Vitamin D with calcium supplementation and risk of atrial fibrillation in postmenopausal women

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Background Atrial fibrillation (AF) is the most common arrhythmia in adults. Although vitamin D deficiency is associated with AF risk factors, retrospective studies of association with AF have shown mixed results. We sought to determine the efficacy of calcium and vitamin D (CaD) supplementation for AF prevention in a randomized trial."

Methods We performed a secondary analysis of the Women's Health Initiative trial on CaD supplementation versus placebo. We linked participants to their Medicare claims to ascertain incident AF."

Results Among 16,801 included participants, there were 1,453 (8.6%) cases of incident AF over an average of 4.5 years, at an average rate of 19.9 events per 1,000 person-years. We found no significant difference in incident AF rates between the CaD and placebo arms (hazard ratio 1.02 for CaD vs placebo, 95% CI 0.92-1.13). After multivariate adjustment, there was no significant association between baseline 25-hydroxyvitamin D serum levels and incident AF (hazard ratio 0.92 for lowest subgroup vs highest subgroup, 95% CI 0.66-1.28)."

Conclusions We present the first analysis of a large randomized trial of daily vitamin D supplementation for AF prevention. We found that CaD had no effect on incidence of AF in Women's Health Initiative CaD trial participants. We also found that baseline serum 25-hydroxyvitamin D level was not predictive of long-term incident AF risk." (Am Heart J 2019;209:68-78.)

Atrial fibrillation (AF), the most common cardiac arrhythmia in adults, affects 3 million people in the United States and is an important contributor to stroke risk and mortality.[1,2] Although AF is less common in women than in men, women with AF are at a higher risk for these complications.[3] Additionally, because the risk of AF increases with age, postmenopausal women share a significant burden of morbidity from the disease.[1,4]

Despite the prevalence of AF, there are few preventative measures that are recommended on a population level. Supplementation of vitamin D emerged as one potential option, as vitamin D deficiency is associated with known AF risk factors such as hypertension[5] and diabetes.[6] Vitamin D deficiency may also be proarrhythmic through mechanisms affecting inflammation and cardiac remodeling.[7,8] Studies of the association between vitamin D status and risk of AF, however, have shown mixed results. Although some case-control studies report a higher risk of AF in patients with vitamin D deficiency,[9-11] several cohort studies report no association.[12-14]

There have been no reported randomized trials on daily vitamin D supplementation as a preventative intervention for AF. Vitamin D administration has primarily been studied in the context of musculoskeletal health and was recently studied in a randomized trial assessing nonarrhythmic cardiovascular outcomes.[15] In the Women's Health Initiative (WHI) calcium and vitamin D (CaD) trial, postmenopausal women were randomized to either receive CaD supplements or placebo.[16] Women who were randomized to CaD had a small improvement in hipbone density but no significant reduction in hip fractures. Later, the trial was reanalyzed to examine the effect of the intervention on incident cardiovascular events.[17] CaD showed no reduction in incident coronary heart disease or stroke, but AF was not included as an outcome. In this study, our aim was to measure an association between incident AF and randomization to CaD. We secondarily examined effects of baseline serum 25-hydroxyvitamin D [25(OH)D] level on response to CaD assignment and as a predictor of incident AF. We hypothesized that assignment to CaD supplementation would reduce the risk of incident AF compared to assignment to placebo.

