogens (**Table 2**).^{4,6}

Except for the expected correlations between lipids and lipoproteins, correlations between baseline biomarkers were weak. Cases and controls in the present analyses demonstrated ORs of 1.43 (95% CI, 0.98-2.08) for conjugated equine estrogens plus medroxyprogesterone vs placebo and 1.20 (0.75-1.90) for conjugated equine estrogens vs placebo.

ASSOCIATIONS OF BASELINE BIOMARKERS WITH INCIDENT CHD

In models adjusted only for treatment assignment, several biomarkers were associated with CHD and to a similar degree in both trials (**Table 2**). The genetic polymorphism GpIIIa leu33pro of the platelet glycoprotein IIa/IIIb complex (GpIIa-IIIb) fibrinogen receptor, but not the other 6 candidate polymorphisms, was associated with CHD risk. In multivariate analyses adjusting for treatment assignment and baseline characteristics (including prevalent CVD, statin treatment, and diabetes mellitus), some inflammatory biomarkers (IL-6, MMP-9, and leukocyte count), lipids (HDL, low-density lipoprotein [LDL], and total cholesterol and triglycerides), thrombotic and other biomarkers (D-dimer, factor VIII, vWF, homocysteine, and fasting insulin), and the GpIIIa leu33pro polymorphism remained significantly associated with CHD (**Table 3**). The associations of biomarkers with CHD varied by time since randomization. Certain biomarkers were significantly associated with CHD risk in the first 2 years after randomization but not after 2 years, including MMP-9, HDL cholesterol, triglycerides, fibrinogen, leukocyte count, and insulin. Factor VIII was associated with CHD in both periods but significantly more so in the first 2 years, and LDL cholesterol, total cholesterol, D-dimer, vWF, and the GpIIIa leu33pro polymorphism were related to risk in both periods. Homocysteine was more strongly associated with CHD after 2 years. Higher levels of E-selectin were associated with lower CHD risk in the first 2 years but higher risk in the second 2-year period.

INTERACTIONS OF BASELINE BIOMARKERS WITH HORMONE EFFECTS ON INCIDENT CHD

In the combined trial data, the baseline level of LDL cholesterol interacted significantly with treatment assignment, with greater risks of CHD during HT in women with higher levels of LDL cholesterol (**Table 4**) (overall $P = .03$ for interaction). This finding depended on the trial of conjugated equine estrogens plus medroxyprogesterone ($P = .006$ for interaction) rather than on the trial of conjugated equine estrogens ($P = .84$ for interaction). A trend in the opposite direction was seen for HDL cholesterol, but the interaction was not significant ($P = .08$). There were no other significant interactions of biomarkers or polymorphisms on CHD with treatment. In additional analyses stratified by time since randomization, the interaction of treatment with LDL cholesterol was significant in both periods, with $P = .05$ in the first 2 years and $P = .01$ in the second 2 years (data not shown). There was also a significant interaction with homocysteine in the second 2 years ($P = .03$) but not in the first 2 years

Table 1. Baseline Characteristics of Women in the Nested Case-Control Study

	CEE + MPA Trial		CEE Trial		P Value ^a
	Control	CHD	Control	CHD	
Ethnicity, No. (%)					
White	424 (88.0)	183 (89.3)	253 (75.5)	117 (76.0)	.87
Black	28 (5.8)	12 (5.9)	58 (17.3)	26 (16.9)	
Other	30 (6.2)	10 (4.9)	24 (7.2)	11 (7.1)	
Smoking status, No. (%)					
Never	268 (56.4)	90 (45.5)	169 (51.8)	72 (47.7)	<.001
Past	171 (36.0)	66 (33.3)	128 (39.3)	48 (31.8)	
Current	36 (7.6)	42 (21.2)	29 (8.9)	31 (20.5)	
Alcohol use, No. (%)					
Nondrinker	221 (46.3)	107 (52.5)	174 (52.1)	102 (67.5)	.006
≤1 drink/d	193 (40.5)	78 (38.2)	133 (39.8)	42 (27.8)	
>1 drink/d	63 (13.2)	19 (9.3)	27 (8.1)	7 (4.6)	
Physical activity, No. (%)					
Inactive	61 (14.7)	40 (23.1)	68 (22.9)	33 (24.3)	.01
<5 METs	89 (21.4)	43 (24.9)	73 (24.6)	44 (32.4)	
5-12 METs	104 (25.0)	43 (24.9)	65 (21.9)	26 (19.1)	
>12 METs	162 (38.9)	47 (27.2)	91 (30.6)	33 (24.3)	
Treated diabetes mellitus, No. (%)	22 (4.6)	28 (13.7)	20 (6.0)	36 (23.4)	<.001
History of hypertension, No. (%)					
Never	274 (65.9)	86 (50.0)	172 (58.7)	55 (41.0)	<.001
Untreated	37 (8.9)	20 (11.6)	18 (6.1)	15 (11.2)	
Treated	105 (25.2)	66 (38.4)	103 (35.2)	64 (47.8)	
History of high cholesterol, No. (%)	66 (16.0)	43 (25.3)	54 (18.6)	36 (27.3)	<.001
LVH on electrocardiography, No. (%)	23 (4.8)	11 (5.4)	23 (6.9)	22 (14.6)	.02
History of CVD, No. (%)	53 (11.1)	48 (24.2)	59 (17.9)	42 (28.0)	<.001
Baseline aspirin use, No. (%)	105 (21.8)	56 (27.3)	75 (22.4)	50 (32.5)	.007
Baseline statin use, No. (%)	35 (7.3)	34 (16.6)	35 (10.4)	21 (13.6)	<.001
Age at screening, No., mean (SD), y	482, 66.7 (6.9)	205, 66.1 (7.54)	335, 66.4 (6.6)	154, 67.4 (6.2)	.84
BMI, No., mean (SD)	478, 27.8 (5.5)	205, 29.0 (5.7)	334, 29.4 (5.6)	154, 30.2 (5.7)	.004
Height, No., mean (SD), cm	480, 161.0 (6.7)	205, 161.2 (6.5)	334, 161.4 (6.4)	154, 160.8 (6.2)	.80
Weight, No., mean (SD), kg	480, 72.2 (15.3)	205, 75.5 (16.0)	335, 76.6 (15.3)	154, 78.2 (16.1)	.008
Waist to hip ratio, No., mean (SD)	479, 0.8 (0.1)	205, 0.8 (0.1)	335, 0.8 (0.1)	154, 0.9 (0.1)	<.001
Waist, No., mean (SD), cm	480, 86.8 (13.7)	205, 90.9 (13.8)	335, 90.9 (13.2)	154, 95.1 (13.9)	<.001
Systolic BP, No., mean (SD), mm Hg	482, 129.7 (17.8)	205, 134.2 (18.5)	335, 130.2 (16.7)	154, 139.6 (19.0)	<.001
Diastolic BP, No., mean (SD), mm Hg	482, 74.9 (9.3)	205, 76.9 (10.3)	334, 75.9 (9.00)	154, 76.3 (10.8)	.02

