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Tissue factor pathway inhibitor, activated protein C resistance, and risk of ischemic stroke due to postmenopausal hormone therapy

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Abstract

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Background and Purpose—To test whether changes in plasma tissue factor pathway inhibitor (TFPI) levels or activated protein C resistance (normalized APC resistance ratio, nAPCsr) modify the increased risk of ischemic stroke due to postmenopausal hormone therapy (PHT).

Methods—Nested case-control study of 455 cases of ischemic stroke and 565 matched controls in the Women's Health Initiative trials of PHT.

Results—Baseline free TFPI was associated with ischemic stroke risk, OR (95% CI) per SD increase = 1.17 (1.01, 1.37, p=0.039, but baseline nAPCsr was not, OR per SD increase = 0.89 (0.75, 1.05), p=0.15. Baseline TFPI levels and nAPCsr did not modify the effect of PHT on ischemic stroke. Treatment-induced mean changes of -28% in free TFPI and +65% in nAPCsr did not change the risk of ischemic stroke (interaction p = 0.452 and 0.971 respectively). In subgroup analyses baseline nAPCsr was inversely associated with lacunar strokes, OR per SD increase = 0.74 (0.57, 0.96), p=0.025, and baseline free TFPI interacted with treatment to increase large vessel atherosclerotic strokes, p=0.008.

Conclusions—Pro-coagulant changes in TFPI or nAPCsr do not modify the increased ischemic stroke risk due to PHT.

Keywords

Cerebrovascular Accident; Estrogen; Hemostasis; Menopause; Randomized Controlled Trials

INTRODUCTION

The Women's Health Initiative (WHI) trials of showed an increased risk of ischemic stroke for postmenopausal hormone therapy (PHT) compared to placebo.[1,2] Baseline levels of several hemostatic markers and genotypic Factor V Leiden (FVL) status did not identify women at increased risk of ischemic stroke on PHT in the WHI trials.[3] PHT increased D-dimer and plasmin-antiplasmin levels and decreased fibrinogen and PAI-1, and change in D-dimer interacted with PHT to increase risk of stroke.[3] The fact that PHT increased the risk of venous thrombo-embolism (VTE) in WHI, especially in subjects with FVL is consistent with a possible role for hemostatic factors in explaining the increased risk of stroke also.[4,5]

Oral PHT increases markers of activated coagulation, reduces coagulation inhibitors, and induces an acquired resistance to the natural anticoagulant activated protein C (APC).[6,7,8,9] Reduction in tissue factor pathway inhibitor (TFPI) and protein S are thought to be important mechanisms underlying the activation of coagulation and acquired protein C resistance associated with oral contraceptives (OCs), and both have been implicated in VTE.[10] Cross-sectional studies suggest that low levels of TFPI or increased APC resistance may play a role in childhood ischemic stroke, but their roles in adult strokes are unclear.[11,12,13] Higher TFPI levels in subjects with subclinical carotid and coronary atherosclerosis and with increased arterial stiffness may reflect endothelial dysfunction.[14,15, 16]

Here we report the first prospective study of the associations of acquired APC resistance and of TFPI levels with stroke, and we examine whether PHT-induced changes in these factors are associated with stroke risk in the WHI trials. We also examine associations with major subgroups of stroke.

METHODS

Details of the design, recruitment, randomization, data collection, intervention, and outcomes ascertainment procedures in the WHI PHT trials, including CONSORT diagrams, have been published previously.[3,17,18]

Study population and interventions

The WHI hormone trials enrolled 27 347 postmenopausal women aged 50-79 years 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. 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.[1,2]

Follow-up and outcome ascertainment

Stroke outcomes were identified by semi-annual questionnaires followed by review of medical records and classification by centrally adjudicated by stroke-trained neurologists blinded to treatment assignment. This report is based on 565 centrally adjudicated strokes with measurements of APC resistance or TFPI at baseline and controls matched 1:1 on age, race, randomization date, hysterectomy status, and self-reported prevalent stroke or transient ischemic attack at baseline. Strokes were classified into ischemic (N=455), hemorrhagic (N=82), other (N=4), cause of death only (N=21), and missing (N=3).). Ischemic strokes were subclassified according to the Trial of Org 10172 in Acute Stroke Therapy (TOAST).[19] The major identifiable subgroups were large artery atherosclerosis N=43, cardioembolism N=66, and small vessel occlusion (lacune) N=117. The remaining 229 ischemic strokes had insufficient information to make a determination or had 2 or more potential etiologies.

