

# Women's Health Initiative clinical trials: potential interactive effect of calcium and vitamin D supplementation with hormonal therapy on cardiovascular disease

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## Abstract

**Objective:** Data in humans and nonhuman primates have suggested a possible synergistic effect of vitamin D and calcium (CaD) and estrogen on the cardiovascular disease (CVD) risk factors. Using randomized trial data we explored whether the effect of menopausal hormone therapy (HT) on CVD events is modified by CaD supplementation.

**Methods:** A prospective, randomized, double-blind, placebo-controlled trial was implemented among postmenopausal women in the Women's Health Initiative. A total of 27,347 women were randomized to the HT trials (0.625 mg/d of conjugated equine estrogens [CEE] alone for women without a uterus vs placebo; or 0.625 mg of CEE in addition to 2.5 mg of medroxyprogesterone acetate daily [CEE + MPA] for women with a uterus vs placebo). After 1 year, 16,089 women in the HT trial were randomized to the CaD trial and received either 1,000 mg of elemental calcium carbonate and 400 IU of vitamin D3 daily or placebo. The mean (SD) duration of follow-up after CaD randomization was 6.2 (1.3) years for the CEE trial and 4.6 (1.1) years for the CEE + MPA trial. CVD and venous thromboembolism events evaluated in this subgroup analysis included coronary heart disease, stroke, pulmonary embolism, all-cause mortality, plus select secondary endpoints (total myocardial infarction, coronary revascularization, deep venous thrombosis, cardiovascular death, and all CVD events). Time-to-event methods were used and models were fit with a Cox proportional hazards regression model.

**Results:** In the CEE trial, CaD significantly modified the effect of CEE on stroke ( $P$  interaction = 0.04). In the CaD-placebo group, CEE's effect on stroke was harmful (hazard ratio [95% confidence interval] = 2.19[1.34-3.58]);

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however, it was neutral in the CaD-supplement group (hazard ratio [95% confidence interval] = 1.07[0.66-1.73]). We did not observe significant CEE-CaD interactions for coronary heart disease, total CVD events, or any of the remaining endpoints. In the CEE + MPA trial, there was no evidence that the effect of CEE + MPA on any of CVD endpoints was modified by CaD supplementation.

**Conclusions:** CaD did not consistently modify the effect of CEE therapy or CEE + MPA therapy on CVD events. However, the increased risk of stroke due to CEE therapy appears to be mitigated by CaD supplementation. In contrast, CaD supplementation did not influence the risk of stroke due to CEE + MPA.

**Key Words:** Calcium and vitamin D – Cardiovascular disease – Hormonal therapy – Menopause – Postmenopausal women – Women’s Health Initiative.

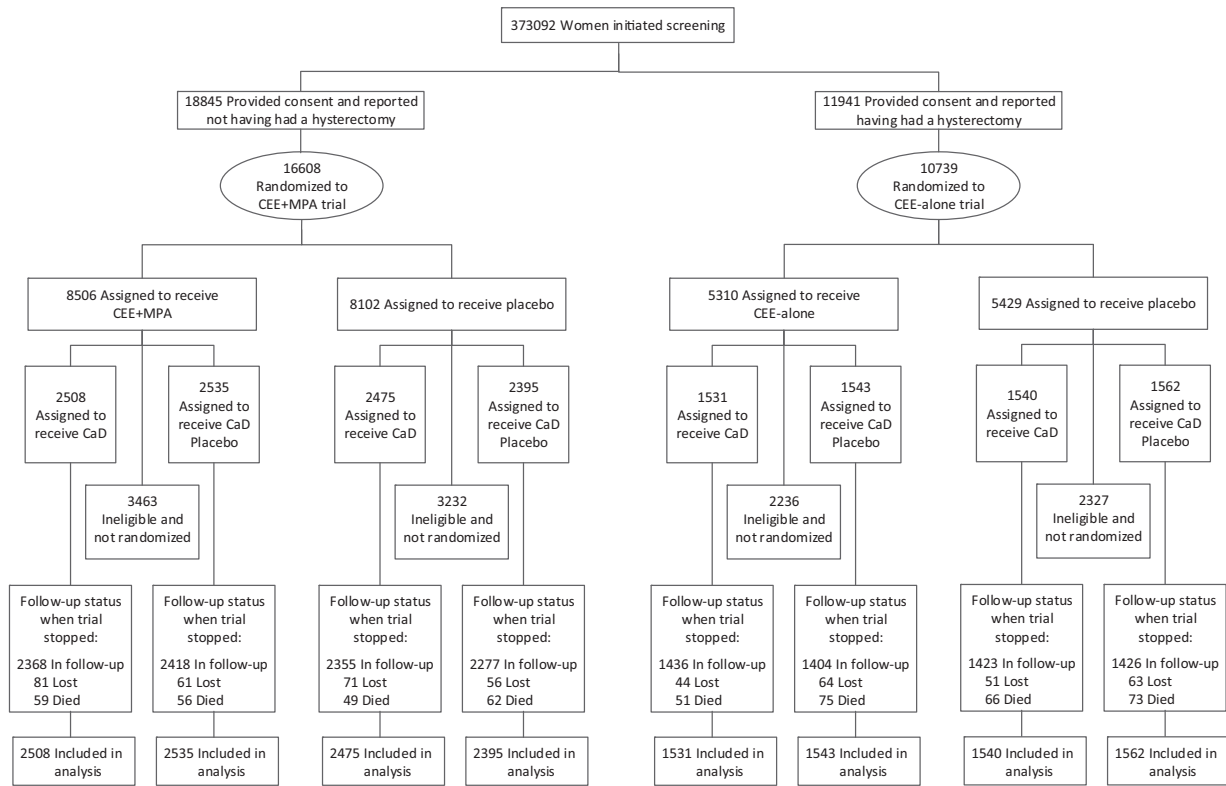
**A**therosclerosis is the most common cause of cardiovascular disease (CVD), and the impact of vitamin D supplementation and estrogen replacement therapy on development of CVD is not known. Data in humans and nonhuman primates have suggested a possible synergistic effect of calcium and vitamin D (CaD) and hormone therapy (HT) on atherosclerosis and CVD risk factors.<sup>1-3</sup> Research data in the rhesus macaque suggest when ovariectomized nonhuman primates were fed an atherogenic diet, and randomized to conjugated equine estrogen (CEE) 0.45 mg/day and had greater percent increases in their plasma 25-hydroxy vitamin D (25OHD) concentrations over 20 months, they had significantly lower plaque formation in coronary arteries and greater coronary artery remodeling compared to nonhuman primates who received no CEE and had minimal increases in their plasma 25OHD concentrations.<sup>1</sup> The biological rationale of the interaction between vitamin D and estrogen in the cardiovascular system may be explained by the structural and functional similarity between vitamin D and estrogen, which may be due to participation at different junctures of the same mechanistic pathway, or perhaps crosstalk between the two hormones and their receptors.<sup>1,3</sup>