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); BP, blood pressure; CEE, conjugated equine estrogens; CHD, coronary heart disease; CVD, cardiovascular disease; LVH, left ventricular hypertrophy; METs, metabolic equivalents; MPA, medroxyprogesterone.

^aThe P values quantify the marginal association of each baseline characteristic with incident CHD and are obtained from a logistic regression model adjusted for treatment assignment (CEE, CEE placebo, CEE + MPA, or CEE + MPA placebo) using a 1-*df* test for association except for the categorical variables ethnicity, smoking status, alcohol use, physical activity, and use of hypertension medication.

(*P* = .54). Finally, a potential interaction of treatment with E-selectin in the first 2 years was noted (*P* = .05), with lower risk in women with higher levels of E-selectin.

BIOMARKER CHANGES FROM BASELINE TO YEAR 1

Hormone therapy in both trials increased CRP and MMP-9 levels, decreased E-selectin levels, and had no effect on IL-6 levels (Table 5). There were also significant increases in HDL cholesterol and triglyceride levels and decreases in LDL and total cholesterol levels. Hormone therapy increased levels of plasmin-antiplasmin complex and decreased fibrinogen, plasminogen activator inhibitor 1 antigen, homocysteine, glucose, and insulin levels but had no effect on D-dimer, factor VIII, prothrombin fragment 1.2, thrombin-activatable fibrinolysis inhibitor, and vWF levels. None of the changes seemed to be associated with change in the risk of CHD after the first year (data not shown).

INTERACTIONS OF BIOMARKER CHANGES WITH HORMONE EFFECTS ON INCIDENT CHD

None of the changes in biomarkers significantly affected the risk of CHD due to hormones after the first year (Table 6). The interaction of change in E-selectin level with treatment assignment had a value of *P* = .08; however, this possible interaction was not in the expected direction, with higher ORs in participants with the most decrease in E-selectin levels. Changes in individual biomarker levels did not seem to be an intermediate outcome in the pathway of hormone effects on CHD (data not shown).

COMMENT

The primary purpose of this case-control study was to seek mechanistic explanations for the early increase in CHD events found in trials of HT, which included women with and without previous CVD. Hence, the focus was

