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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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Abstract

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

Objectives—To evaluate a range of inflammatory, lipid, thrombotic, and genetic markers for their association with CHD and to assess whether any of these markers modified or mediated the initially increased risk associated with hormone therapy

Design—Nested case-control study of biomarkers and genetic variants in the Women's Health Initiative randomized, controlled trials of hormone therapy in postmenopausal women aged 50–79 years at baseline.

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Interventions—Conjugated equine estrogens 0.625 mg daily or placebo in 10,739 hysterectomized women, and the same estrogen plus medroxy-progesterone acetate 2.5 mg daily in 16,608 women with an intact uterus.

Main outcome measures—Associations between putative biomarkers and genetic markers, hormone treatment, and CHD events during the first 4 years after randomization.

Results—In multivariable-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 hormone treatment (particularly with CEE+MPA), so that women with higher levels were at higher risk of CHD when given hormone therapy (p for interaction = 0.03). There was a non-significant interaction of baseline high-density lipoprotein cholesterol with hormone therapy on CHD (p = 0.08). The levels of several biomarkers were changed by hormone therapy, but these changes did not appear to be associated with future CHD events.

Conclusions—The study confirmed that several thrombotic, inflammatory, and lipid biomarkers were associated with CHD events in postmenopausal women, however only low-density cholesterol (an established risk factor) modified the effect of hormone therapy. Further research is needed to identify the mechanisms by which hormone therapy increases the risk of CHD.

Keywords

biomarkers; risk prediction; hormone therapy; estrogen; medroxy-progesterone; coronary heart disease; stroke; mortality; clinical trials; age; menopause

Introduction

The Women's Health Initiative (WHI) trials of postmenopausal hormone therapy (HT) tested whether estrogen alone or in combination with a progestin would reduce the risk of coronary heart disease (CHD) in predominantly healthy postmenopausal women. The trial of conjugated equine estrogens (CEE) plus medroxy-progesterone (MPA) in women with an intact uterus was stopped early because of an increase in cardiovascular events (CHD, stroke, and venous thrombo-embolism) and of breast cancer.[1] The parallel trial of CEE alone in hysterectomized women was also stopped early because of increased strokes and lack of benefit for CHD.[2] Following publication of these findings, current recommendations state that HT should not be started or continued for the prevention of CHD.[3]

In the CEE+MPA trial the cumulative hazard ratio (HR) for CHD after an average of 5.6 years follow-up was 1.24, with a 95% confidence interval (CI) of 1.00, 1.54; however the initial risks were higher, with HRs in years 1 through ≥6 of 1.81, 1.34, 1.27, 1.25, 1.45, and 0.70 respectively (p for trend = 0.02).[4] Similar trends for higher initial risks were found in the Heart and Estrogen/Progestin Replacement Study of CEE+MPA.[5] In the WHI trial of CEE there was no overall effect on CHD, with a cumulative of HR 0.95, CI 0.79, 116 after 7.1 years. CHD risks 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 (p for trend = 0.14).[6]

To elucidate the mechanisms by which HT might initially increase the risk for CHD, the WHI investigators conducted a nested case-control study which included all centrally-adjudicated

cases of CHD occurring in the first 4 years of the study. The possible influence of lipids, lipoproteins, and coagulation factors on trial results were pre-specified in the study protocol. Other laboratory markers were chosen based on a priori knowledge from other studies of their relationship to CHD, with a focus on those affected by HT. In this contribution, 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 on CHD.

Methods

Details of the design, recruitment, randomization, data collection, intervention, and outcomes ascertainment procedures in the WHI HT trials, including CONSORT diagrams, have been published previously.[1,2]

Study population and interventions

The WHI hormone trials enrolled 27 347 postmenopausal women aged 50–79 from 1993 to 1998 at 40 US clinical centers based on hysterectomy status: 16 608 without hysterectomy in a trial of CEE+MPA; 10 739 with hysterectomy in a trial of CEE alone. At baseline, women completed screening and baseline questionnaires by interview and self-report and a physical examination was done. Blood specimens were collected at baseline and the one-year visit. The study was approved by the human subjects review committee at each participating institution, and all participants provided written informed consent.

Participants were randomly assigned to take a single daily tablet containing a placebo or active medication: women without hysterectomy took 0.626 mg CEE plus 2.5 mg MPA (Prempro), and women with hysterectomy took 0.625 mg CEE (Premarin). Study drugs and placebo were supplied by Wyeth-Ayerst, St. Davids, PA. The planned end-date of the trials was March 31, 2005 for a total follow up of 8.4 years; however, CEE+MPA trial medications were stopped on July 7, 2002 and CEE was 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 venous thromboembolism (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 at baseline. Matching on prevalent disease was specific to the case type, so that cases of CHD were matched on prevalent myocardial infarction, cases of stroke on prevalent stroke, and cases of VTE on prevalent VTE. All controls for the three 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. Among the 359 participants with CHD, 11 also had a stroke, 9 had a VTE, and 1 had all 3 events. Analyses involving year one biomarker data involved 236 cases who experienced their CHD event after the year one 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 semi-annual questionnaires and classified by centrally-trained local adjudicators following medical record review. All locally-adjudicated cases of CHD were reviewed by central adjudicators. CHD included nonfatal myocardial infarction (MI), CHD death, and silent MI. Definite and probable nonfatal MI required overnight hospitalization and was defined according to an algorithm based on standardized criteria using cardiac pain, cardiac enzymes and troponin levels, and electrocardiographic findings, and included MI occurring during surgery and aborted MI. CHD death was defined as death consistent with underlying cause of CHD plus one or more of the following: hospitalization

for myocardial infarction within 28 days prior to death, previous angina or myocardial infarction, death due to a procedure related to CHD, or a death certificate consistent with underlying cause of atherosclerotic CHD. Definite silent myocardial infarction was diagnosed from baseline and year 3 and 6 electrocardiograms (Novacode 5.1 and 5.2).[8]