Consequently, a synergistic effect of estrogen and CaD on the risk of CVD events in menopausal women may exist. The Women’s Health Initiative (WHI) clinical trials offer a unique opportunity to study this potential relationship. The WHI CaD/HT trial was a double-blinded, randomized, placebo-controlled study with overlapping arms for HT, and CaD, addressing multiple health outcomes in menopausal women. In the 7-year CaD trial, the risk of myocardial infarction (MI), death due to coronary heart disease (CHD) and stroke were similar for women randomized to 1,000 mg/day of calcium carbonate and 400 IU/day of vitamin D3 and those randomized to placebo.<sup>4</sup> In the HT trials, estrogen and progestin therapy (CEE plus medroxyprogesterone acetate [CEE + MPA]) did not show benefit for the development of CHD,<sup>5,6</sup> although there was a suggestion of lower CHD risk with CEE alone among women 50 to 59 years of age at baseline randomization.<sup>6,7</sup> Both formulations of HT, regardless of age or years since menopause, significantly increased the risk of stroke by more than 30%.<sup>6,8</sup> Use of oral estrogens has been declining steadily since the 2002 and 2004 release of these WHI trials results, but oral estrogens remain the most common route of administration for postmenopausal women older than 50 years.<sup>9</sup>

In the WHI, low-density lipoprotein cholesterol (LDL-C) reduction was greatest among women randomized to both CaD and HT (CEE with or without MPA) compared with those randomized to either CaD alone or HT alone, with a synergistic effect on LDL-C reduction observed between CaD and HT.<sup>2</sup> Data investigating the role of combined vitamin D supplementation and HT on various health outcomes has, however, been sparse. The results of a study from the WHI reported fewer hip fractures in women randomized to both CaD and HT (both CEE alone and CEE + MPA) than in those randomized to only one active treatment.<sup>10</sup> Little is, however, known if combined CaD supplementation and HT have additive or synergistic effects on CVD outcomes. To our knowledge no study in humans has investigated the relationship of combined CaD supplementation and HT with CVD events. Given the available aforementioned data on nonhuman primates and humans, we hypothesized that those who received both estrogens and vitamin D would have fewer CVD and venous thromboembolism (VTE) events. The objective of this study is to compare the incidence of CVD and VTE events in a cohort of postmenopausal women randomized to CaD, HT, both CaD and HT, and both placebos.

## METHODS

The WHI clinical trials were designed to evaluate the risks and benefits of dietary modification (DM), HT, and CaD. A total of 68,132 postmenopausal women, aged 50 to 79 years, were recruited at 40 clinical sites in the United States from September 1993 to October 1998 and were randomized in the WHI-DM trial, WHI-HT trials or both. Each clinical site obtained institutional review board approval and written informed consent was obtained from each participant. Detailed information about the study population, recruitment methods, study regimens, randomization, blinding, follow-up, data and safety monitoring and quality assurance has been published previously.<sup>11-13</sup> A total of 27,347 women were randomized to the HT trials: 16,608 women with a uterus were randomized to oral CEE (0.625 mg/d) plus MPA (2.5 mg/d) or placebo; 10,739 women with prior hysterectomy were randomized to oral CEE (0.625 mg/d) alone or placebo (Fig. 1). During the HT trials, at either the first or second annual follow-up visit, 16,089 women were enrolled and randomized in the CaD trial. The WHI CaD trial was a double-blind, randomized, placebo-controlled study designed to test the effects of CaD supplementation (1,000 mg of



**FIG. 1.** Flow diagram of the Women’s Health Initiative (WHI) trials of menopause hormone therapy (HT) and calcium plus vitamin D (CaD). There were 27,347 women randomized in the menopause HT trials, and 16,089 participants were subsequently randomized in the CaD trial. CEE, conjugated equine estrogen; MPA, medroxyprogesterone acetate.

elemental calcium carbonate and 400 IU of vitamin D3 daily) on multiple health outcomes in menopausal women. The eligibility criteria to be enrolled in the CaD trial included many safety parameters (eg, no previous hypercalcemia or renal calculi) and no competing risk indicators (eg, no medical condition associated with survival of less than 3 years). Women were able to participate in the CaD trial even if they were taking their own supplemental calcium and/or vitamin D, provided their personal daily vitamin D supplementation did not exceed 600 IU/day (later changed to 1,000 IU/d). The amount of dietary calcium and vitamin D was determined via a baseline questionnaire.