Table 2. Baseline Biomarkers and Gene Polymorphisms

	No., Median (IQR) ^a				P Value ^b
	CEE + MPA Trial		CEE Trial		
	Control	CHD	Control	CHD	
C-reactive protein, mg/L	464, 1.8 (3.5)	198, 2.9 (4.2)	326, 2.6 (3.6)	149, 3.7 (5.5)	<.001
E-selectin, ng/mL	471, 43.0 (26.0)	196, 45.5 (27.5)	329, 45.0 (27.0)	147, 47.0 (34.0)	.08
IL-6, pg/mL	471, 2.8 (2.2)	196, 3.4 (2.3)	324, 2.8 (2.3)	152, 3.7 (3.7)	<.001
MMP-9, ng/mL	482, 217.5 (148.0)	205, 228.0 (168.0)	335, 217.0 (148.0)	154, 235.0 (177.0)	.01
HDL cholesterol, mg/dL	481, 54 (20.0)	202, 48 (15.0)	334, 52 (17.0)	152, 47 (17.0)	<.001
LDL cholesterol, mg/dL	473, 138 (44.0)	194, 151 (41.0)	327, 140 (47.0)	146, 149 (46.0)	<.001
Total cholesterol, mg/dL	482, 221 (49.0)	204, 235 (44.5)	335, 228 (51.0)	154, 233 (52.0)	<.001
Triglycerides, mg/dL	482, 130 (82.0)	204, 145 (107.5)	335, 142 (90.0)	154, 162 (101.0)	<.001
Lipoprotein(a), mg/dL	459, 18 (28.0)	194, 22 (37.0)	320, 23 (34.0)	147, 22 (35.0)	.15
D-dimer, µg/mL	481, 0.3 (0.3)	202, 0.4 (0.4)	334, 0.3 (0.3)	153, 0.4 (0.4)	<.001
Fibrinogen, mg/dL	481, 305 (112.0)	202, 317 (123.0)	334, 314 (125.0)	153, 332 (128.0)	<.001
Factor VIII, %	480, 105.0 (61.0)	202, 120.0 (74.0)	334, 103.0 (68.0)	154, 117.5 (77.0)	<.001
PAI-1 antigen, ng/mL	454, 35 (46.4)	187, 41 (47.4)	308, 44 (57.4)	142, 48 (51.7)	.54
Prothrombin fragment 1.2, nmol/L	448, 1.3 (0.4)	185, 1.3 (0.5)	306, 1.3 (0.5)	142, 1.4 (0.6)	.09
PAP, nmol/L	453, 4.5 (2.5)	187, 4.5 (2.3)	308, 4.2 (2.1)	142, 4.3 (2.4)	.51
TAFI, µg/mL	470, 5.1 (2.5)	200, 4.9 (2.6)	327, 5.2 (2.7)	146, 5.2 (1.9)	.35
von Willebrand factor, %	479, 93.0 (54.0)	202, 97.0 (64.0)	333, 89.0 (52.0)	153, 100.0 (61.0)	<.001
Leukocyte count, cells/µL	482, 5.8 (1.9)	202, 6.3 (2.3)	335, 5.7 (2.1)	153, 6.4 (2.3)	<.001
Platelet count, ×10 ³ /µL	481, 246.0 (80.0)	202, 246.5 (75.0)	335, 242.0 (69.0)	153, 244.0 (82.0)	.76
Hematocrit, %	482, 40.3 (3.7)	200, 41.4 (3.7)	334, 40.6 (3.8)	152, 41.0 (4.5)	.04
Homocysteine, mg/L	481, 1.10 (3.4)	204, 1.14 (3.9)	335, 1.12 (3.9)	154, 1.15 (4.0)	.003
Glucose, mg/dL	480, 97 (16.0)	202, 100 (24.0)	332, 98 (18.0)	153, 102 (32.0)	<.001
Insulin, µIU/mL	451, 7.1 (6.7)	191, 9.2 (8.0)	316, 8.5 (8.4)	136, 10.0 (9.6)	<.001
Estrogen receptor β-A1730G, No. (%)					
C/C	176 (38.7)	67 (34.2)	118 (37.1)	52 (36.1)	.67
C/T	216 (47.5)	101 (51.5)	155 (48.7)	71 (49.3)	
T/T	63 (13.8)	28 (14.3)	45 (14.2)	21 (14.6)	
Factor V Leiden, No. (%)					
G/G	430 (95.3)	192 (96.0)	315 (96.0)	144 (97.3)	.39
G/A	21 (4.7)	8 (4.0)	13 (4.0)	4 (2.7)	
Factor XIII val34Leu, No. (%)					
Val/val	244 (54.1)	111 (55.5)	196 (59.8)	89 (59.7)	.62
Val/leu	179 (39.7)	77 (38.5)	117 (35.7)	48 (32.2)	
Leu/leu	28 (6.2)	12 (6.0)	15 (4.6)	12 (8.1)	
GP1bα-Thr145Met, No. (%)					
C/C	386 (84.6)	165 (84.2)	237 (74.3)	118 (82.5)	.42
C/T	62 (13.6)	30 (15.3)	76 (23.8)	21 (14.7)	
T/T	8 (1.8)	1 (0.5)	6 (1.9)	4 (2.8)	
MTHFR, No. (%)					
C/C	192 (42.3)	96 (48.0)	162 (49.4)	65 (43.3)	.99
C/T	198 (43.6)	83 (41.5)	139 (42.4)	65 (43.3)	
T/T	64 (14.1)	21 (10.5)	27 (8.2)	20 (13.3)	
PAI-1, No. (%)					
4G/4G	111 (25.1)	51 (26.0)	65 (20.1)	38 (26.6)	.39
4G/5G	236 (53.3)	107 (54.6)	170 (52.5)	70 (49.0)	
5G/5G	96 (21.7)	38 (19.4)	89 (27.5)	35 (24.5)	
Prothrombin 20210, No. (%)					
G/G	434 (96.0)	195 (98.0)	321 (97.9)	147 (99.3)	.10
A/G	18 (4.0)	4 (2.0)	7 (2.1)	1 (0.7)	
Glycoprotein IIIa leu33pro, No. (%) ^c					
C/C	13 (2.8)	2 (1.0)	10 (3.1)	2 (1.4)	<.001
C/T	93 (20.4)	69 (35.2)	83 (26.0)	47 (32.4)	
T/T	351 (76.8)	125 (63.8)	226 (70.8)	96 (66.2)	