Genetic and biomarker analysis

Blood samples were collected from all participants at baseline and 1 year and stored at −70° Celsius. Analyses were run in single batches including both cases and controls and 10% blind duplicates within 8 years of collection. Lipid profile (analyzed in EDTA plasma with high density lipoprotein (HDL) precipitation by heparin manganese (Dade-Behring, Deerfield Illinois, United States), interleukin-6 (IL-6, ultra-sensitive ELISA, R&D Systems, Minneapolis, Minnesota, United States), E-selectin, matrix metalloproteinase-9 (MMP-9), homocysteine and Lp(a) were measured at Medical Research Laboratories (Highland Heights, Kentucky, United States). C-reactive protein (N-High Sensitivity CRP, Dade-Behring, Deerfield, Illinois, United States), fibrinogen (clot rate assay: Diagnostica Stago, Parsippany, New Jersey, United States), factor VIII activity (clotting time on mixing with factor VIII deficient plasma using STA-Deficient VIII; Diagnostica Stago, Parsippany, New Jersey, United States), von Willebrand factor activity and fibrin D-dimer (immunoturbidometric assays: Liatest von Willebrand factor, Liatest D-Di; Diagnostica Stago, Parsippany, New Jersey, United States), plasminogen activator inhibitor-1 antigen (PAI-1) and plasmin-antiplasmin complex plasmin-antiplasmin complex (PAP, both by in-house immunoassay, prothrombin fragment 1.2 (ELISA, Dade-Behring, Deerfield Illinois, United States) and thrombin activatable fibrinolysis inhibitor (TAFI; immunoassay with antibodies from Affinity Biologicals, Ancaster, Ontario, Canada) were measured at the Laboratory for Clinical Biochemistry Research, University of Vermont (Burlington, Vermont, United States). Complete blood count was performed in clinics' local laboratories. Genetic polymorphisms were assayed at Wake Forest University, Winston Salem, North Carolina, United States (Estrogen receptor β-A1730G (rs4986938), GP1bα-Thr145Met (rs6065), GPIIIa leu33pro (rs 5918)), and at the Leiden University, Netherlands (Factor V Leiden, prothrombin 20210, thermolabile variant of methylene-tetrahydrofolate reductase (MTHFR), PAI-1 4G/5G).

Statistical methods

All baseline marker values were log-transformed due to skewed distributions and for consistency; differences from baseline to year one were analyzed on the original scale. Logistic regression models were controlled for age and trial, BMI, waist-hip ratio, smoking, alcohol consumption, physical activity, history of diabetes, history of high blood cholesterol, prevalent cardiovascular disease (other than myocardial infarction), LVH on electrocardiogram, systolic blood pressure, use of antihypertensive medication, 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 out of 31 instances (1–2 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 response, correcting for risk factors. Since linearity was rejected for CRP, IL-6, Factor VIII and leukocyte count we employed quadratic models for these biomarkers. While 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 per standard deviation increase. Thus, there is no one-to-one correspondence between p-values for models <0.05 and confidence intervals for odds ratios not containing 1. For the interaction of change in biomarker levels at one year we show the odds ratios 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 scatter plots; 1 result for Factor VIII, 7 for hematocrit, and 2 for von Willebrand factor were deemed to be outliers and were excluded from the analyses.

We tested for nominal statistical significance at $p<0.05$ 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 (1–2 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 (1–2 expected) and stratified by years since randomization 4 out of 62 tests were significant (3 expected); 23 tests of interaction of change in biomarker levels at one year with treatment assignment on CHD risk of which none was significant (1 expected). Statistical analyses were performed using SAS version 9 (SAS Institute Inc, Cary, NC).

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 CEE placebo group than the CEE+MPA placebo group, in keeping with the higher baseline risks of CHD in the trial of CEE (Table 2).[4,6] With the exception of the expected correlations between lipids and lipoproteins, correlations between baseline biomarkers were weak. Cases and controls in the current analyses demonstrated odds ratios of 1.43 ((95% confidence interval 0.98, 2.08) for CEE+MPA versus placebo and 1.20 (0.75, 1.90) for CEE versus 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/III3b fibrinogen receptor, but not the other 6 candidate polymorphisms, was associated with CHD risk. In multivariate analyses adjusting for trial assignment and baseline characteristics (including prevalent CVD, statin treatment, and diabetes) some inflammatory biomarkers (IL-6, MMP-9, leukocyte count,), lipids (HDL-C, LDL-C, total cholesterol, and triglycerides), thrombotic and other biomarkers (d-dimer, factor VIII, von Willebrand factor, 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; these included MMP-9, HDL-cholesterol, triglycerides, fibrinogen, leukocyte count, and insulin. Factor VIII was associated with CHD in both time periods, but significantly more so in the first 2 years, while LDL-cholesterol, total cholesterol, d-dimer, von Willebrand factor, as well as the GPIIIa leu33pro polymorphism were related to risk in both time 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 on HT in women with higher levels of LDL-