The current study analyses were not protocol prespecified. The primary endpoint of this analysis is CHD, which is a composite outcome defined as an MI requiring an overnight hospital stay, death due to CHD, or a silent MI diagnosed on serial electrocardiography. Further CVD events analyzed in this analysis include stroke, pulmonary embolism, all-cause mortality, plus select secondary endpoints (total MI, coronary revascularization defined as a documented coronary artery bypass graft or percutaneous coronary intervention, deep venous thrombosis, CVD death, and all CVD events). The CVD outcomes were chosen to correspond to a previously published WHI report.<sup>6</sup> The adjudication methods and outcomes ascertainment in the WHI have been described previously.<sup>14</sup>

Due to trial sequence, analysis of the 2 × 2 partial factorial design of the HT and CaD trials focuses on whether the effect of CEE or CEE + MPA (CEE vs CEE placebo, or CEE + MPA vs CEE + MPA placebo) is enhanced by CaD, with results for the influence of CEE, alone or with MPA, stratified by CaD randomization group. This approach simplifies the presentation of results and leverages prior reports that suggested HT was more influential than CaD.<sup>2,4,6</sup>

For each HT trial all randomized participants were included, and the time-scale began at HT randomization using time-to-event methods based on the intention-to-treat principle. Summary statistics were estimated using Cox proportional hazards models stratified by age, prior disease (if appropriate), and randomization status in the WHI-DM trial. To account for subsequent CaD randomization, time-dependent strata were used, along with an indicator variable for the interaction between time-dependent CaD strata and HT randomization arm. Models were constructed for each clinical end point with women contributing follow-up time until the end of the intervention (July 7, 2002 for the CEE + MPA trial and February 29, 2004 for the CEE trial), date of their first relevant clinical event, death, or last follow-up, whichever came first.

Subgroup analyses, further stratified by age, are reported for all outcomes; corresponding significance tests are based on 1 degree of freedom for whether age-related trends differed

by CaD strata. Post-hoc subgroup analyses, further stratified by select baseline characteristics, explored the significant CEE-CaD interaction for stroke. Since pre-existing data were analyzed for this study, power calculations were not performed. All statistical tests were two-sided and nominal *P* values of 0.05 or lesser were considered statistically significant. *P* values should, however, be interpreted cautiously due to multiple comparisons.

## RESULTS

The baseline characteristics for both the HT trials with respect to CaD randomization revealed well balanced subject demographics and disease risk factors (Tables 1 and 2). Compared with CEE + MPA participants, women in the CEE alone trial were further from the onset of menopause and were more likely to have taken HT before the onset of the trial. The mean (SD) duration of follow-up after CaD randomization was 6.2 (1.3) years for the CEE trial and 4.6 (1.1) years for the CEE + MPA trial.

There were 43 versus 57 (CEE vs placebo) CHD events among women randomized to CaD, and 48 versus 52 (CEE vs placebo) CHD events among women randomized to CaD-placebo. The corresponding hazard ratios (HRs, 95% confidence interval [CI]) were 0.76 (0.51-1.13) and 1.02 (0.69-1.51), respectively (Fig. 2), and the interaction was not statistically significant (*P* = 0.30).

There was evidence that CaD modified the effect of CEE on stroke (*P* interaction = 0.04). There were 35 versus 34 (CEE vs placebo) events among women randomized to CaD (HR 1.07 [0.66-1.73]) and 48 versus 24 (CEE vs placebo) events for those randomized to CaD-placebo (HR 2.19 [1.34-3.58], Fig. 2). The ratio of hazard ratios (rHRs), 1.07 divided by 2.19, provides a comparison for the effect of CEE on stroke with and without CaD (rHR [95% CI] = 0.49 [0.25-0.97]).

Randomization to CaD also significantly modified the effect of CEE on CVD death (*P* interaction = 0.03, Fig. 2), but estimates were too imprecise to quantify the benefit or risk among women randomized to CaD (HR [95% CI] = 0.57 [0.29-1.10]), or those randomized to placebo (HR [95% CI] = 1.45 [0.85-2.47]).

There was no evidence that CaD modified the effect of CEE + MPA on any of the events studied (Fig. 3) including CHD.

The influence of CEE tended to be more favorable for younger rather than older women (Fig. 4), and trends were similar between CaD randomization groups. In particular, while the risk of stroke associated with CEE increased with older age groups, CaD supplementation attenuated the risk of stroke similarly across all age groups (*P* value for three-way interaction = 0.77).

To explore the significant interaction on stroke, the effect of CEE (active vs placebo) by CaD randomization group on stroke was examined by the subgroups: age, race/ethnicity, body mass index, smoking, hypertension, total vitamin D intake, and total calcium intake. A single significant interaction (*P* interaction = 0.04) suggested that smoking status modified the effect of CEE-CaD on stroke (Fig. 5). Data

were, however, sparse, and combining past and current smokers yielded *P*-int = 0.19; HR (95% CI) = 1.16 (0.60-2.24) among the CaD strata (19 [CEE-alone] vs 18 [placebo]), and HR (95% CI) = 4.15 (1.79-9.62) among CaD placebo (26 [CEE-alone] vs 7 [placebo]). Subgroup analyses for CHD and all CVD events were decidedly null (*P* interaction > 0.20).