Abbreviations: CEE, conjugated equine estrogens; CHD, coronary heart disease; CVD, cardiovascular disease; HDL, high-density lipoprotein; IL-6, interleukin 6; IQR, interquartile range; LDL, low-density lipoprotein; MMP-9, matrix metalloproteinase 9; MPA, medroxyprogesterone; MTHFR, methylenetetrahydrofolate reductase; PAI-1, plasminogen activator inhibitor 1; PAP, plasmin-antiplasmin complex; TAFI, thrombin-activatable fibrinolysis inhibitor.

SI conversion factors: To convert C-reactive protein to nanomoles per liter, multiply by 9.524; HDL, LDL, and total cholesterol to millimoles per liter, multiply by 0.0259; D-dimer to nanomoles per liter, multiply by 5.476; fibrinogen to micromoles per liter, multiply by 0.0294; glucose to millimoles per liter, multiply by 0.0555; hematocrit to a proportion of 1.0, multiply by 100; homocysteine to micromoles per liter, multiply by 7.397; insulin to picomoles per liter, multiply by 6.945; leukocyte count to ×10⁹ per liter, multiply by 0.001; lipoprotein(a) to micromoles per liter, multiply by 0.0357; PAI-1 to picomoles per liter, multiply by 19.231; platelet count to ×10⁹ per liter, multiply by 1; triglycerides to millimoles per liter, multiply by 0.0113.

^aInterquartile range (75th percentile-25th percentile) is a nonparametric measure of data variability.

^bThe P values quantify the marginal association of each biomarker with incident CHD and were obtained from a logistic regression model adjusted for treatment assignment (CEE, CEE placebo, CEE + MPA, or CEE + MPA placebo) using a 1-*df* test for the association of biomarkers (log scale) and a 1- to 2-*df* test for polymorphisms.

^cBecause of rounding, percentages may not total 100.

Table 3. Adjusted CHD Risk (per Standard Deviation Increase) Associated With Biomarkers^a

	Overall (N=359)		Within 2 y (n=202)		After 2 y (n=157)	
	OR (95% CI) ^b	P Value ^c	OR (95% CI) ^b	P Value ^c	OR (95% CI) ^b	P Value ^c
Inflammatory markers						
C-reactive protein	1.17 (0.99-1.38)	.20	1.20 (0.97-1.47)	.16	1.13 (0.90-1.42)	.18
E-selectin	0.96 (0.83-1.11)	.55	0.80 (0.67-0.96)	.01	1.27 (1.03-1.57)	.04
Interleukin 6	1.17 (1.00-1.36)	.05	1.19 (0.99-1.44)	.06	1.16 (0.94-1.43)	.31
MMP-9	1.16 (1.01-1.34)	.04	1.25 (1.05-1.50)	.009	1.08 (0.89-1.31)	.63
Lipids						
HDL cholesterol	0.81 (0.69-0.95)	.007	0.72 (0.59-0.88)	.002	0.90 (0.72-1.11)	.22
LDL cholesterol	1.44 (1.23-1.69)	<.001	1.52 (1.24-1.85)	<.001	1.38 (1.11-1.71)	.002
Total cholesterol	1.37 (1.18-1.59)	<.001	1.42 (1.18-1.76)	<.001	1.33 (1.08-1.64)	.003
Triglycerides	1.18 (1.02-1.36)	.02	1.29 (1.08-1.54)	.005	1.07 (0.88-1.31)	.33
Thrombosis and other blood markers						
D-dimer	1.38 (1.18-1.61)	<.001	1.44 (1.18-1.76)	<.001	1.35 (1.09-1.66)	.007
Fibrinogen	1.12 (0.97-1.29)	.18	1.24 (1.03-1.50)	.03	1.00 (0.82-1.21)	.94
Factor VIII	1.27 (1.09-1.47)	<.001	2.47 (1.93-3.17)	<.001	0.80 (0.66-0.96)	.02
Prothrombin fragment 1.2	1.01 (0.88-1.17)	.59	1.06 (0.90-1.26)	.25	0.98 (0.78-1.22)	.98
von Willebrand factor	1.19 (1.03-1.38)	.01	1.23 (1.02-1.47)	.02	1.18 (0.96-1.44)	.05
Leukocyte count	1.20 (1.04-1.39)	.01	1.26 (1.05-1.51)	.01	1.18 (0.97-1.44)	.11
Hematocrit	1.01 (0.88-1.17)	.84	1.11 (0.93-1.32)	.27	0.88 (0.73-1.07)	.17
Homocysteine	0.23 (1.07-1.41)	.002	1.02 (0.86-1.21)	.86	1.57 (1.29-1.90)	<.001
Glucose	1.09 (0.94-1.25)	.50	1.15 (0.97-1.37)	.22	1.05 (0.85-1.28)	.97
Insulin	1.22 (1.02-1.46)	.04	1.41 (1.13-1.77)	.003	1.02 (0.80-1.31)	.94
Gene polymorphisms (C/C + C/T vs T/T)						
Glycoprotein IIIa leu33pro	1.58 (1.15-2.16)	.005	1.51 (1.02-2.24)	.03	1.61 (1.06-2.45)	.02

Abbreviations: See Table 2; CHD, coronary heart disease; CI, confidence interval; OR, odds ratio.