C (Table 4, overall P for interaction = 0.03). This finding depended on the trial of CEE+MPA (P for interaction=0.006) rather than the trial of CEE (P for interaction=0.0.84). A trend in the opposite direction was seen for HDL-cholesterol, but the interaction was not statistically significant (P=0.08). 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 time periods, with P=0.05 in the first 2 years and P=0.01 in the second 2 years (data not shown). There was also a significant interaction with homocysteine in the second two years (p=0.03) but not in the first 2 years (P=0.54). Finally, a potential interaction of treatment with E-selectin (p=0.05) in the first 2 years was noted, with lower risk in women on CEE+MPA in women with higher levels; however, there was no such trend in the CEE trial.

Biomarker Changes from Baseline to Year 1

Hormone therapy in both trials increased CRP and MMP-9, decreased E-selectin, and had no effect on IL-6 (Table 5). There were also significant increases in HDL-C and triglycerides, and decreases in LDL-C and total cholesterol. Hormone therapy increased levels of PAP, and decreased fibrinogen, PAI-1 antigen, homocysteine, glucose, and insulin, but had no effect on d-dimer, factor VIII, prothrombin F1.2, TAFI, or von Willebrand factor. None of the changes appeared 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 influenced the risk of CHD due to hormones after the first year (Table 6). The interaction of change in E-selectin with treatment assignment had a p-value of 0.08; however this possible interaction was not in the expected direction, with higher ORs in the participants with least decrease in E-selectin. Changes in individual biomarker levels did not appear 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 hormone therapy, which included women with and without prior CVD. Hence, the focus was on various inflammatory, thrombotic, lipid, and genetic markers potentially associated with CHD risk, and on biomarkers which are affected by hormone therapy. We hypothesized that such biomarkers would modify or mediate the effect of hormone therapy on CHD. In adjusted analyses, 12 of the 23 biomarkers (and one of the 8 candidate genetic polymorphisms) studied were associated with CHD. It is noteworthy that baseline CRP did not emerge as a strong independent risk factor in these analyses. Intriguingly, several biomarkers appeared 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 appeared 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 both early and later CHD events, including LDL-cholesterol, total cholesterol, d-dimer, and von Willebrand factor, while homocysteine was more strongly associated with later events. Previous studies 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 PAP with CHD risk tend to be stronger for early events.[9–12] An association of the common glycoprotein variant GPIIIa leu33pro with CHD risk has been described previously, and may be of clinical relevance since its presence may modify the effectiveness of platelet glycoprotein IIb/IIIa inhibitors used for prevention of acute coronary syndromes.[13]

Baseline LDL-cholesterol appeared to modify significantly the effect of hormone therapy such that women with higher levels of LDL-cholesterol were at higher risk of CHD (particularly for CEE+MPA). This interaction was significant overall, and in each 2 year period after randomization. A weaker (non-significant) interaction in a protective direction was seen for HDL-cholesterol. As previously reported, these interactions with baseline lipids appeared to be stronger in the trial of CEE+MPA than in the trial of CEE[4,6] It is not known why hormones should interact with lipid levels in this manner, since 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 or low HDL-cholesterol levels have more sub-clinical coronary artery disease and a consequently more adverse response to hormone therapy. Diseased arteries may have decreased expression of estrogen receptors, decreased vasodilatation, increased inflammatory activation and plaque instability in response to estrogen.[14] Recent animal data suggest that elevated levels of endogenous oxysterols associated with high cholesterol levels inhibit binding of estrogen to its receptors, and block the potentially beneficial effects of estrogen on healthy arteries.[15] 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, since 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 hormone therapy, 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 one-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 hormone therapy. Hence, though many biomarkers were associated with CHD, and many of these change on hormone therapy, we were unable to demonstrate that these changes mediate hormone effects on CHD risk. The observation that favorable changes in LDL-cholesterol and HDL-cholesterol did not reduce subsequent CHD risk over 4.6 and 6.1 years may be due to changes in lipoprotein metabolism not reflected in these standard measurements, 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, while the decrease in levels on hormone therapy appeared to be associated with a trend towards higher risk of CHD ($p=0.08$ in the main analysis, $p=0.03$ in analysis excluding prevalent CVD).

The study may have been underpowered to demonstrate interactions between biomarker change and hormone effects on CHD. By design, the analysis of mediation 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 two 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 hormone therapy 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, HDL3-cholesterol, d-dimer, and TAFI).[7] However, only baseline PAP levels interacted significantly with treatment assignment and then it was in a paradoxical fashion such that higher levels were associated with increased risk in the placebo group but not in the CEE+MPA group. Similar paradoxical trends were observed for baseline IL-6, d-dimer, and leukocyte count. Unlike the null findings for CHD, one-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 take postmenopausal hormone therapy. Further research is needed to better individualize hormone therapy. However, it might be useful to measure the lipid profile prior to prescribing hormone therapy, since high LDL-cholesterol levels (and perhaps low HDL-cholesterol levels) are associated with increased risk of CHD for women starting hormone therapy. The presence of other risk factors such as older age, persistent vasomotor symptoms, cigarette smoking, high blood pressure, diabetes, prior cardiovascular disease, inactivity, and overweight puts women at higher risk, and that risk would be increased in an additive manner if they also take hormones.[4,6,16] The decision to recommend hormone therapy needs to take into account the severity of the vasomotor symptoms (the main current indication for hormone therapy) as well as the individual risk profile.