Biomarker Analyses

Blood samples were collected into tubes containing 1.8% sodium citrate and centrifuged within 2 hours at 1300xg for 10 minutes at 4° C and stored at -70°C. Assays were run in duplicate in single batches including cases and controls and 10% blind duplicates within 8 years of collection. The endogenous thrombin potential-based activated protein C resistance test (ETP-based APC resistance test) was performed at the Department of Biochemistry at the University of Maastricht, the Netherlands, as described earlier.[20] The test result is expressed as the ratio of thrombin generation without and with added APC, normalized against pooled normal plasma (nAPC-sr). In this assay higher nAPCsr values indicate increasing APC resistance. Total and free TFPI antigen were assayed using the same batch of Asserochrom ELISA (Stago) kits for each, and TFPI activity was measured in citrated plasma by an in-house chromogenic substrate activity assay at the Department of Hematology, Oslo University Hospital, Oslo.[21,22] Inter-and intra-assay coefficients of variation were 3-5% and 1-3% for all assays. In 150 blind duplicates from the current study the intra-class correlations for nAPCsr and TFPI (total, free, and activity) were 0.73, 0.83, 0.44, and 0.84 respectively.

Statistical Analyses

The primary analysis tested the hypothesis that PHT-induced increases in APC resistance and reductions in free TFPI levels increase the risk of ischemic stroke. Cases of ischemic stroke (N=455) and all controls (N=565) were used to study associations of baseline values with ischemic stroke; associations of change in TFPI values were examined in 317 cases

occurring after year 1 and in 465 controls while change in nAPCsr values were examined in 220 cases and 330 controls with both baseline and year 1 values available. Lacunar and non-lacunar ischemic stroke, hemorrhagic stroke, and total stroke were examined in secondary analyses. We combined the trial data since their effects on stroke were similar.[1,2] Sensitivity analyses excluded women not adherent to their study medication 6 months before their stroke.

Biomarkers were examined on a continuous (linear) scale, after log-transformation for biomarkers that had skewed distributions. We used markers linearly to assess significance but reported odds ratios (OR) and 95% confidence intervals (CI) per standard deviation (SD) increase. Thus, there was not one-to-one correspondence between p-values below 0.05 and CIs for OR not containing 1. For the interaction of change in biomarkers levels from baseline to year 1, we computed p-values from logistic coefficients for change as a continuous variable but showed ORs by tertiles of change. We also examined whether changes in individual biomarkers were intermediates in the pathway of HT effects on stroke by comparing regression models with and without terms for biomarker change covariates.

We tested for nominal significance at $p<0.05$ without adjustment for multiple testing. Statistical analyses were performed on SAS statistical software (version 9; SAS Institute Inc, Cary, North Carolina).

RESULTS

In this case-control dataset PHT compared to placebo yielded overall multivariable-adjusted ORs (95% CI) for total, ischemic, and hemorrhagic stroke of 1.60 (1.23, 2.09), 1.81(1.36, 2.40), and 0.87 (0.52, 1.46) respectively. Cases of ischemic stroke were more likely to be smokers, non-drinkers, inactive, diabetic, hypertensive, have left ventricular hypertrophy, or a history of prior cardiovascular disease, higher body mass index, waist-to-hip ratio, systolic and diastolic blood pressure compared to controls (Table 1). Due to matching factors race/ethnicity and age did not differ between cases and controls. In unadjusted analyses baseline free TFPI levels were significantly higher in ischemic stroke cases than controls ($p=0.002$) but total TFPI, TFPI activity, and nAPCsr did not differ between cases and controls. In multivariable-adjusted models baseline free TFPI was positively associated with ischemic stroke, OR per SD increase = 1.17 (1.01, 1.37, $p=0.037$, but baseline nAPCsr was not associated with ischemic stroke, OR per SD increase = 0.89 (0.75, 1.05), $p=0.15$ (Table 2). Baseline TFPI and nAPCsr did not modify the effect of hormone therapy on stroke risk (Table 3).