^aOnly results that were statistically significant in this analysis or that had a $P < .10$ in Table 2 are shown.

^bOdds ratio for incident CHD compared with all controls per standard deviation increase in log-transformed biomarker from a logistic regression model adjusted for treatment assignment (CEE, CEE placebo, CEE + MPA, and CEE + MPA placebo), age, body mass index, waist to hip ratio, smoking status, alcohol consumption, physical activity, diabetes mellitus, history of cardiovascular disease, left ventricular hypertrophy on electrocardiography, history of high cholesterol requiring medication, systolic blood pressure, and use of antihypertensive medications, aspirin, or statins.

^c P values for biomarkers are based on a logistic regression model using a 1- df test for biomarkers (log scale) and a 1- df test for polymorphisms. Covariate adjustment is as in the preceding footnote.

on various inflammatory, thrombotic, lipid, and genetic markers potentially associated with CHD risk and on biomarkers that are affected by HT. We hypothesized that such biomarkers modify or mediate the effect of HT on CHD. In adjusted analyses, 12 of the 23 biomarkers (and 1 of the 8 candidate genetic polymorphisms) studied were associated with CHD. Baseline CRP did not emerge as a strong independent risk factor in these analyses. Several biomarkers seemed to be more strongly related to CHD within 2 years than after 2 years, including MMP-9, fibrinogen, factor VIII, and leukocyte count, all of which may be thought of as potential markers of plaque destabilization or an acute-phase reaction. Some components of the metabolic syndrome, such as HDL cholesterol, triglycerides, and fasting insulin, also seemed to be more strongly related to CHD in the first 2 years. Other markers, some of which may be related to an ongoing atherosclerotic process, were associated with early and later CHD events, including LDL and total cholesterol, D-dimer, and vWF, whereas homocysteine was more strongly associated with later events. Previous studies⁹⁻¹² in elderly men have suggested that fibrinogen may be more closely related to death close to the baseline measurement, and in elderly women the associations of CRP, D-dimer, and plasmin-antiplasmin complex with CHD risk tend to be stronger for early events. An association of the common glycoprotein variant GpIIIa leu33pro with

CHD risk has been described previously¹³ and may be of clinical relevance because its presence may modify the effectiveness of platelet GpIIb-IIIa inhibitors used for the prevention of acute coronary syndromes.

Baseline LDL cholesterol seemed to modify significantly the effect of HT such that women with higher levels of LDL cholesterol were at higher risk for CHD (particularly for the trial of conjugated equine estrogens plus medroxyprogesterone). This interaction was significant overall and in each 2-year period after randomization. A weaker (nonsignificant) interaction in a protective direction was seen for HDL cholesterol. As previously reported,^{4,6} these interactions with baseline lipids seemed to be stronger in the trial of conjugated equine estrogens plus medroxyprogesterone than in the trial of conjugated equine estrogens. It is not known why hormones should interact with lipid levels in this manner because the lipid-modifying effects of hormones might have been more beneficial in participants with high baseline levels. It is plausible that women with high LDL cholesterol levels or low HDL cholesterol levels have more subclinical coronary artery disease and a consequently more adverse response to HT. Diseased arteries may have decreased the expression of estrogen receptors, decreased vasodilation, and increased inflammatory activation and plaque instability in response to estrogen.¹⁴ Recent animal data¹⁵ suggest that elevated levels of en-

Table 4. Associations of Baseline Biomarker Levels and Gene Polymorphisms With CHD Risk by Treatment Assignment