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References

1. Writing Group for the Women's Health Initiative Investigators. Risks and benefits of estrogen plus progestin for healthy postmenopausal women. JAMA 2002;288:321–333. [PubMed: 12117397]
2. The Women's Health Initiative Steering Committee. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy. 2004;291:1701–1712.
3. Mosca L, et al. Evidence-based guidelines for cardiovascular disease prevention in women. Circulation 2006;109:672–693. [PubMed: 14761900]
4. Manson JE, Hsia J, Johnson kC, et al. Estrogen plus progestin and risk of coronary heart disease. N Engl J Med 2003;349:253–234.
5. Hulley S, Grady D, Bush T, et al. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. JAMA 1998;280:605–613. [PubMed: 9718051]
6. Hsia J, Langer D, Manson JE, et al. Conjugated equine estrogens and the risk of coronary heart disease. Arch Int Med 2006;166:357–365. [PubMed: 16476878]
7. Kooperberg C, Cushman M, Hsia J, Robinson JG, Aragaki AK, Lynch JK, Baird AE, Johnson KC, Kuller LH, Beresford SA, Rodriguez B. Can biomarkers identify women at increased stroke risk? The Women's Health Initiative Hormone Trials. PLoS Clin Trials 2007;2:e28. [PubMed: 17571161]
8. Rautaharju PM, Park LP, Chaitman BR, Rautaharju F, Zhang ZM. The Novacode criteria for classification of ECG abnormalities and their clinically significant progression and regression. J Electrocardiol 1998;31:157–187. [PubMed: 9682893]
9. Yano K, Grove JS, Chen R, Rodriquez BL, Curb JD, Tracy RP. Plasma fibrinogen as a predictor of total and cause-specific mortality in elderly Japanese-American men. Arterioscl Thromb Vasc Biol 2001;21:1065–1070. [PubMed: 11397721]
10. Tracy RP, Arnold Am, Ettinger W, Fried L, Meilahn E, Savage P. The relationship of fibrinogen and factors VII and VIII to incident cardiovascular disease and death in the elderly. Results from the Cardiovascular Health Study. Arterioscl Thromb Vasc Biol 1999;19:1776–1783. [PubMed: 10397698]

11. Tracy RP, Lemaitre RN, Psaty BM, Ives DG, Evans RW, Cushman M, Meilahn EN, Kuller LH. Relationship of C-reactive protein to risk of cardiovascular disease in the elderly. Arterioscl Thromb Vasc Biol 1997;17:1121–1127. [PubMed: 9194763]

12. Cushman M, Lemaitre RN, Kuller LH, Psaty BM, Macy EM, Sharrett RA, Tracy RP. Fibrinolytic activation markers predict myocardial infarction in the elderly. The Cardiovascular Health Study. Arterioscl Thromb Vasc Biol 1999;19:493–498. [PubMed: 10073948]

13. Weiss EJ, Bray PF, Tayback M, Schulman SP, Kickler TS, Becker LC, Weiss JL, Gerstenblith G, Goldscmidt-Clermont PJ. A polymorphism of a platelet glycoprotein receptor as an inherited risk factor for coronary thrombosis. N Engl J Med 1996;334:1090–1094. [PubMed: 8598867]

14. Mendelsohn M, Karas R. Molecular and cellular basis of cardiovascular gender differences. Science 2005;308:1583–1587. [PubMed: 15947175]

15. Umetani M, Domoto H, Gormley A, Yuhanna IS, Cummins CL, Javitt NB, Korach KS, Shaul PW, Mangelsdorf DJ. 27-Hydroxycholesterol is an endogenous SERM that inhibits the cardiovascular effects of estrogen. Nature Medicine. 2007 Sep 16;[Epub ahead of print]

16. Rossouw JE, Prentice RL, Manson JE, Wu L, Barad D, Barnabei VM, Ko M, LaCroix AZ, Margolis KL, Stefanick ML. Postmenopausal hormone therapy and risk of cardiovascular disease by age and years since menopause. JAMA 2007;297:1465–1477. [PubMed: 17405972]

Table 1

Baseline Characteristics of Women in the Hormone Trials

	CEE+MPA Trial				CEE Trial				
	Control		CHD		Control		CHD		
	N	%	N	%	N	%	N	%	P-value[1]
Ethnicity									0.87
White	424	88.0	183	89.3	25 3	75.5	11 7	76.0	
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									<0.001
Never	268	56.4	90	45.5	16 9	51.8	72	47.7	
Past	171	36.0	66	33.3	12 8	39.3	48	31.8	
Current	36	7.6	42	21.2	29	8.9	31	20.5	
Alcohol use									0.006
Non drinker	221	46.3	107	52.5	17 4	52.1	10 2	67.5	
≤1 drink/day	193	40.5	78	38.2	13 3	39.8	42	27.8	
>1 drink/day	63	13.2	19	9.3	27	8.1	7	4.6	
Physical activity, METs									0.01
Inactive	61	14.7	40	23.1	68	22.9	33	24.3	
<5	89	21.4	43	24.9	73	24.6	44	32.4	
5–12	104	25.0	43	24.9	65	21.9	26	19.1	
≥12	162	38.9	47	27.2	91	30.6	33	24.3	
Treated diabetes	22	4.6	28	13.7	20	6.0	36	23.4	<0.001
History of hypertension									<0.001
Never	274	65.9	86	50.0	17 2	58.7	55	41.0	