## DISCUSSION

This study observed that for women in the CEE trial, regardless of age, subsequent randomization to CaD was associated with lowering risk of stroke by half (rHR [95% CI] = 0.49 [0.25-0.97]). This is an intriguing result since the CEE trial reported a significant overall increase of 35% for stroke risk,<sup>6</sup> whereas the CaD trial reported a nonsignificant 5% reduction.<sup>4</sup> Paradoxically, mean systolic blood pressure was significantly increased in both WHI-HT trials and subsequent randomization to CaD did not mitigate the overall influence of HT.<sup>2</sup> The association between CEE and coronary artery calcification was also not modified by randomization to CaD for a subsample of WHI participants aged 50 to 59 years (*n* = 754) that were randomized to both CEE and CaD trials.<sup>15</sup> A significant synergistic effect between CaD and HT on LDL-C reduction was, however, reported among women with low total intake of vitamin D (*P* = 0.03).<sup>2</sup>

Additional analyses from the WHI should be planned to corroborate the observed reduction in stroke risk. 2018 ACC/AHA guideline stated that a coronary artery calcium score predicts atherosclerotic cardiovascular disease events in a graded fashion and is independent of other risk factors, such as age, sex, and ethnicity.<sup>16</sup> A systematic review and meta-analysis showed that the presence and severity of coronary artery calcification were associated with incident stroke over mid-long-term follow-up, in asymptomatic patients without apparent CVDs.<sup>17</sup> Therefore, a secondary analysis of coronary artery calcified plaque measurements that includes more than 300 women who were not randomized to CaD, but leverages dietary sources of calcium and vitamin D may be informative. Other non-WHI randomized trials should include subgroup analysis of CaD in their investigations of HT and CVD. If replicated, women taking CEE for the treatment of vasomotor symptoms may consider concurrently taking CaD supplementation to possibly attenuate their increased risk of stroke due to CEE.

Data have suggested a cardiovascular benefit when HT is initiated in younger menopausal women. This “timing hypothesis” has been shown in the Early versus Late Intervention Trial with Estradiol in which the change in carotid artery intima media thickness was less in women randomized to HT within 6 years of the onset of menopause.<sup>18</sup> An attempt was made in this study to look into the effect of CEE and CaD on clinical end points stratified by age subgroup. Although the number of events trended toward lower number of cases in CEE and CaD active arm from older (70-79 years) to younger (50-59 years) age subgroups, CaD did not modulate the age trends for CEE that have been previously reported for CHD (*P* = 0.08), all-cause mortality (*P* = 0.04), total MI (*P* = 0.02), and coronary artery bypass graft/percutaneous coronary

TABLE 1. Baseline characteristics of the conjugated equine estrogen-alone trial

Characteristic	CaD-supplement group				CaD-placebo group			
	CEE-alone (n = 1,531)		Placebo (n = 1,540)		CEE-alone (n = 1,543)		Placebo (n = 1,562)	
	N	%	N	%	N	%	N	%
Age at screening, y, mean (SD)	63.1	(7.2)	63.1	(7.3)	62.7	(7.2)	63.3	(7.3)
Age group at screening, y, %								
50-59	496	32.4	526	34.2	541	35.1	518	33.2
60-69	698	45.6	696	45.2	674	43.7	677	43.3
70-79	337	22.0	318	20.6	328	21.3	367	23.5
Race/ethnicity, %								
White	1,204	78.6	1,167	75.8	1,180	76.5	1,200	76.8
Black	182	11.9	231	15.0	205	13.3	232	14.9
Hispanic	85	5.6	100	6.5	100	6.5	78	5.0
American Indian	12	0.8	7	0.5	14	0.9	11	0.7
Asian/Pacific Islander	23	1.5	16	1.0	25	1.6	20	1.3
Unknown	25	1.6	19	1.2	19	1.2	21	1.3
Years since menopause, y, %								
<10	237	18.2	243	18.4	283	21.7	249	18.8
10-<20	440	33.8	451	34.2	425	32.6	434	32.7
≥20	624	48.0	624	47.3	596	45.7	644	48.5
Hormone use, %								
Never	785	51.3	760	49.4	793	51.4	813	52.0
Past	563	36.8	573	37.2	510	33.1	527	33.7
Current <sup>a</sup>	183	12.0	206	13.4	240	15.6	222	14.2
Vasomotor symptoms, %								
None	882	58.1	850	55.7	832	54.2	871	56.5
Mild	376	24.8	423	27.7	445	29.0	414	26.9
Moderate or severe	259	17.1	252	16.5	257	16.8	256	16.6
Body mass index, kg/m <sup>2</sup> , mean (SD)	30.3	(6.2)	30.2	(6.0)	30.1	(6.1)	30.3	(6.4)
Body mass index, kg/m <sup>2</sup> , %								
<25	291	19.1	275	18.0	344	22.4	302	19.5
25-29	521	34.1	560	36.6	495	32.2	528	34.0
≥30	714	46.8	697	45.5	698	45.4	721	46.5
Systolic BP, mm Hg, mean (SD)	130.0	(17.2)	129.2	(17.3)	129.4	(17.0)	129.6	(17.2)
Diastolic BP, mm Hg, mean (SD)	76.9	(9.2)	76.5	(9.4)	76.5	(8.9)	76.5	(9.1)
Smoking, %								
Never	793	52.4	783	51.3	801	52.3	795	51.4
Past	578	38.2	575	37.7	582	38.0	612	39.5
Current	142	9.4	167	11.0	148	9.7	141	9.1
Never pregnant/no term pregnancy, %	136	9.8	119	8.6	143	10.4	141	10.0
Age at first birth (among those ever pregnant), y, %								
<20	352	25.3	356	25.6	348	25.3	341	24.1
20-29	848	60.9	837	60.3	809	58.9	854	60.4
≥30	57	4.1	76	5.5	73	5.3	78	5.5
Age at hysterectomy, y, %								
<40	579	38.1	602	39.3	637	41.5	627	40.4
40-49	695	45.8	647	42.3	626	40.8	657	42.3
50-54	132	8.7	167	10.9	162	10.5	162	10.4
≥55	113	7.4	114	7.5	111	7.2	106	6.8
Bilateral oophorectomy, %	567	40.2	591	41.2	563	39.1	606	42.0
Medical treatment, %								
Treated for diabetes	113	7.4	102	6.6	106	6.9	114	7.3
Treated for hypertension or BP ≥140/90	763	52.8	725	50.3	731	50.8	738	50.7
Elevated cholesterol levels requiring medication	223	14.6	232	15.1	202	13.1	227	14.5
Statin use at baseline	120	7.8	116	7.5	108	7.0	113	7.2
Aspirin use (≥80 mg/d) at baseline	314	20.5	302	19.6	297	19.2	334	21.4
Medical history, %								
Myocardial infarction	46	3.0	47	3.1	31	2.0	43	2.8
Angina	108	7.1	103	6.7	104	6.8	101	6.5
CABG/PCI	40	2.6	36	2.4	26	1.7	27	1.7
Stroke	15	1.0	25	1.6	29	1.9	27	1.7
Deep vein thrombosis or pulmonary embolism	19	1.2	24	1.6	30	1.9	26	1.7
Fracture at age ≥55	192	16.8	166	15.1	183	16.5	177	15.7
Family history of breast cancer	250	17.3	247	17.0	263	18.2	271	18.5
>High school/GED, %	1,041	68.9	1,042	68.3	1,028	67.3	1,071	68.9
Family income ≥\$50,000, %	347	23.9	332	22.9	348	23.9	362	24.4