Biomarker	Odds Ratio per SD (95% CI) ^a				P Value for Interaction ^b
	CEE + MPA	CEE + MPA Placebo	CEE	CEE Placebo	
Inflammation					
C-reactive protein	1.10 (0.85-1.43)	1.44 (1.03-2.01)	1.34 (0.97-1.85)	0.86 (0.61-1.23)	.84
E-selectin	0.92 (0.72-1.17)	0.99 (0.73-1.36)	0.77 (0.57-1.05)	1.20 (0.89-1.62)	.09
Interleukin 6	1.03 (0.81-1.33)	1.20 (0.89-1.60)	1.47 (1.02-2.13)	1.19 (0.88-1.61)	.94
MMP-9	1.31 (1.02-1.69)	1.04 (0.79-1.37)	1.13 (0.84-1.53)	1.12 (0.79-1.58)	.32
Lipids					
HDL cholesterol	0.70 (0.53-0.92)	0.95 (0.71-1.27)	0.73 (0.52-1.02)	0.88 (0.64-1.22)	.08
LDL cholesterol	1.97 (1.47-2.63)	1.05 (0.77-1.45)	1.52 (1.09-2.12)	1.50 (1.06-2.14)	.03
Total cholesterol	1.76 (1.34-2.32)	1.17 (0.87-1.58)	1.37 (1.00-1.87)	1.37 (0.99-1.90)	.13
Triglycerides	1.22 (0.95-1.57)	1.26 (0.96-1.66)	1.24 (0.91-1.68)	1.03 (0.75-1.42)	.70
Thrombosis and other blood markers					
D-dimer	1.47 (1.12-1.92)	1.21 (0.91-1.61)	1.31 (0.96-1.80)	1.65 (1.15-2.38)	.19
Fibrinogen	1.03 (0.80-1.32)	1.11 (0.81-1.53)	1.14 (0.84-1.53)	1.19 (0.88-1.62)	.62
Factor VIII	1.35 (1.03-1.76)	1.22 (0.90-1.65)	1.17 (0.88-1.56)	1.41 (1.01-1.97)	.04
Prothrombin fragment 1.2	0.93 (0.72-1.20)	0.96 (0.69-1.33)	1.15 (0.84-1.56)	1.22 (0.90-1.67)	.61
von Willebrand factor	1.18 (0.93-1.51)	1.14 (0.85-1.54)	1.43 (1.04-1.99)	1.17 (0.84-1.61)	.51
Leukocyte count	1.27 (0.98-1.64)	1.12 (0.85-1.49)	1.39 (1.02-1.89)	1.05 (0.78-1.42)	.18
Hematocrit	1.32 (1.01-1.73)	0.96 (0.71-1.29)	0.89 (0.67-1.17)	0.90 (0.68-1.19)	.25
Homocysteine	1.49 (1.16-1.91)	1.09 (0.82-1.45)	1.21 (0.91-1.62)	1.11 (0.84-1.48)	.12
Glucose	0.96 (0.77-1.20)	1.14 (0.88-1.47)	1.10 (0.84-1.43)	1.07 (0.83-1.38)	.45
Insulin	1.38 (1.04-1.84)	1.23 (0.88-1.72)	1.24 (0.90-1.70)	0.95 (0.68-1.33)	.21
Gene polymorphisms (C/C + C/T vs T/T)					
Glycoprotein IIIa leu33pro	2.07 (1.21-6.23)	1.71 (0.86-3.39)	1.72 (0.86-3.43)	1.49 (0.74-3.00)	.61

Abbreviations: See Table 2; CHD, coronary heart disease; CI, confidence interval; SD, standard deviation.

^aFrom a logistic regression model adjusted for trial, age, body mass index, waist to hip ratio, smoking status, alcohol consumption, physical activity, diabetes mellitus, history of cardiovascular disease, left ventricular hypertrophy on electrocardiography, history of high cholesterol requiring medication, systolic blood pressure, and use of antihypertensive medications, aspirin, or statins.

^bP values for the interaction of active treatment/placebo × biomarker on CHD risk based on a 1-*df* test for biomarkers (log scale).

Table 5. Change in Biomarkers From Baseline to Year 1

Biomarker	Median (Interquartile Range) ^a				P Value for Change ^b
	CEE + MPA	CEE + MPA Placebo	CEE	CEE Placebo	
Inflammation					
C-reactive protein, mg/L	1.1 (3.6)	-0.0 (1.5)	2.2 (4.4)	0.1 (2.3)	<.001
E-selectin, ng/mL	-7.0 (9.0)	0.0 (10.0)	-7.0 (12.0)	-1.0 (9.0)	<.001
Interleukin 6, pg/mL	0.2 (1.5)	0.1 (1.7)	0.3 (1.6)	0.1 (1.8)	.44
MMP-9, ng/mL	53.0 (154.0)	-3.00 (111.0)	28.0 (154.0)	-11.5 (140.0)	<.001
Lipids, mg/dL					
HDL cholesterol	4.0 (8.0)	0.0 (9.0)	7.0 (12.0)	0.0 (6.0)	<.001
LDL cholesterol	-20.0 (30.0)	-1.0 (27.0)	-23.0 (32.0)	1.0 (29.5)	<.001
Total cholesterol	-16.0 (33.0)	-2.0 (33.0)	-12.5 (35.0)	0.0 (33.0)	<.001
Triglycerides	14.0 (56.0)	1.0 (49.0)	17.5 (69.0)	0.0 (50.0)	<.001
Thrombosis and other blood markers					
D-dimer, µg/mL	0.0 (0.3)	0.0 (0.2)	0.0 (0.3)	0.0 (0.2)	.16
Fibrinogen, mg/dL	-26.5 (79.0)	-7.5 (68.0)	-10.0 (100.0)	-5.0 (80.0)	.02
Factor VIII, %	-2.0 (34.0)	0.0 (30.0)	1.0 (29.0)	2.5 (34.0)	.27
PAI-1 antigen, ng/mL	-6.2 (25.4)	-0.6 (28.9)	-9.9 (34.0)	-0.6 (30.2)	<.001
Prothrombin fragment 1.2, nmol/L	0.1 (0.4)	0.0 (0.4)	0.1 (0.5)	0.0 (0.4)	.13
PAP, nmol/L	0.7 (2.0)	0.1 (1.5)	0.7 (1.9)	0.2 (1.3)	<.001
TAFI, µg/mL	-0.0 (0.7)	-0.1 (0.7)	0.2 (0.7)	0.0 (0.9)	.07
von Willebrand factor, %	0.0 (33.0)	3.0 (31.0)	0.00 (33.0)	0.0 (37.0)	.62
Homocysteine, mg/L	-0.4 (2.2)	-0.3 (2.4)	-0.4 (2.3)	0.0 (2.7)	.01
Glucose, mg/dL	-3.0 (13.0)	-1.0 (12.0)	-3.0 (15.0)	1.0 (16.0)	.002
Insulin, µIU/mL	-1.0 (3.3)	0.1 (3.8)	-1.1 (4.8)	0.8 (3.6)	<.001