	CEE+MPA Trial				CEE Trial				
	Control		CHD		Control		CHD		
	N	%	N	%	N	%	N	%	P-value[1]
Untreated	37	8.9	20	11.6	18	6.1	15	11.2	
					10				
Treated	105	25.2	66	38.4	3	35.2	64	47.8	
History of high cholesterol	66	16.0	43	25.3	54	18.6	36	27.3	<0.001
LVH on electrocardiogram	23	4.8	11	5.4	23	6.9	22	14.6	0.02
History of CVD	53	11.1	48	24.2	59	17.9	42	28.0	<0.001
Baseline aspirin use	105	21.8	56	27.3	75	22.4	50	32.5	0.007
Baseline statin use	35	7.3	34	16.6	35	10.4	21	13.6	<0.001
	N	Mean (Std)	N	Mean (Std)	N	Mean (Std)	N	Mean (Std)	
					33		15		
Age at screening, years	482	66.7 (6.9)	205	66.1 (7.54)	5	66.4 (6.6)	4	67.4 (6.2)	0.84
Body-mass index, kg/m^2	478	27.8 (5.5)	205	29.0 (5.7)	334	29.4 (5.6)	154	30.2 (5.7)	0.004
Height, cm	480	161.0 (6.7)	205	161.2 (6.5)	334	161.4 (6.4)	154	160.8 (6.2)	0.80
Weight, kg	480	72.2 (15.3)	205	75.5 (16.0)	335	76.6 (15.3)	154	78.2 (16.1)	0.008
Waist to hip ratio	479	0.8 (0.1)	205	0.8 (0.1)	335	0.8 (0.1)	154	0.9 (0.1)	<0.001
Waist, cm	480	86.8 (13.7)	205	90.9 (13.8)	335	90.9 (13.2)	154	95.1 (13.9)	<0.001
Systolic BP	482	129.7 (17.8)	205	134.2 (18.5)	335	130.2 (16.7)	154	139.6 (19.0)	<0.001
Diastolic BP	482	74.9 (9.3)	205	76.9 (10.3)	334	75.9 (9.00	154	76.3 (10.8)	0.02

[1] P-value quantifies marginal association of baseline characteristic with incident CHD. Obtained from a logistic regression model, adjusted for treatment assignment (CEE, CEE placebo, E+P, E+P placebo) using a 1 degree of freedom test for association; except for the categorical variables ethnicity, smoking, alcohol use, physical activity, and use of hypertension medication.

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Table 2

Baseline Biomarkers and Gene Polymorphisms

	CEE+MPA Trial						CEE Trial						
	Control			CHD			Control			CHD			P-value[2]
	N	Median	IQR[1]	N	Median	IQR	N	Median	IQR	N	Median	IQR	
C-reactive protein (ug/ml)	464	1.8	3.5	198	2.9	4.2	326	2.6	3.6	149	3.7	5.5	<0.001
E-selectin (ng/ml)	471	43.0	26.0	196	45.5	27.5	329	45.0	27.0	147	47.0	34.0	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	<0.001
MMP-9 (ng/ml)	482	217.5	148.0	205	228.0	168.0	335	217.0	148.0	154	235.0	177.0	0.01
HDL cholesterol (mg/dl)	481	54.0	20.0	202	48.0	15.0	334	52.0	17.0	152	47.0	17.0	<0.001
LDL cholesterol (mg/dl)	473	138.0	44.0	194	151.0	41.0	327	140.0	47.0	146	149.0	46.0	<0.001
Total cholesterol (mg/dl)	482	221.0	49.0	204	235.0	44.5	335	228.0	51.0	154	232.5	52.0	<0.001
Triglyceride (mg/dl)	482	130.0	82.0	204	145.0	107.5	335	142.0	90.0	154	162.0	101.0	<0.001
Lp (a) (mg/dl)	459	18.0	28.0	194	22.0	37.0	320	23.0	34.0	147	22.0	35.0	0.15
D-dimer (ug/ml)	481	0.3	0.3	202	0.4	0.4	334	0.3	0.3	153	0.4	0.4	<0.001
Fibrinogen (mg/dl)	481	305.0	112.0	202	317.0	123.0	334	313.5	125.0	154	332.0	128.0	<0.001
Factor VIII (%)	480	105.0	61.0	202	120.0	74.0	334	103.0	68.0	154	117.5	77.0	<0.001
PAI – 1 antigen (ng/ml)	454	35.4	46.4	187	41.3	47.4	308	43.6	57.4	142	47.8	51.7	0.54
Prothrombin F1.2 (nmol/L)	448	1.3	0.4	185	1.3	0.5	306	1.3	0.5	142	1.4	0.6	0.09
PAP (nmol/L)	453	4.5	2.5	187	4.5	2.3	308	4.2	2.1	142	4.3	2.4	0.51
TAFI conc (ug/ml)	470	5.1	2.5	200	4.9	2.6	327	5.2	2.7	146	5.2	1.9	0.35
vWF (%)	479	93.0	54.0	202	97.0	64.0	333	89.0	52.0	153	100.0	61.0	<0.001
Leukocyte count (Kcell/ml)	482	5.8	1.9	202	6.3	2.3	335	5.7	2.1	153	6.4	2.3	<0.001
Platelet count (Kcell/ml)	481	246.0	80.0	202	246.5	75.0	335	242.0	69.0	153	244.0	82.0	0.76
Hematocrit (%)	482	40.3	3.7	200	41.4	3.7	334	40.6	3.8	152	41.0	4.5	0.04
Homocysteine (umol/L)	481	8.1	3.4	204	8.4	3.9	335	8.3	3.9	154	8.5	4.0	0.003
Glucose (mg/dl)	480	96.5	16.0	202	100.0	24.0	332	98.0	18.0	153	102.0	32.0	<0.001
Insulin (UIU/ml)	451	7.10	6.7	191	9.20	8.0	316	8.5	8.4	136	10.0	9.6	<0.001
	N	%		N	%		N	%		N	%		