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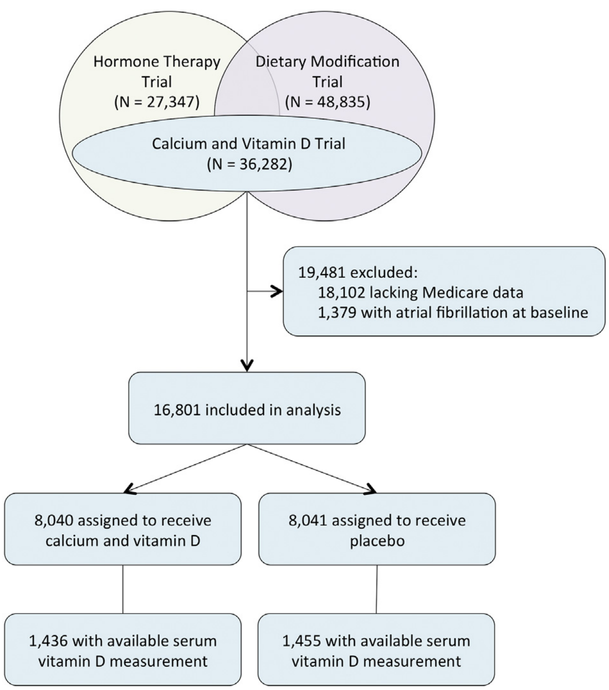
Figure 1



Description of study cohort.

Methods

Participants and study design

Details of the WHI trial design have previously been reported.[18] From 1993 to 1998, WHI enrolled 68,133 postmenopausal women between the ages of 50 and 79 years at 40 US medical centers to participate in their clinical trials.[18,19] Participants enrolled in the hormone therapy trial, the dietary modification trial, or both. The women in these trials were also eligible to participate in the CaD trial (Figure 1). Women were ineligible to participate in WHI if they were unlikely to survive or remain in the vicinity of a WHI clinic for 3 years or if they had alcoholism, drug dependency, or dementia.

In a double-blind fashion, the CaD trial randomized 36,282 WHI participants either to receive both vitamin D and calcium supplementation or to receive placebo.[16] Those in the active arm were instructed to take 1,000 mg/d of elemental calcium and 400 IU/d of vitamin D3. Participants in either trial arm were permitted to continue any personal supplemental calcium (up to 1,000 mg/d) and supplemental vitamin D (up to 1,000 IU/d). Exclusion criteria for the CaD trial included hypercalcemia, renal calculi, corticosteroid use, and calcitriol use. In this study, we included only the subset of WHI CaD participants for which claims data could be obtained from Medicare. We excluded women without Medicare data as well as women with baseline AF.

Data collection

Baseline characteristic definitions for the WHI have been described and were obtained by questionnaires and clinical measurements.[19] Baseline AF was determined by a self-reported doctor diagnosis or by presence of AF on a 12-lead electrocardiogram during baseline clinic visit, and used for exclusion from this study. Data collection and follow-up for the CaD trial were also previously reported.[16] In brief, total daily vitamin D intake at baseline was defined as the sum of dietary vitamin D and vitamin D supplement use prior to randomization. Blood specimens were collected at randomization visit for all participants. Levels of 25(OH)D were measured in the baseline blood specimens for a subset of participants using the DiaSorin Liaison chemiluminescent immunoassay system at DiaSorin headquarters (Stillwater, MN) as part of a nested case-control study where cases were women who suffered fractures during follow-up. Patients were assessed every 6 months by telephone or clinic visit, with at least 1 clinic visit annually. Adherence to medication was assessed by weighing pill bottles at clinic visits.

Ascertainment of outcomes

Ascertainment of incident AF in WHI participants has been described[20,21] and, for this study, was based solely on Medicare claims. WHI data were linked with Medicare data using social security numbers, birth dates, and death dates, with 97% of Medicare-eligible WHI participants successfully linked. AF incidence was defined as having at least a single *International Classification of Diseases, Ninth Revision,* diagnosis code of 427.31 from inpatient, outpatient, or physician diagnosis while the participant was enrolled in Medicare Fee For Service Parts A and B (FFS A+B). Participants enrolled in FFS A+B at WHI enrollment entered the risk set at WHI baseline, whereas participants who enrolled in FFS A+B after WHI enrollment were evaluated with a 2-year look-back period to assess for preexisting AF at the time of entering the risk set. Participants who were AF-free for the duration of the look-back period entered the risk set at the time of completion of the look-back period. Participants who left FFS A+B and then returned for a subsequent FFS A+B interval were not required to undergo a look-back period, as they had been established as AF free on their initial entry into the risk set. Ultimately, the average available follow-up for all participants was 9.1 years if we allowed time beyond AF events.