Abbreviations: See Table 2.

SI conversion factors: See Table 2.

^aNumbers of CHD cases and controls for CEE+MPA were 79 and 180, respectively; for CEE+MPA placebo, 55 and 148, respectively; for CEE, 55 and 120, respectively; and for CEE placebo, 47 and 112, respectively.

^bP values are from a paired *t* test (per participant) of change in biomarker during hormone treatment compared with placebo controlling for the same variables as in Table 3.

Table 6. Effect of Hormone Therapy on Adjusted CHD Risk by Change in Biomarkers

Biomarker	First Tertile of Change		Second Tertile of Change		Third Tertile of Change		P Value for Interaction ^a
	Change Value	OR (95% CI)	Change Value	OR (95% CI)	Change Value	OR (95% CI)	
Inflammation							
C-reactive protein, mg/L	<-0.1	0.75 (0.39-1.45)	-0.1 to <1.7	1.40 (0.72-2.69)	≥1.7	1.39 (0.66-2.93)	.33
E-selectin, ng/mL	<-8	1.24 (0.57-2.72)	-8 to <-1	1.38 (0.70-2.70)	≥-1	0.77 (0.40-1.47)	.08
MMP-9, ng/mL	<-25	0.90 (0.49-1.64)	-25 to <63	0.98 (0.50-1.90)	≥63	1.33 (0.71-2.49)	.16
Lipids, mg/dL							
HDL cholesterol	<0	1.06 (0.53-2.11)	0 to <6	1.34 (0.72-2.49)	≥6	1.29 (0.60-2.78)	.44
LDL cholesterol	<-24	0.69 (0.33-1.43)	-24 to <-2	1.13 (0.55-2.31)	≥-2	1.37 (0.70-2.70)	.46
Total cholesterol	<-21	0.96 (0.50-1.84)	-21 to <3	0.84 (0.43-1.62)	≥3	1.61 (0.84-3.10)	.19
Triglycerides	<-7	0.79 (0.42-1.50)	-7 to <25	2.31 (1.16-4.59)	≥25	0.70 (0.38-1.32)	.65
Thrombosis and other blood markers							
Fibrinogen, mg/dL	<-39	1.13 (0.59-2.17)	-39 to <12	1.01 (0.55-1.85)	≥12	1.27 (0.69-2.33)	.57
PAI-1 antigen, ng/mL	<-12.2	1.24 (0.63-2.45)	-12.2 to <4.2	0.75 (0.37-1.52)	≥4.2	0.90 (0.48-1.68)	.24
PAP, nmol/L	<-0.1	0.72 (0.37-1.39)	-0.1 to <1	1.29 (0.64-2.61)	≥1	0.89 (0.45-1.75)	.90
Homocysteine, mg/L	<-1.1	1.29 (0.70-2.40)	-1.1 to <0.4	1.31 (0.69-2.49)	≥0.4	0.84 (0.45-1.55)	.29
Glucose, mg/dL	<-6	0.85 (0.44-1.66)	-6 to <3	1.10 (0.57-2.12)	≥3	1.38 (0.78-2.43)	.32
Insulin, μIU/mL	<-1.6	0.89 (0.46-1.70)	-1.6 to <0.9	1.25 (0.63-2.49)	≥0.9	1.09 (0.57-2.07)	.53

Abbreviations: See Table 2; CHD, coronary heart disease; CI, confidence interval; OR, odds ratio.

^aP values for interaction of active treatment/placebo × biomarker change are based on a 1-df test for change in biomarker controlling for the same variables as in Table 3.

ogenous oxysterols associated with high cholesterol levels inhibit binding of estrogen to its receptors and block the potentially beneficial effects of estrogen on healthy arteries. There was also a significant interaction of treatment with homocysteine in the second 2 years and possibly with E-selectin in the first 2 years. It is possible that these interactions could have occurred by chance because multiple statistical tests were performed, and the number of observed significant findings for interaction did not exceed the number expected by chance.