Free TFPI was inversely related to nAPCsr at baseline (linear regression β (SE) -0.84 (0.18), $p<0.0001$) and at 1 year (β -1.41 (0.19), $p<0.0001$). TFPI was correlated with lipoproteins but adding lipoprotein covariates to the models did not alter any results. PHT variably decreased TFPI levels and increased nAPCsr compared to no changes in the placebo group, with mean net decreases of −19, -28, and -22% respectively for total, free and TFPI activity and an increase of 65% for nAPCsr (Table 4 and Figure 1). In the subset with known FVL carrier status baseline mean (SD) nAPCsr values were 4.9 (2.2) in the 25 FVL heterozygotes and 7.2 in the single homozygote, compared to a mean of 3.0 (1.9) in non-carriers. Baseline and year 1 nAPCsr exceeded 2.0 in all but one of the heterozygotes; however, their values were overlapped completely by those of known non-carriers.

Change in free TFPI did not modify stroke risk (interaction $p=0.452$, Table 5). Though women in upper and lower tertiles of change in nAPCsr appeared to be at higher risk of ischemic stroke on HT, the interaction was not significant (interaction $p=0.971$ in linear models, $p=0.38$ in quadratic models). Models with change in TFPI or nAPCsr added as

covariate showed no evidence that change in these biomarkers mediated the effect of PHT on ischemic stroke risk.

Secondary analyses restricted to adherent participants yielded results similar to those for the overall study. Models comparing the extreme deciles of TFPI or nAPCsr with values below or above the median, respectively, did not yield significant associations with ischemic stroke for baseline values or for change at 1 year. In other secondary analyses the findings for total stroke were similar in direction and strength to those for ischemic stroke, while hemorrhagic stroke showed no evidence of association with TFPI or nAPCsr. Subtypes of stroke by TOAST criteria indicated that lacunar strokes were inversely associated with baseline nAPCsr, OR 0.74 (0.57, 0.96), p= 0.025; however, nAPCsr did not interact with treatment on ischemic stroke. Higher baseline levels of free TFPI were not associated with large artery atherosclerotic strokes, but interacted with treatment to increase risk (p=0.008).

DISCUSSION

Even though PHT induced substantial increases in nAPCsr and decreases in TFPI, these changes were not related to the increased risks of ischemic stroke observed in the WHI trials. The mechanisms underlying the increased stroke risks on postmenopausal hormone therapy remain elusive, though hemostatic mechanisms remain the most likely culprits with increases in D-dimer implicated in prior analyses of a subset of WHI data.[3] Without knowledge of a mechanism it is difficult to design strategies to prevent this complication of oral PHT therapy. Transdermal estradiol does not appear to share the pro-thrombotic potential of oral estrogens with respect to venous thrombosis or stroke, but randomized trial evidence is lacking.[23,24]

The relationships of nAPCsr and TFPI to ischemic stroke risk are complex. Contrary to our hypothesis that reduced anti-coagulant function would increase risk of ischemic stroke, in this study baseline free TFPI levels were higher in cases compared to controls while no significant differences were observed for nAPCsr. The findings for TFPI are consistent with previous studies of carotid atherosclerosis, arterial stiffness, and ischemic heart disease.[14,15,16, 25] Previous case-control studies did not find convincing evidence that FVL is associated with stroke.[26]

In the subset of lacunar strokes nAPCsr values were significantly lower in stroke cases than in controls. Lacunar strokes result from pathology of the small perforating arteries supplying the deep subcortical areas of the brain. Previous work has suggested that lacunar arteriopathy may differ from occlusive atherothombosis of the large arteries responsible for other ischemic strokes, and that the risk factor profile of lacunar stroke may differ from that of non-lacunar stroke.[27] Higher free TFPI levels increased the risk of large vessel atherosclerotic stroke due to hormone therapy. However, these subgroup findings may have occurred by chance due to the multiple testing performed.