CABG/PCI, coronary artery bypass graft/percutaneous coronary intervention; CEE, conjugated equine estrogens; GED, General Educational Development.  
<sup>a</sup>Required a 3-month washout before randomization.

**TABLE 2.** Baseline characteristics of the conjugated equine estrogen-medroxyprogesterone acetate trial

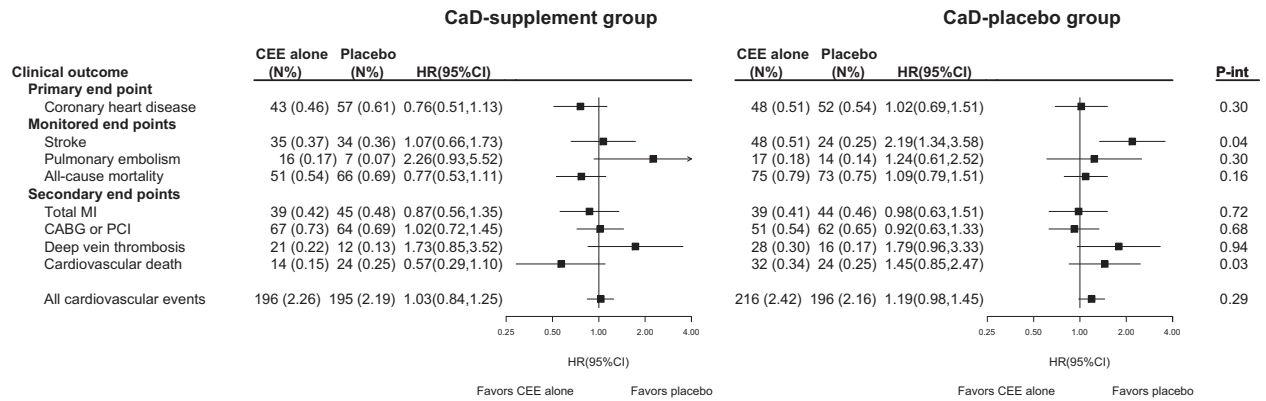
Characteristic	CaD-supplement group				CaD-placebo group			
	CEE+MPA (n = 2,508)		Placebo (n = 2,475)		CEE+MPA (n = 2,535)		Placebo (n = 2,395)	
	N	%	N	%	N	%	N	%
Age at screening, y, mean (SD)	62.4	(7.0)	62.8	(7.1)	62.9	(7.0)	63.0	(7.0)
Age group at screening, y, %								
50-59	937	37.4	886	35.8	881	34.8	824	34.4
60-69	1,114	44.4	1,116	45.1	1,159	45.7	1,083	45.2
70-79	457	18.2	473	19.1	495	19.5	488	20.4
Race/ethnicity, %								
White	2,132	85.0	2,084	84.2	2,167	85.5	2,051	85.6
Black	149	5.9	176	7.1	158	6.2	151	6.3
Hispanic	144	5.7	129	5.2	117	4.6	106	4.4
American Indian	9	0.4	9	0.4	4	0.2	7	0.3
Asian/Pacific Islander	47	1.9	48	1.9	55	2.2	50	2.1
Unknown	27	1.1	29	1.2	34	1.3	30	1.3
Years since menopause, y, %								
<10	930	41.1	882	38.4	871	37.7	825	37.0
10-<20	890	39.4	916	39.9	915	39.6	870	39.0
≥20	441	19.5	497	21.7	523	22.7	533	23.9
Hormone use, %								
Never	1,866	74.5	1,847	74.7	1,847	72.9	1,778	74.3
Past	462	18.4	441	17.8	498	19.7	472	19.7
Current <sup>a</sup>	178	7.1	186	7.5	189	7.5	144	6.0
Vasomotor symptoms, %								
None	1,465	59.1	1,515	61.7	1,552	61.8	1,431	60.3
Mild	671	27.1	664	27.0	643	25.6	639	26.9
Moderate or severe	341	13.8	278	11.3	317	12.6	303	12.8
Body mass index, kg/m <sup>2</sup> , mean (SD)	28.7	(5.9)	28.7	(6.0)	28.5	(5.8)	28.7	(5.9)
Body mass index, kg/m <sup>2</sup> , %								
<25	720	28.8	719	29.3	766	30.4	687	28.8
25-29	855	34.2	864	35.2	880	34.9	871	36.6
≥30	923	36.9	871	35.5	875	34.7	824	34.6
Systolic BP, mm Hg, mean (SD)	126.6	(17.0)	127.1	(17.0)	127.7	(17.9)	127.4	(17.5)
Diastolic BP, mm Hg, mean (SD)	75.6	(9.0)	75.9	(9.0)	75.7	(9.0)	76.0	(9.2)
Smoking, %								
Never	1,257	50.6	1,200	49.1	1,311	52.0	1,255	53.1
Past	981	39.5	996	40.7	969	38.5	871	36.9
Current	244	9.8	249	10.2	239	9.5	236	10.0
Never pregnant/no term pregnancy, %	239	10.6	254	11.3	259	11.2	246	11.3
Age at first birth (among those ever pregnant), y, %								
<20	346	15.3	360	16.0	339	14.7	334	15.3
20-29	1,465	64.7	1,431	63.7	1,499	64.8	1,431	65.6
≥30	215	9.5	200	8.9	215	9.3	169	7.8
Bilateral oophorectomy, %	6	0.2	7	0.3	12	0.5	4	0.2
Medical treatment, %								
Treated for diabetes	106	4.2	95	3.8	103	4.1	105	4.4
Treated for hypertension or BP ≥140/90	897	39.1	983	41.8	1,013	43.0	926	40.8
Elevated cholesterol levels requiring medication	262	10.4	307	12.4	281	11.1	275	11.5
Statin use at baseline	158	6.3	169	6.8	157	6.2	137	5.7
Aspirin use (≥80 mg/d) at baseline	491	19.6	483	19.5	466	18.4	469	19.6
Medical history, %								
Myocardial infarction	38	1.5	38	1.5	43	1.7	39	1.6
Angina	80	3.2	81	3.3	84	3.3	83	3.5
CABG/PCI	25	1.0	27	1.1	24	1.0	29	1.2
Stroke	11	0.4	20	0.8	15	0.6	16	0.7
Deep vein thrombosis or pulmonary embolism	21	0.8	22	0.9	28	1.1	19	0.8
Fracture at age ≥55	278	15.5	270	14.6	279	14.9	306	16.8
Family history of breast cancer	391	16.4	348	15.0	388	16.2	357	15.7
>High school/GED, %	1,854	74.4	1,796	73.1	1,893	75.0	1,762	74.0
Family income ≥\$50,000, %	753	31.4	768	32.8	730	30.4	722	31.6