Statistical analysis

All participants with any AF-free follow-up during the CaD trial period were included in the main analyses. Baseline characteristics are presented with means and SDs for continuous variables and with frequencies and percentages for categorical variables. *P* values for comparisons of characteristics between intervention arms were from *t* tests for continuous variables or χ^2

Table I. Characteristics at CaD trial baseline* by intervention assignment

	All participants (N = 16081)		CaD (n = 8040)		Placebo (n = 8041)	
Variable	n	%	n	%	n	%
Age (y), mean (SD)	66.9	(5.4)	66.9	(5.4)	66.9	(5.4)
50-59	932	5.8	456	5.7	476	5.9
60-69	10074	62.6	5016	62.4	5058	62.9
>70	5075	31.6	2568	31.9	2507	31.2
Race/ethnicity						
White	13986	87.0	6993	87.0	6993	87.0
African American	1271	7.9	628	7.8	643	8.0
Hispanic	370	2.3	199	2.5	171	2.1
Other/unknown	454	2.8	220	2.7	234	2.9
Education						
≤High school	4071	25.3	2047	25.5	2024	25.2
School after high school	6326	39.3	3135	39.0	3191	39.7
College graduate	5610	34.9	2826	35.1	2784	34.6
Region by latitude						
Southern (<35° N)	4298	26.7	2148	26.7	2150	26.7
Middle (35-40° N)	4471	27.8	2231	27.7	2240	27.9
Northern (>40° N)	7312	45.5	3661	45.5	3651	45.4
BMI (kg/m^2), mean (SD)	28.7	(5.8)	28.7	(5.8)	28.7	(5.8)
Total recreational physical activity (MET-h/wk), mean (SD)	11.2	(13.0)	11.4	(13.3)	11.1	(12.7)
Smoking						
Never	8465	52.6	4223	52.5	4242	52.8
Past	6394	39.8	3219	40.0	3175	39.5
Current	1069	6.6	529	6.6	540	6.7
Alcohol (drinks/wk), mean (SD)	2.0	(4.2)	2.0	(4.2)	2.0	(4.2)
0	6851	42.6	3427	42.6	3424	42.6
>0-<7	7664	47.7	3836	47.7	3828	47.6
≥7	1513	9.4	754	9.4	759	9.4
Medical history						
Hypertension	7343	45.7	3693	45.9	3650	45.4
Treated diabetes	898	5.6	445	5.5	453	5.6
Treated hyperlipidemia	2041	12.7	1032	12.8	1009	12.5
Statin use	1600	9.9	801	10.0	799	9.9
Coronary heart disease	447	2.8	225	2.8	222	2.8
Myocardial infarction	346	2.2	174	2.2	172	2.1
CABG/PTCA	240	1.5	126	1.6	114	1.4
Stroke	204	1.3	90	1.1	114	1.4
Peripheral artery disease	272	1.7	138	1.7	134	1.7
Heart failure	94	0.6	49	0.6	45	0.6
Any personal calcium supplement use	8998	56.0	4515	56.2	4483	55.8
Any personal vitamin D supplement use	7980	49.6	4026	50.1	3954	49.2
Total vitamin D intake (IU/d), mean (SD)	378.9	(271.2)	380.4	(268.3)	377.5	(274.1)
<200	5670	35.3	2760	34.3	2910	36.2
200-<400	3021	18.8	1552	19.3	1469	18.3
400-<600	3946	24.5	1998	24.9	1948	24.2
≥600	3180	19.8	1595	19.8	1585	19.7
Hormone therapy trial arm						
Not randomized	8581	53.4	4291	53.4	4290	53.4
Active	3755	23.4	1862	23.2	1893	23.5
Placebo	3745	23.3	1887	23.5	1858	23.1
Dietary modification trial arm						
Not randomized	5276	32.8	2669	33.2	2607	32.4
Intervention	4124	25.6	2015	25.1	2109	26.2
Comparison	6681	41.5	3356	41.7	3325	41.4
25(OH)D data available	2686	16.7	1328	16.5	1358	16.9
25(OH)D level (ng/mL), mean (SD)	18.9	(9.0)	19.0	(9.1)	18.7	(8.9)

CABG, Coronary artery bypass grafting; *PTCA*, percutaneous transluminal coronary angioplasty.
*Race/ethnicity, education, and region by latitude, as well as variables missing at year 1 for individual participants, were defined at WHI baseline.

tests for categorical variables. *P* values for characteristics across subgroups of baseline serum 25(OH)D were from models for linear trend.