The 5.7% prevalence of FVL ascertained by genotype is similar to that reported in the literature[9] but nAPCsr did not clearly discriminate FVL carriers from non-carriers. This may be due to high overall nAPCsr values in this study compared to values reported in the literature, making the window for discrimination smaller. The high values are likely caused by the fact that APCsr determined with the ETP-based APC resistance test is normalized by dividing through the APCsr measured in normal pooled plasma.[20] Differences in pre-analytical variables such as venipuncture, citrate concentration, centrifugation, plasma handling, and storage of the WHI plasma and the normal pooled plasma used in the laboratory may result in high sensitivity of pooled plasma for APC compared to WHI plasma.

Strengths of the current study include its setting in the context of a randomized controlled clinical trial, which allows for an unbiased assessment of treatment effects on hemostatic factors and stroke. Limitations include the relatively small number of strokes overall and the large number of strokes of undetermined etiology. Only large interactions of treatment with biomarkers could have been detected; however the primary analysis of interaction by change in biomarker offered no hint of potential effect modification. Another limitation is the variability in the laboratory measurements, especially for free TFPI. Clinical trial participants may differ from the general population and the results may not be generalizable.

We conclude that changes nAPCsr and TFPI in response to PHT do not modify or mediate the increased ischemic stroke risk due to hormone therapy. Higher baseline free TFPI was associated with overall risk of ischemic stroke and with increased risk of large vessel atherosclerotic strokes but the direction of these associations was the opposite of what one might have expected from studies of venous thrombo-embolism. Future studies are needed to confirm the apparent protective association of nAPCsr with lacunar stroke and to explore the biology of this association.

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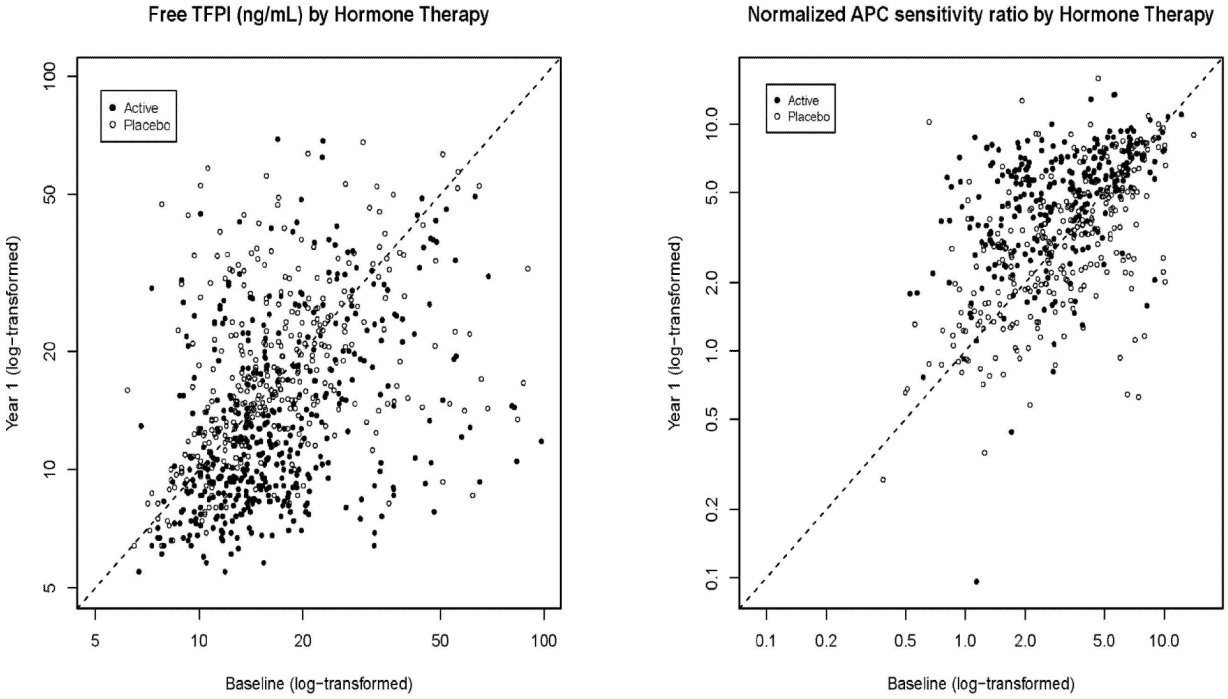