CABG/PCI, coronary artery bypass graft/percutaneous coronary intervention; CEE+MPA, conjugated equine estrogens plus medroxyprogesterone acetate; GED, General Educational Development.

<sup>a</sup>Required a 3-month washout before randomization.

intervention ( $P = 0.06$ )<sup>6</sup>; the  $P$  values for interactions between CEE and age and CaD for all study end points were not significant (Fig. 4). One of the intriguing findings of this study is, however, on the CEE-age interaction for stroke. Per study findings by Manson et al<sup>6</sup> in 2013, there was no evidence of a

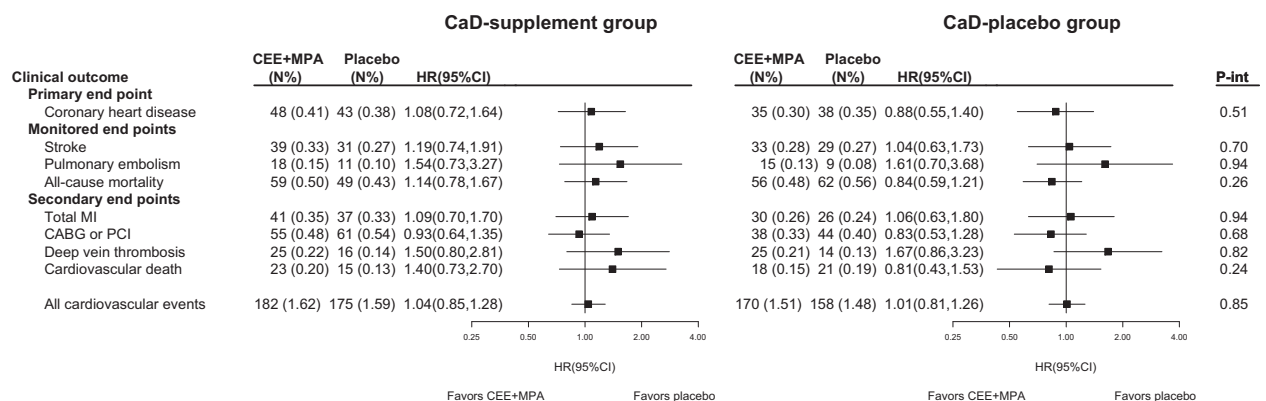
CEE-age interaction for stroke ( $P = 0.77$ ). In the current study, although limiting CEE trial women to those who also participated in the CaD trial, a post-hoc test for CEE-age interaction for stroke yields  $P = 0.03$  with HR (95% CI) = 0.77 (0.30, 1.95), 1.35 (0.84, 2.18), and 2.36 (1.31,