Because we present several models on a subset of participants for whom serum 25(OH)D data are available, we used inverse probability weighting to ensure that the correct balance between treatment arms was well approximated. Using the full sample of CaD trial participants, a logistic model was fit to predict the probability of having 25 (OH)D serum data available for each participant as a function of selected variables including age, race/ethnicity, latitude region, CaD trial assignment, trial assignments in the overlapping WHI hormone and dietary modification trials,[18,19] postrandomization fracture outcomes, and postrandomization colorectal cancer outcomes. From this logistic model, the probability of having 25(OH)D serum data for participants in the subset was pulled and inverted to create weights. Models that were adjusted for baseline serum 25(OH)D level were also adjusted for the laboratory source of the serum measurement.

Our main outcome was the intention-to-treat effect of assignment to CaD supplementation on incident AF during the trial period. Events and annualized rates are presented with both weighted and unweighted results. The hazard ratios (HRs) and associated *P* values were computed from proportional-hazards models with incident AF as a function of CaD intervention arm, stratified within the model by intervention assignment for the overlapping WHI hormone and dietary modification trials.[18,19] Kaplan-Meier plots were used to examine the cumulative incidence of AF over time, with censoring for death or loss of Medicare follow-up.

For determination of interaction, a series of proportional hazards models were run with incident AF as a function of CaD intervention arm, the variables of interest, and their interaction product. For subgroups with cut points from continuous distributions, *P* values were derived from a separate model evaluating the interaction between intervention arm and the continuous subgroup variable. All interaction models were weighted to the original CaD trial sample.

The relationship between AF and baseline serum 25(OH)D was evaluated in a series of hierarchal models. At each adjustment level, a proportional-hazards model was fit with AF as a function of subgroups of serum 25(OH)D. *P* values at each level are presented from a separate model with AF as a function of linear trend across subgroups of serum 25(OH)D. These models are all weighted to the full CaD trial sample using the weighting described above and are stratified within the model for serum study sources as well as WHI hormone, dietary modification, and CaD interventions.

Sensitivity analyses

We conducted several sensitivity analyses for the main outcome of risk of incident AF by intervention arm. In 1 sensitivity analysis, we used a stricter definition of incident AF for ascertainment of outcomes. This definition required either 1 inpatient diagnosis or 2 outpatient or physician diagnoses within 1 year. In another sensitivity analysis, we estimated an as-treated effect, where participants were censored for becoming nonadherent, defined as discontinuing the assigned intervention or taking less than 80% of their assigned doses. For our third sensitivity analysis, death was considered a competing risk rather than a censored event. Using a Fine-Gray competing risks regression model, we better estimated the cumulative incidence of AF among the entire cohort rather than only among those who survive up to a given time point. Finally, in a fourth sensitivity analysis, we extended the follow-up period for the main outcome beyond the trial period using all available follow-up data to look for any delayed effects of treatment that was given during the trial.