Figure 1.

Free TFPI and normalized APC sensitivity ratio at baseline and year 1 in women randomized to active hormone therapy or placebo therapy (N=782 with repeat measures of TFPI, N=550 of nAPCsr).

Table 1

Baseline characteristics of women in the nested case control study of ischemic stroke

	Estrogen Trial				Estrogen + Progestin Trial				P-Value*
	Control (N=297)		Ischemic Stroke (N=239)		Control (N=268)		Ischemic Stroke (N=216)		
Race/ethnicity									0.22
White	229	77.1	176	73.6	232	86.6	180	83.3	
Black	45	15.2	49	20.5	18	6.7	19	8.8	
Other/Unspecified	23	7.7	14	5.9	18	6.7	17	7.9	
Smoking status									0.007
Never	151	51.5	116	49.4	152	57.6	96	44.7	
Past	119	40.6	93	39.6	91	34.5	87	40.5	
Current	23	7.8	26	11.1	21	8.0	32	14.9	
Alcohol use									0.003
Non drinker	134	45.3	150	62.8	115	43.2	100	46.5	
≤ 1 drink/day	120	40.5	68	28.5	113	42.5	91	42.3	
>1 drink/day	42	14.2	21	8.8	38	14.3	24	11.2	
Total expenditure from physical activity (MET-hrs/wk)									0.01
Inactive (0)	43	16.3	61	28.2	43	18.0	42	22.0	
<5	69	26.1	59	27.3	56	23.4	44	23.0	
5-<12	62	23.5	34	15.7	48	20.1	47	24.6	
≥12	90	34.1	62	28.7	92	38.5	58	30.4	
Treated diabetes	21	7.1	35	14.6	7	2.6	25	11.6	<0.001
History of hypertension									<0.001
Never hypertensive	149	56.4	61	29.3	159	66.3	98	52.7	
Untreated hypertensive	23	8.7	36	17.3	19	7.9	23	12.4	
Treated hypertensive	92	34.8	111	53.4	62	25.8	65	34.9	
Treated for high cholesterol	50	19.2	36	17.1	34	14.5	38	20.7	0.49
Left ventricular hypertrophy on ECG	22	7.6	36	15.1	11	4.2	19	8.9	<0.001

	Estrogen Trial				Estrogen + Progestin Trial				P-Value*
	Control (N=297)		Ischemic Stroke (N=239)		Control (N=268)		Ischemic Stroke (N=216)		
Aspirin use	79	26.6	62	25.9	60	22.4	51	23.6	0.96
Statin use	27	9.1	17	7.1	15	5.6	18	8.3	0.86
History of CVD†	47	16.3	64	27.6	34	12.8	31	14.8	0.005