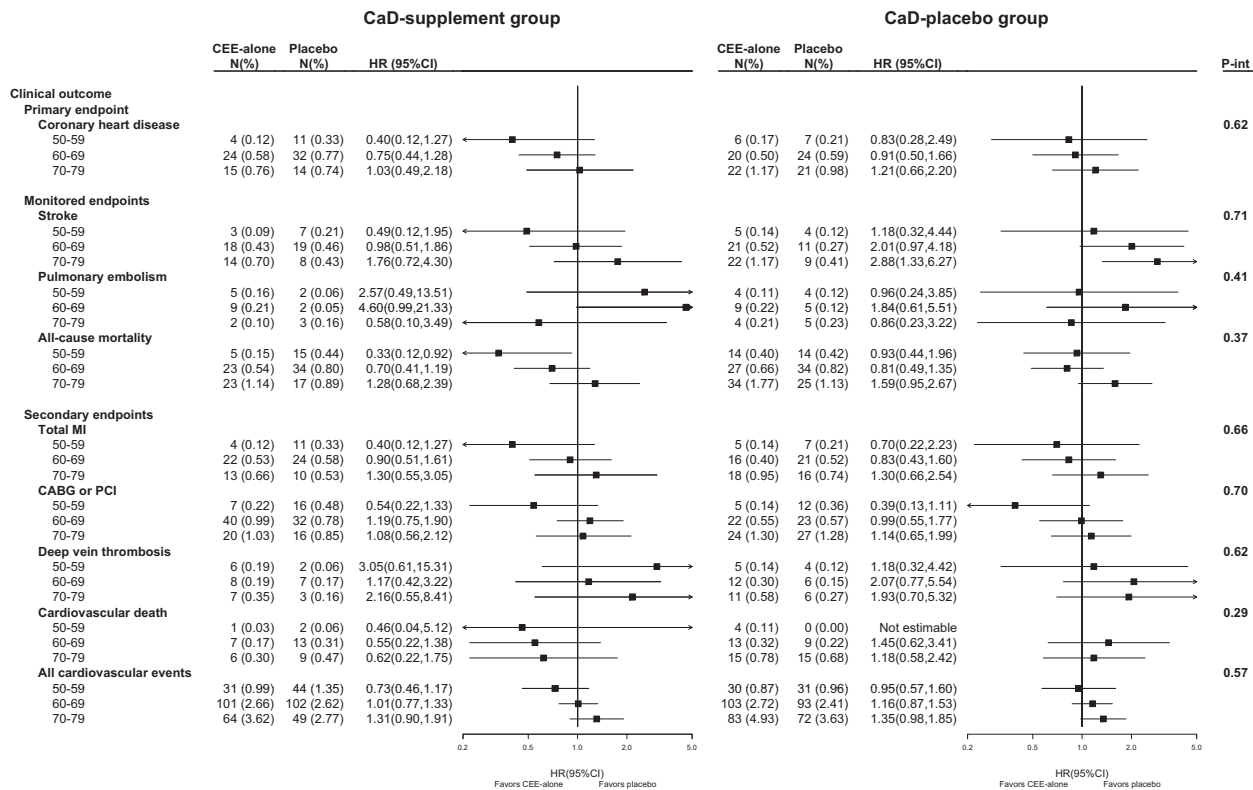
**FIG. 2.** Forest plot of the number of events (annualized %) and hazard ratios (95% CI) for cardiovascular disease (CVD) outcomes in Women’s Health Initiative (WHI) CEE trial, during intervention phase, stratified by CaD trial randomization group. P-int is the significance level for the interaction between CEE and CaD. A significant interaction ( $P\text{-int} \leq 0.05$ ) suggests that CaD randomization group modified the effect of CEE. CABG, coronary artery bypass graft; CEE, conjugated equine estrogen; CI, confidence interval; HR, hazard ratio; MI, myocardial infarction; PCI, percutaneous coronary intervention.

4.22) among women aged 50 to 59, 60 to 69, and 70 to 79 years, respectively; in other words, there appears to be an age trend for CEE on stroke after stratification by CaD. It is worth further exploring the role of CaD and CEE interaction in younger postmenopausal women, since the initial findings of WHI in 2002 are now being used inappropriately in treatment decision making for women in their 40s and 50s who have distressing vasomotor symptoms.<sup>19</sup> The results of WHI-HT trial have shown that the safety profile for CEE is more favorable than CEE + MPA,<sup>19</sup> the significant CEE-CaD interaction for stroke, in addition to CEE-age interaction as previously reported, may make the safety profile for CEE even more favorable in young or early menopausal women with no endometrial protection required. Although low number of events in this study may lead to an inadequate power to confirm the “timing hypothesis” in younger menopausal women taking both CEE and CaD, further research may be needed to examine the vascular effect of estrogen and CaD treatment in different age groups of postmenopausal women.