		CEE+MPA Trial						CEE Trial						
		Control			CHD			Control			CHD			P-value[2]
		N	Median	IQR[1]	N	Median	IQR	N	Median	IQR	N	Median	IQR	
ER Beta - 1730 A/G	CC	176	38.7		67	34.2		118	37.1		52	36.1		0.67
	CT	216	47.5		101	51.5		155	48.7		71	49.3		
	TT	63	13.8		28	14.3		45	14.2		21	14.6		
Factor V Leiden	GG	430	95.3		192	96.0		315	96.0		144	97.3		0.39
	GA	21	4.7		8	4.0		13	4.0		4	2.7		
Factor XIII val34Leu	val/val	244	54.1		111	55.5		196	59.8		89	59.7		0.62
	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		
GP1bαThr145Met	CC	386	84.6		165	84.2		237	74.3		118	82.5		0.42
	CT	62	13.6		30	15.3		76	23.8		21	14.7		
	TT	8	1.8		1	0.5		6	1.9		4	2.8		
MTHFR	CC	192	42.3		96	48.0		162	49.4		65	43.3		0.99
	CT	198	43.6		83	41.5		139	42.4		65	43.3		
	TT	64	14.1		21	10.5		27	8.2		20	13.3		
Plasminogen Activator Inhibitor	4G4G	111	25.1		51	26.0		65	20.1		38	26.6		0.39
	4G5G	236	53.3		107	54.6		170	52.5		70	49.0		
	5G5G	96	21.7		38	19.4		89	27.5		35	24.5		
Prothrombin 20210	GG	434	96.0		195	98.0		321	97.9		147	99.3		0.10
	AG	18	4.0		4	2.0		7	2.1		1	0.7		
Glycoprotein IIIa leu33pro	CC	13	2.8		2	1.0		10	3.1		2	1.4		
	CT	93	20.4		69	35.2		83	36.0		47	32.4		<0.001
	TT	351	76.8		125	63.8		226	70.8		96	66.2		

[1] Inter-quartile range (75th percentile-25h percentile) is a non-parametric measure of data variability.

[2] P-value quantifies marginal association of biomarker with incident CHD. Obtained from a logistic regression model, adjusted for treatment assignment (CEE, CEE placebo, E+P, E+P placebo, using a 1 degree-of-freedom test for association of biomarkers (log scale) and 1–2 degrees-of-freedom test for polymophisms.

Table 3

Adjusted CHD Risk (per SD increase) Associated with Biomarkers

	Overall (N=359 cases)				Within 2 Years (N=202 cases)				After 2 Years (N=157 cases)			
	Odds Ratio[1] (95% CI)			P-value[2]	Odds Ratio (95% CI)			P-value	Odds Ratio[4] (95% CI)			P-value
Inflammatory markers												
C-reactive protein	1.17	(0.99,	1.38)	0.20	1.20	(0.97,	1.47)	0.16	1.13	(0.90,	1.42)	0.18
E-selectin	0.96	(0.83,	1.11)	0.55	0.80	(0.67,	0.96)	0.01	1.27	(1.03,	1.57)	0.04
Interleukin-6	1.17	(1.00,	1.36)	0.05	1.19	(0.99,	1.44)	0.06	1.16	(0.94,	1.43)	0.31
MMP-9	1.16	(1.01,	1.34)	0.04	1.25	(1.05,	1.50)	0.009	1.08	(0.89,	1.31)	0.63
Lipids												
HDL-cholesterol	0.81	(0.69,	0.95)	0.007	0.72	(0.59,	0.88)	0.002	0.90	(0.72,	1.11)	0.22
LDL-cholesterol	1.44	(1.23,	1.69)	<0.001	1.52	(1.24,	1.85)	<0.001	1.38	(1.11,	1.71)	0.002
Total Cholesterol	1.37	(1.18,	1.59)	<0.001	1.42	(1.18,	1.76)	<0.001	1.33	(1.08,	1.64)	0.003
Triglyceride	1.18	(1.02,	1.36)	0.02	1.29	(1.08,	1.54)	0.005	1.07	(0.88,	1.31)	0.33
Thrombosis and other blood markers												
D-dimer	1.38	(1.18,	1.61)	<0.001	1.44	(1.18,	1.76)	<0.001	1.35	(1.09,	1.66)	0.007
Fibrinogen	1.12	(0.97,	1.29)	0.18	1.24	(1.03,	1.50)	0.03	1.00	(0.82,	1.21)	0.94
Factor VIII	1.27	(1.09,	1.47)	<0.001	2.47	(1.93,	3.17)	<0.001	0.80	(0.66,	0.96)	0.02
Prothrombin F1.2	1.01	(0.88,	1.17)	0.59	1.06	(0.90,	1.26)	0.25	0.98	(0.78,	1.22)	0.98
von Willebrand factor	1.19	(1.03,	1.38)	0.01	1.23	(1.02,	1.47)	0.02	1.18	(0.96,	1.44)	0.05
Leukocyte count	1.20	(1.04,	1.39)	0.01	1.26	(1.05,	1.51)	0.01	1.18	(0.97,	1.44)	0.11
Hematocrit	1.01	(0.88,	1.17)	0.84	1.11	(0.93,	1.32)	0.27	0.88	(0.73,	1.07)	0.17
Homocysteine	.23	(1.07,	1.41)	0.002	1.02	(0.86,	1.21)	0.86	1.57	(1.29,	1.90)	<0.001
Glucose	1.09	(0.94,	1.25)	0.50	1.15	(0.97,	1.37)	0.22	1.05	(0.85,	1.28)	0.97
Insulin	1.22	(1.02,	1.46)	0.04	1.41	(1.13,	1.77)	0.003	1.02	(0.80,	1.31)	0.94
	Odds Ratio CC+CTvsTT (95% CI)				Odds Ratio CC+CTvsTT (95% CI)				Odds Ratio CC+CTvsTT (95% CI)			
Gene polymorphisms												
Glycoprotein IIIa leu33pro	1.58	(1.15,	2.16)	0.005	1.51	(1.02,	2.24)	0.03	1.61	(1.06,	2.45)	0.02