	Estrogen Trial						Estrogen + Progestin Trial						
	Control			Ischemic Stroke			Control			Ischemic Stroke			
	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	P-Value*
Age at screening	297	67.3	6.5	239	67.3	6.4	268	67.4	6.7	216	67.6	6.6	0.73
Body-mass index (kg/m^2)	297	28.8	5.8	238	30.1	5.5	266	28.0	5.6	213	28.2	5.0	0.02
Waist/hip ratio	295	0.8	0.1	237	0.9	0.1	266	0.8	0.1	216	0.8	0.1	<0.001
Systolic BP (mm Hg)	297	132.8	17.0	239	139.8	18.9	268	129.2	16.5	216	137.0	18.7	<0.001
Diastolic BP (mm Hg)	297	76.3	9.3	239	77.8	9.2	268	74.4	9.0	216	76.4	10.3	0.002
Total TFPI (ng/mL)	288	86.2	19.2	224	87.8	20.3	250	87.7	20.1	184	89.0	21.0	0.35
Free TFPI (ng/mL)	288	18.6	12.3	224	21.2	12.8	250	18.1	11.0	184	19.0	11.6	0.002
TFPI Activity (%)	288	111.0	24.4	223	110.2	27.7	250	114.1	26.0	184	117.4	28.7	0.86
nAPCsr (ratio)	236	3.8	2.5	181	3.6	2.4	218	3.4	2.0	167	3.2	2.0	0.11

† MI, PCI, CABG, CHF, angina, stroke, or atrial fibrillation

* The p values quantify the marginal association of each baseline characteristic and biomarker with ischemic stroke and are obtained from logistic regression models in combined trials adjusted for treatment assignment (CEE, CEE placebo, CEE+MPA, CEE+MPA placebo) using a 1-df test for association except for the categorical values ethnicity, smoking status, alcohol use, physical activity, and history of hypertension.

Table 2

Adjusted Ischemic Stroke Risk per Standard Deviation Higher Baseline Tissue Factor Pathway Inhibitor and Activated Protein C Resistance (N=455 ischemic strokes, N=565 controls)

	Odds Ratio per SD Increase (95% Confidence Interval)*			
	Estrogen Trial	**Estrogen + Progestin Trial**	**Combined Trials†**	**P-Value‡**
Total TFPI, ng/mL	1.06 (0.86, 1.31)	1.02 (0.82, 1.28)	1.08 (0.93, 1.25)	0.329
Free TFPI, ng/mL	1.37 (1.11, 1.70)	0.95 (0.74, 1.21)	1.17 (1.01, 1.37)	0.039
TFPI Activity, %	0.88 (0.71, 1.08)	1.17 (0.93, 1.47)	1.04 (0.90, 1.21)	0.556
nAPCsr, ratio	0.87 (0.69, 1.10)	0.86 (0.66, 1.11)	0.89 (0.75, 1.05)	0.153

*Odds ratio for ischemic stroke compared with controls per standard deviation of biomarker (in controls) from logistic regression models adjusted for treatment assignment (CEE, CEE placebo, CEE+MPA, CEE+MPA placebo), interaction with treatment assignment, age, race, BMI, waist-hip ratio, smoking, alcohol use, physical activity, diabetes mellitus, prevalent cardiovascular disease (including atrial fibrillation), systolic and diastolic blood pressure, LVH on ECG, use of antihypertensive medications, aspirin, statins, and ever treated for high cholesterol.

†Covariate adjustment for hysterectomy status and as in the preceding footnote.

‡P values are based on logistic regression models in combined trials using a 1-df test for association. Covariate adjustment as in the preceding footnotes.

Table 3

Associations of Baseline Tissue Factor Pathway Inhibitor and Activated Protein C Resistance with Ischemic Stroke Risk by Treatment Assignment