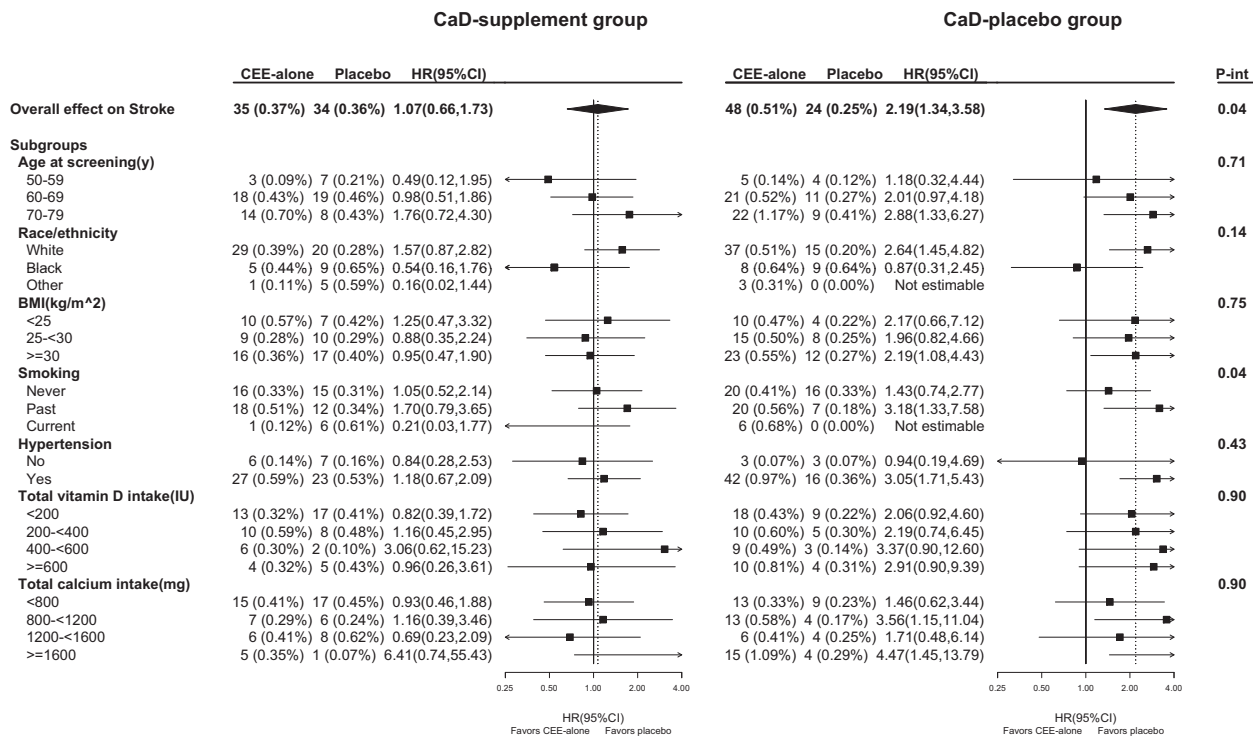
The main strength of this study is the multicentered, randomized, double-blinded, placebo-controlled design in a large cohort of menopausal women. Limitations of this study include that the dosage of vitamin D (400 IU/d), which is typically used for bone health, may be inadequate to lower the incidence or have an effect on most CVD and VTE events. Furthermore, women in both randomization arms were also allowed to continue their own calcium and vitamin D supplements, and at enrollment the overall estimated mean total calcium (1,151 mg) and vitamin D (367 IU) intakes of CaD trial participants<sup>20</sup> were twice the national average<sup>21</sup> and nearly met then existing US dietary recommendations.<sup>22</sup> These high intakes may have limited our ability to detect the effect of CaD with HT on CVD events, although we did examine the effect of HT and CaD in subgroup analyses and observed no significant trends for CEE-CaD by total calcium or vitamin D intake. Serum vitamin D measurements were available on a subset of CaD trial participants ( $n = 567$ ) at baseline and 2 years after randomization. Relatively high



**FIG. 3.** Forest plot of the number of events (annualized %) and hazard ratios (95% CI) for cardiovascular disease (CVD) outcomes in Women’s Health Initiative (WHI) CEE plus MPA trial, during intervention phase, stratified by CaD trial randomization group. P-int is the significance level for the interaction between CEE + MPA and CaD. A significant interaction ( $P\text{-int} \leq 0.05$ ) suggests that CaD randomization group modified the effect of CEE plus MPA. CABG, coronary artery bypass graft; CEE, conjugated equine estrogen; CI, confidence interval; HR, hazard ratio; MI, myocardial infarction; MPA, medroxyprogesterone acetate; PCI, percutaneous coronary intervention.



**FIG. 4.** Forest plot of the effect of CEE (active vs placebo) stratified by CaD randomization group on coronary heart disease (CHD), stroke, all cardiovascular events for age subgroup. P-int is the significance level for the three-way interaction between CEE and CaD and age subgroup. A significant interaction ( $P\text{-int} \leq 0.05$ ) suggests that the interaction between CEE and age subgroup is modified by CaD. CABG, coronary artery bypass graft; CEE, conjugated equine estrogen; CI, confidence interval; HR, hazard ratio; MI, myocardial infarction; PCI, percutaneous coronary intervention.



**FIG. 5.** Forest plot of the effect of CEE (active vs placebo) stratified by CaD randomization group on stroke for select subgroups. P-int is the significance level for the three-way interaction between CEE and CaD and subgroup. A significant interaction ( $P\text{-int} \leq 0.05$ ) suggests that the interaction between CEE and CaD is modified by the corresponding subgroup. BMI, body mass index; CEE, conjugated equine estrogen; CI, confidence interval; HR, hazard ratio.

intakes of total calcium and vitamin D were evident at baseline with nearly half of participants having 25OHD3 concentrations exceeding 20 ng/mL, a concentration deemed adequate by the Institute of Medicine.<sup>23,24</sup> Despite these limitations, randomization to CaD resulted in a 38% (29%-47%) increase in mean (95% CI) serum 25OHD3.<sup>24</sup> Of note, the 38% increase in 25OHD3 was robust across key subgroups with only a nonsignificant suggestion of interaction. Specifically, there were mean increases of 55% versus 28% in serum 25OHD3 among women with total vitamin D consumption less than 100 versus 600 IU or higher, although the trend was not statistically significant among this smaller subsample ( $P = 0.18$ ). Similarly, there were mean increases of 52% versus 27% in serum 25OHD3 among women with measurements during winter versus summer months, but the trend was not significant ( $P = 0.18$ ).<sup>24</sup> Lastly, unlike the analysis of HT plus CaD on fracture,<sup>10</sup> the effects of HT plus CaD on CVD endpoints were not prespecified in the study protocol. Although a significant CEE-CaD interaction was observed for stroke and CVD mortality, the result was not further corroborated by subgroup analyses, and the nominal  $P$  values presented should be interpreted cautiously, as multiple outcomes and subgroups were examined. Therefore, these findings should be considered as hypotheses generating, until assessed with other randomized clinical trials.

## CONCLUSIONS

In conclusion, CaD did not consistently modify the effect of CEE therapy or CEE + MPA therapy on CVD events. The increased risk of stroke seen with CEE was reduced when CEE was given in conjunction with CaD, but these effects were not observed in the CEE + MPA trial.

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