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Results

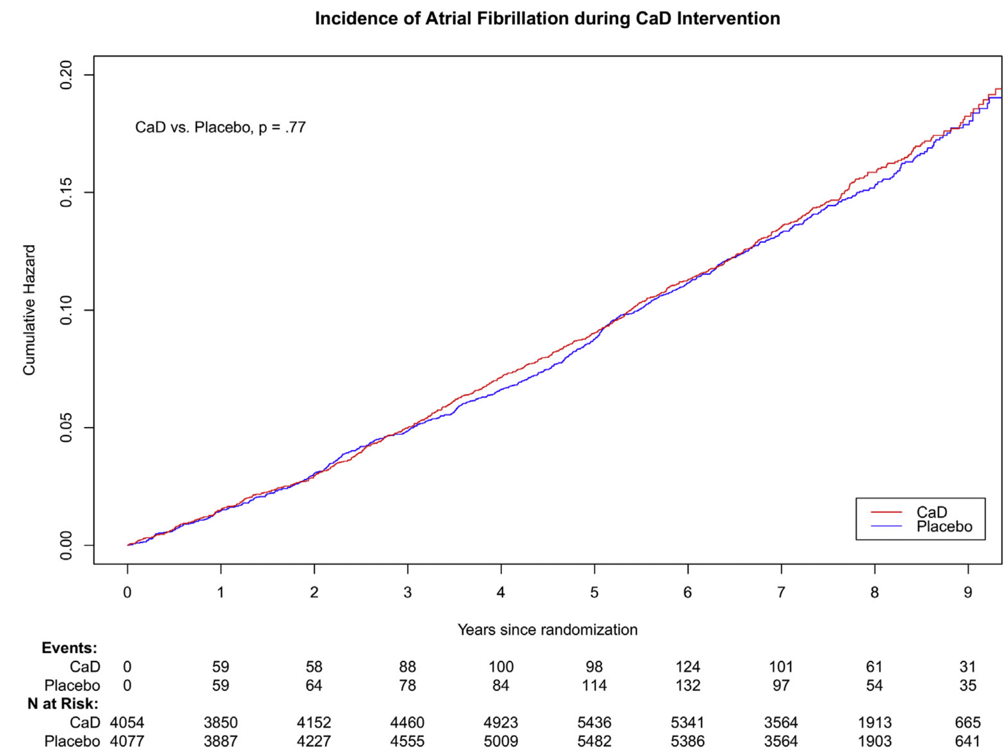
Baseline characteristics

Our inclusion of participants is described in Figure 1. Among the 36,282 participants of the WHI CaD trial, we included 16,801 participants who had Medicare FFS A+B data and were free from AF at baseline. A total of 8,040 of included participants were assigned to receive CaD, and 8,041 were assigned to receive placebo. Baseline characteristics are reported in Table I and are similar for both groups. On average, participants were 66.9 years of age with a body mass index (BMI) of 28.7 kg/m^2, a serum 25 (OH)D level of 18.9 ng/mL, and a total daily vitamin D intake of 378.9 IU/d. Overall, 45.7% had a history of hypertension, 5.6% had a history of treated diabetes, and 2.8% had coronary heart disease. A majority of participants (87%) were white, 7.9% were African American, 2.3% were Hispanic, and 2.8% had other racial/ethnic backgrounds.

CaD and incident AF

The average within-trial follow-up until incident AF or censorship for all included participants was 4.5 years. During the trial period, 1,453 participants (8.6%) developed

Figure 2



Cumulative incidence of atrial fibrillation by CaD intervention assignment (unweighted).

Table II. Atrial fibrillation as a function of CaD intervention assignment

Outcome	CaD	Placebo	*P* value
Atrial fibrillation, all Medicare participants, unweighted			
Events	729	724	
Events per 1000 person-years	20.0	19.7	
Unadjusted, HR (95% CI)	1.02 (0.92-1.13)	1.00 (ref)	.77
Atrial fibrillation, all Medicare participants, weighted to full CaD trial population			
Observed events	729	724	
Weighted events*	1250	1224	
Weighted events per 1000 person-years*	18.4	18.0	
Unadjusted, HR (95% CI)	1.02 (0.92-1.14)	1.00 (ref)	.72
Atrial fibrillation, 25(OH)D subsample, weighted to full CaD trial population			
Observed events	153	155	
Weighted events*	1190	1261	
Weighted events per 1000 person-years*	17.2	17.3	
Unadjusted, HR (95% CI)	0.99 (0.74-1.34)	1.00 (ref)	.96
Adjusted for serum 25(OH)D†, HR (95% CI)	0.96 (0.71-1.28)	1.00 (ref)	.77