	Odds Ratio per SD Increase (95% Confidence Interval)*								
	Estrogen Trial				Estrogen + Progestin Trial				
	CEE		CEE Placebo		CEE+MPA		CEE+MPA Placebo		P Value for Interaction†
	N	OR (CI)	N	OR (CI)	N	OR (CI)	N	OR (CI)	
Total TFPI, ng/mL	264	1.16 (0.88, 1.52)	248	1.00 (0.75, 1.33)	236	1.13 (0.84, 1.49)	198	1.01 (0.72, 1.41)	0.408
Free TFPI, ng/mL	264	1.55 (1.15, 2.08)	248	1.21 (0.91, 1.60)	236	0.81 (0.59, 1.12)	198	1.16 (0.82, 1.63)	0.861
TFPI Activity, %	263	0.93 (0.71, 1.21)	248	0.91 (0.68, 1.22)	236	1.23 (0.92, 1.65)	198	1.18 (0.87, 1.61)	0.946
nAPCsr, ratio	216	1.02 (0.74, 1.39)	201	0.79 (0.58, 1.07)	208	0.99 (0.72, 1.37)	177	0.78 (0.55, 1.10)	0.134

*From logistic regression models adjusted for age, race, BMI, waist-hip ratio, smoking, alcohol use, physical activity, diabetes mellitus, prevalent cardiovascular disease (including atrial fibrillation), systolic and diastolic blood pressure, LVH on EKG, use of antihypertensive medications, aspirin, statins, and ever treated for high cholesterol.

†P values for the interaction in combined trials of active treatment/placebo X biomarker on ischemic stroke risk based on a 1-df test for biomarkers. Covariate adjustment for hysterectomy status and as in the preceding footnote.

Table 4

Change in Tissue Factor Pathway Inhibitor and Activated Protein C Resistance from Baseline to Year 1

	Median (Interquartile Range)								
	Estrogen Trial				Estrogen + Progestin Trial				
	CEE		CEE Placebo		CEE+MPA		CEE+MPA Placebo		P Value for Change*
	N	Median (IQR)	N	Median (IQR)	N	Median (IQR)	N	Median (IQR)	
Total TFPI, ng/mL	226	-18.10 (21.70)	207	-1.40 (16.60)	195	-17.40 (19.00)	154	-1.40 (15.40)	<0.001
Free TFPI, ng/mL	226	-3.60 (8.00)	207	0.20 (6.90)	195	-3.10 (6.40)	154	0.30 (6.10)	<0.001
TFPI Activity, %	225	-27.00 (30.00)	207	-4.00 (22.00)	195	-25.00 (29.00)	154	0.00 (19.00)	<0.001
nAPCsr, ratio	147	1.60 (3.22)	147	-0.16 (2.16)	128	1.45 (2.54)	128	0.14 (1.73)	<0.001

* P values are from a paired t test (per participant) of change in biomarker in combined trials during hormone treatment compared to control adjusted for hysterectomy status, baseline levels of biomarkers, and the same covariates as in Table 3.

Table 5

Effect of Hormone Therapy on Ischemic Stroke Risk by Change in Tissue Factor Pathway Inhibitor and Activated Protein C Resistance

	First Tertile		Second Tertile		Third Tertile		
	Change Value	**OR (95% CI)**	**Change Value**	**OR (95% CI)**	**Change Value**	**OR (95% CI)**	**P Value for Interaction***
Total TFPI, ng/mL	< -17.5	1.38 (0.65, 2.94)	-17.6 - -2.4	2.06 (1.16, 3.64)	>-2.4	2.03 (1.08, 3.81)	0.769
Free TFPI, ng/mL	< -4.1	2.10 (1.08, 4.09)	-4.1 - 0.6	1.27 (0.72, 2.24)	> 0.6	2.62 (1.46. 4.71)	0.452
TFPI Activity, %	< -25.0	1.29 (0.59, 2.80)	-25 - -4	2.07 (1.14, 3.76)	> -4.0	1.74 (0.93, 3.28)	0.714
nAPCsr, ratio	< -0.1	2.29 (1.08, 4.86)	-0.2 - 1.5	1.09 (0.54, 2.22)	> 1.5	2.60 (1.14, 5.90)	0.971

* P values for interaction in combined trials of active treatment/placebo X change in biomarker adjusting for hysterectomy status, baseline level of biomarkers, and the same covariates as in Table 3, based on 317 cases and 465 controls for TFPI and 220 cases and 330 controls for nAPCsr. Tertile cutpoints for change are derived from controls.