Only results that were statistically significant in this analysis or had a p-value < 0.10 in Table 2 are shown.

[1]Odds ratio for incident CHD compared to all controls per SD increase in log-transformed biomarker from a logistic regression model adjusted for treatment assignment (CEE, CEE placebo, E+P, E+P placebo), age, BMI waist-hip ratio, smoking, alcohol consumption, physical activity, diabetes, history of CVD, LVH on electrocardiogram, history of high cholesterol requiring medication, systolic blood pressure, use of antihypertensive medication, aspirin or statins.

[2]P-value for biomarkers based on logistic regression model using a 1 degree-of-freedom test for biomarkers (log-scale) and a 1 degree-of-freedom test for polymorphisms. Covariate adjustment as above.

Table 4

Associations of Baseline Biomarker Level and Gene Polymorphisms with CHD Risk by Treatment Assigment

	CEE+MPA			Placebo			CEE			Placebo			P value for interaction *1*
	Odds Ratio*2*: per SD (95% CI)			Odds Ratio: per SD (95% CI)			Odds Ratio: per SD (95% CI)			Odds Ratio: per SD (95% CI)			
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)	0.84
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)	0.09
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)	0.94
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)	0.32
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)	0.08
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)	0.03
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)	0.13
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)	0.70
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)	0.19
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)	0.62
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)	0.04
Prothrombin F1.2	0.93	(0.72,	1.20)	0.96	(0.69,	1.33)	1.15	(0.84,	1.56)	1.22	(0.90,	1.67)	0.61
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)	0.51
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)	0.18
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)	0.25
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)	0.12
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)	0.45
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)	0.21
	Odds Ratio CC+CTvsTT (95% CI)			Odds Ratio CC+CTvsTT (95% CI)			Odds Ratio CC+CTvsTT (95% CI)			Odds Ratio CC+CTvsTT (95% CI)			
Gene polymorphisms													
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	0.61

[1] P-value for the interaction of active treatment/placebo * biomarker on CHD risk, based on a 1 degree-of-freedom test for biomarkers (log-scale).

[2] From a logistic regression model adjusted for trial, age, BMI, waist-hip ratio, smoking, alcohol consumption, physical activity, diabetes, history of CVD, LVH on electrocardiogram, history of high cholesterol requiring medication, systolic blood pressure, use of antihypertensive medication, aspirin or statins.