All models are stratified by intervention assignment for the WHI Hormone and Dietary Modification trials.
*Weighted event totals and events per 1000 person-years are inverse probability weighted to the full Calcium Vitamin D trial sample.
† Serum 25(OH)D adjusted model additionally adjusted for serum study source.

incident AF at an average rate of 19.9 events per 1,000 person-years. To examine the effect of CaD supplementation on the risk of AF, we plotted the cumulative incidence of AF stratified by treatment arm (Figure 2). Overall, we found no significant difference in incidence rates between the CaD and placebo groups (HR 1.02 for CaD vs placebo, 95% CI 0.92-1.13) (Table II). Our results were unchanged when our study sample was weighted to estimate results for the full

Table III. Atrial fibrillation as a function of CaD intervention assignment by subgroups

	CaD			Placebo			CaD vs placebo	
Subgroup	Observed events	Weighted events*	Weighted events per 1K person-years*	Observed events	Weighted events*	Weighted events per 1K person-years*	HR (95% CI)	Interaction *P* value†
Age (y)								.59
<70	332	707	14.9	343	707	14.8	1.01 (0.86-1.17)	
≥70	397	543	26.4	381	517	25.6	1.03 (0.90-1.19)	
Race								.85
White	666	1108	19.2	657	1089	18.6	1.03 (0.92-1.15)	
Nonwhite	63	142	14.1	67	136	14.1	0.99 (0.68-1.43)	
Region by latitude								.87
Southern (<35° N)	185	341	17.2	118	330	16.9	1.01 (0.82-1.26)	
Middle (35-40° N)	204	351	19.5	199	342	18.3	1.06 (0.87-1.30)	
Northern (>40° N)	340	558	18.5	344	553	18.5	1.00 (0.85-1.17)	
Hypertension								.62
Yes	428	662	19.7	410	588	18.7	1.07 (0.71-1.62)	
No	300	528	15.1	306	661	16.1	0.92 (0.60-1.43)	
Diabetes								.27
Yes	63	104	23.6	55	60	12.5	1.93 (0.57-6.49)	
No	666	1086	16.8	669	1201	17.7	0.95 (0.70-1.29)	
Serum 25(OH)D (ng/mL)‡								.36
<10	21	130	13.7	20	171	18.9	0.69 (0.30-1.61)	
≥10-<20	77	525	16.4	70	564	16.0	1.04 (0.67-1.61)	
≥20	55	534	19.4	65	527	18.5	1.05 (0.66-1.67)	
Background calcium supplement use								.81
Yes	403	689	18.3	406	664	17.7	1.03 (0.89-1.19)	
No	326	561	18.5	318	560	18.4	1.01 (0.85-1.18)	
Background vitamin D supplement use								.36
Yes	378	647	19.2	366	594	17.9	1.07 (0.92-1.25)	
No	351	603	17.6	358	631	18.1	0.97 (0.83-1.13)	

All models are inverse probability weighted to the full Calcium Vitamin D trial sample.
* Weighted event totals and events per 1000 person-years are inverse probability weighted to the full CaD trial sample.
† For continuous/ordinal markers (age, region by latitude, serum 25[OH]D), interaction is from a separate model with atrial fibrillation as a function of trial, continuous marker, and their interaction.
‡ The subgroup analysis based on serum 25(OH)D levels was limited to a minority of participants for whom the measurements were available.