The levels of 14 biomarkers changed in response to HT, including 7 for which baseline levels were associated with incident CHD (MMP-9, HDL cholesterol, LDL cholesterol, total cholesterol, triglycerides, homocysteine, and fasting insulin). However, the 1-year changes in biomarker levels were not associated with CHD in the subsequent years, and there were no significant interactions between changes in biomarkers and CHD risk due to HT. Hence, although many biomarkers were associated with CHD, and many of these change during HT, we did not demonstrate that these changes mediate hormone effects on CHD risk. The observation that favorable changes in LDL and HDL cholesterol levels did not reduce subsequent CHD risk in 4.6 and 6.1 years, respectively, may be due to changes in lipoprotein metabolism not reflected in these standard measurements¹⁶ or could reflect changes in inflammation or coagulation that offset any benefit. The results for E-selectin are complex and run counter to its role as an adhesion molecule and marker of endothelial dysfunction.¹⁴ Higher baseline levels were associated with lower CHD risk and possibly interacted with treatment assignment in the first 2 years, whereas the decrease in levels on HT seemed to be associated with a trend toward higher risk of CHD (P for interaction = .08).

This study may have been underpowered to demonstrate interactions between biomarker change and hormone effects on CHD. By design, the analysis of media-

tion of hormone effect by change in biomarker levels excluded CHD events occurring in the first year, and, hence, fewer CHD events were available for analysis. Variability in individual responses and measurement error of biomarkers at 2 points in time would also decrease power. In addition, this part of the study may have missed a critical period of increased risk due to biomarker change during the first few months of the first year. It is also possible that the effects of HT are mediated through mechanisms that were not studied here. A parallel exploration of biomarkers and stroke risk in the hormone trials found that several biomarkers were associated with stroke risk (including CRP, IL-6, MMP-9, LDL cholesterol, HDL cholesterol, D-dimer, and thrombin-activatable fibrinolysis inhibitor).⁷ However, only baseline plasmin-antiplasmin complex levels interacted significantly with treatment assignment, and then it was in a paradoxical manner such that higher levels were associated with increased risk in the placebo group but not in the conjugated equine estrogens plus medroxyprogesterone group. Similar paradoxical trends were observed for baseline IL-6, D-dimer, and leukocyte count. Unlike the null findings for CHD, 1-year increases in D-dimer levels were associated with increased stroke risk.

This investigation did not identify any novel biomarkers or gene polymorphisms that might be clinically useful for identifying women at increased risk if they undergo postmenopausal HT. Further research is needed to better individualize HT. However, it might be useful to measure the lipid profile before prescribing HT because high LDL cholesterol levels (and perhaps low HDL cholesterol levels) are associated with increased risk of CHD for women starting HT. The presence of other risk factors, such as older age, persistent vasomotor symptoms, cigarette smoking, high blood pressure, diabetes mellitus, previous CVD, inactivity, and overweight, puts women at higher risk, and that risk would be increased in an additive manner if they also take hormones.^{4,6,17} The

decision to recommend HT needs to take into account the severity of the vasomotor symptoms (the main current indication for HT) and the individual risk profile.

Accepted for Publication: April 28, 2008.

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Author Contributions: Dr Rossouw had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Rossouw, Cushman, Greenland, Lloyd-Jones, Kooperberg, and Robinson. *Acquisition of data:* Cushman, Greenland, Robinson, Hendrix, and Hsia. *Analysis and interpretation of data:* Rossouw, Greenland, Bray, Kooperberg, Pettinger, Robinson, and Hsia. *Drafting of the manuscript:* Rossouw and Kooperberg. *Critical revision of the manuscript for important intellectual content:* Cushman, Greenland, Lloyd-Jones, Bray, Kooperberg, Pettinger, Robinson, Hendrix, and Hsia. *Statistical analysis:* Kooperberg and Pettinger. *Obtained funding:* Rossouw, Cushman, Greenland, and Hendrix. *Administrative, technical, and material support:* Cushman, Bray, Hendrix, and Hsia. *Study supervision:* Rossouw and Hsia.

Financial Disclosure: Dr Robinson has received grants from Abbott, Aegerion, Bristol-Myers Squibb, Daiichi Seiko, Hoffman-La Roche, Merck, Pfizer, Schering-Plough, and Takeda; has received honoraria from Merck/Schering-Plough; and serves on advisory boards for AstraZeneca and Merck.

Funding/Support: The WHI program is funded by contracts N01WH22110, 24152, 32100-2, 32105-6, 32108-9, 32111-13, 32115, 32118-32119, 32122, 42107-26, 42129-32, and 44221 from the National Heart, Lung, and Blood Institute, National Institutes of Health.

Role of the Sponsor: The National Institutes of Health had input into the design and conduct of the study; the collection, management, analysis, and interpretation of the data; and the preparation of the manuscript. Wyeth Research did not participate in any of the aforementioned aspects of the study.

Disclaimer: Dr Greenland is the editor of the *Archives of Internal Medicine*. He was not involved in the clinical evaluation or decision to accept this article for publication.

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