Table 5

Change in Biomarkers from Baseline to Year 1

	CEE+MPA Trial		CEE Trial		
	CEE+MPA	Placebo	CEE	Placebo	
CHD/controls	79/180	55/148	55/120	47/112	
	Median (Interquartile Range)				P value[1]
Inflammation					
C-reactive protein (ug/ml)	1.1 (3.6)	−0.0 (1.5)	2.2 (4.4)	0.1 (2.3)	<0.001
E-selectin (ng/ml)	−7.0 (9.0)	0.0 (10.0)	−7.0 (12.0)	−1.0 (9.0)	<0.001
Interleukin-6 (pg/ml)	0.2 (1.5)	0.1 (1.7)	0.3 (1.6)	0.1 (1.8)	0.44
MMP-9 (ng/ml)	53.0 (154.0)	−3.00 (111.0)	28.0 (154.0)	−11.5 (140.0)	<0.001
Lipids					
HDL- cholesterol (mg/dl)	4.0 (8.0)	0.0 (9.0)	7.0 (12.0)	0.0 (6.0)	<0.001
LDL-cholesterol (mg/dl)	−20.0 (30.0)	−1.0 (27.0)	−23.0 (32.0)	1.0 (29.5)	<0.001
Total cholesterol (mg/dl)	−16.0 (33.0)	−2.0 (33.0)	−12.5 (35.0)	0.0 (33.0)	<0.001
Triglycerides (mg/dl)	14.0 (56.0)	1.0 (49.0)	17.5 (69.0)	0.0 (50.0)	<0.001
Thrombosis and other blood markers					
D-dimer (ug/ml)	0.0 (0.3)	0.0 (0.2)	0.0 (0.3)	0.0 (0.2)	0.16
Fibrinogen (mg/dl)	−26.5 (79.0)	−7.5 (68.0)	−10.0 (100.0)	−5.0 (80.0)	0.02
Factor VIII (%)	−2.0 (34.0)	0.0 (30.0)	1.0 (29.0)	2.5 (34.0)	0.27
PAI-1 antigen (ng/ml)	−6.2 (25.4)	−0.6 (28.9)	−9.9 (34.0)	−0.6 (30.2)	<0.001
Prothrombin F1.2 (nmol/L)	0.1 (0.4)	0.0 (0.4)	0.1 (0.5)	0.0 (0.4)	0.13
PAP (nmol/L)	0.7 (2.0)	0.1 (1.5)	0.7 (1.9)	0.2 (1.3)	<0.001
TAFI (ug/ml)	−0.0 (0.7)	−0.1 (0.7)	0.2 (0.7)	0.0 (0.9)	0.07
von Willebrand factor (%)	0.0 (33.0)	3.0 (31.0)	0.00 (33.0)	0.0 (37.0)	0.62
Homocysteine (umol/L)	−0.4 (2.2)	−0.3 (2.4)	−0.4 (2.3)	0.0 (2.7)	0.01
Glucose (mg/dl)	−3.0 (13.0)	−1.0 (12.0)	−3.0 (15.0)	1.0 (16.0)	0.002
Insulin (UIU/ml)	−1.0 (3.3)	0.1 (3.8)	−1.1 (4.8)	0.8 (3.6)	<0.001

	CEE+MPA Trial		CEE Trial		
	CEE+MPA	Placebo	CEE	Placebo	
CHD/controls	79/180	55/148	55/120	47/112	
	Median (Interquartile Range)				P value[1]

[1] P-value from a paired t-test (per participant) of change in biomarker on hormone treatment compared to placebo, controlling for the same variables as in Table 3.

Table 6

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

	First Tertile of Change				Second Tertile of Change				Third Tertile of Change				
	Change Value	OR (95% CI)			Change Value	OR (95% CI)			Change Value	OR (95% CI)			P-value for interaction[1]
Inflammation													
C-reactive protein (ug/ml)	< −0.1	0.75	(0.39	1.45)	−0.1 - < 1.7	1.40	(0.72	2.69)	≥1.7	1.39	(0.66	2.93)	0.33
E-selectin (ng/ml)	<−8	1.24	(0.57	2.72)	−8 - < −1	1.38	(0.70	2.70)	≥−1	0.77	(0.40	1.47)	0.08
MMP-9 (ng/ml)	<−25	0.90	(0.49	1.64)	−25 - < 63	0.98	(0.50	1.90)	≥63	1.33	(0.71	2.49)	0.16
Lipids													
HDL-cholesterol (mg/dl)	<0	1.06	(0.53	2.11)	0 - < 6	1.34	(0.72	2.49)	≥6	1.29	(0.60	2.78)	0.44
LDL-cholesterol (mg/dl)	<−24	0.69	(0.33	1.43)	−24 - < −2	1.13	(0.55	2.31)	≥ −2	1.37	(0.70	2.70)	0.46
Total cholesterol (mg/dl)	<−21	0.96	(0.50	1.84)	−21 - < 3	0.84	(0.43	1.62)	≥ 3	1.61	(0.84	3.10)	0.19
Triglycerides (mg/dl)	<−7	0.79	(0.42	1.50)	−7 - < 25	2.31	(1.16	4.59)	≥ 25	0.70	(0.38	1.32)	0.65
Thrombosis and other blood markers													
Fibrinogen (mg/dl)	<−39	1.13	(0.59	2.17)	−39 - < 12	1.01	(0.55	1.85)	≥ 12	1.27	(0.69	2.33)	0.57
PAI-1 antigen (ng/ml)	<−12.2	1.24	(0.63	2.45)	−12.2 - < 4.2	0.75	(0.37	1.52)	≥ 4.2	0.90	(0.48	1.68)	0.24
PAP (nmol/L)	<−0.1	0.72	(0.37	1.39)	−0.1 - < 1	1.29	(0.64	2.61)	≥ 1	0.89	(0.45	1.75)	0.90
Homocysteine (umol/L)	<−1.1	1.29	(0.70	2.40)	−1.1 - < 0.4	1.31	(0.69	2.49)	≥ 0.4	0.84	(0.45	1.55)	0.29
Glucose (mg/dl)	<−6	0.85	(0.44	1.66)	−6 - < 3	1.10	(0.57	2.12)	≥ 3	1.38	(0.78	2.43)	0.32
Insulin (UIU/ml)	<−1.6	0.89	(0.46	1.70)	−1.6 - < 0.9	1.25	(0.63	2.49)	≥ 0.9	1.09	(0.57	2.07)	0.53

Only biomarkers with significant change in Table 5 are shown.

[1] P-value for interaction of active treatment/placebo * biomarker change is based on a 1 degree-of-freedom test for change in biomarker controlling for the same variables as in Table 3.