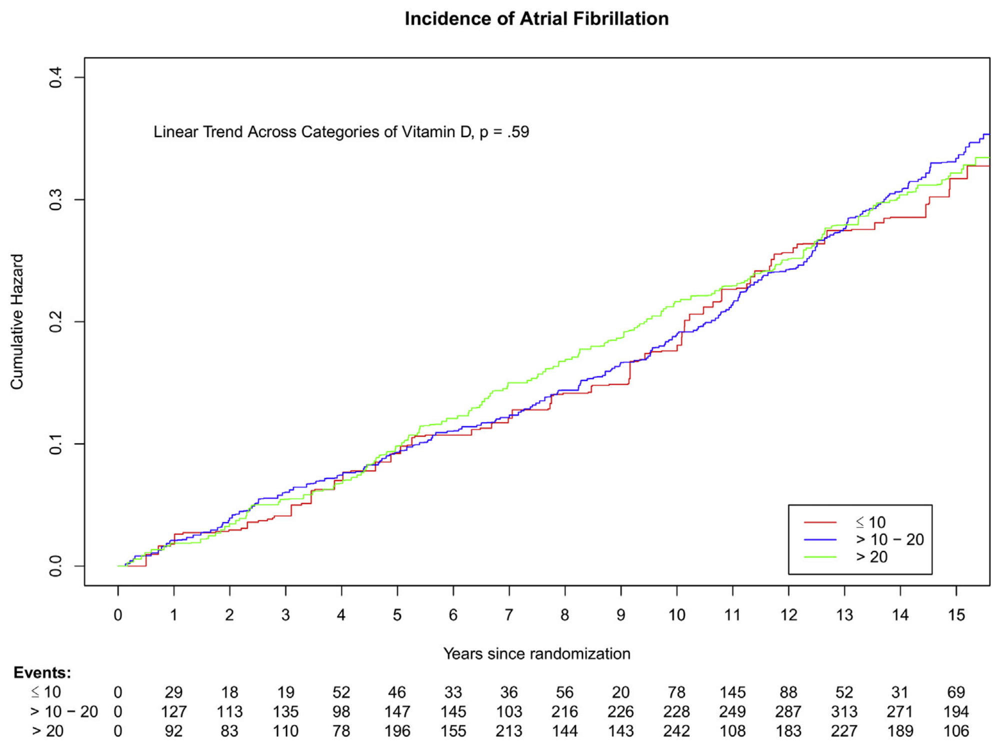
CaD trial population (Table II). When we restricted our analysis to include only participants with available baseline 25(OH)D measurements, the results were again similar. This subset included 2,891 participants with 308 incident AF events (10.7%) during the trial period at an average rate of 22.9 events per 1,000 person-years of follow-up. We

Table IV. Characteristics at CaD baseline* by serum 25(OH)D level (ng/mL)

	<10 (n = 430)		10-<20 (n = 1369)		≥20 (n = 1092)		
Variable	n	%	n	%	n	%	*P* value†
Age (y), mean (SD)	65.7	(7.4)	66.7	(6.8)	65.9	(6.7)	.37
50-59	106	24.7	246	18.0	232	21.2	
60-69	175	40.7	591	43.2	502	46.0	
>70	149	34.7	532	38.9	358	32.8	
Race/ethnicity							<.001‡
White	307	71.4	1200	87.7	992	90.8	
African American	89	20.7	107	7.8	43	3.9	
Hispanic	22	5.1	30	2.2	27	2.5	
Other/unknown	12	2.8	32	2.3	30	2.7	
Education							.36
≤High school	100	23.3	334	24.4	258	23.6	
School after high school	176	40.9	530	38.7	407	37.3	
College graduate	154	35.8	505	36.9	427	39.1	
Region by latitude							.31
Southern (<35° N)	101	23.5	326	23.8	277	25.4	
Middle (35-40° N)	140	32.6	405	29.6	339	31.0	
Northern (>40° N)	189	44.0	638	46.6	476	43.6	
BMI (kg/m^2), mean (SD)	30.5	(6.4)	29.1	(5.6)	27.4	(5.5)	<.001
Total recreational physical activity (MET-h/wk), mean (SD)	8.2	(10.9)	9.4	(11.5)	13.7	(14.8)	<.001
Smoking							.02§
Never	236	54.9	748	54.6	541	49.5	
Past	151	35.1	522	38.1	480	44.0	
Current	43	10.0	99	7.2	71	6.5	
Alcohol (drinks/wk)	1.6	(3.6)	2.0	(4.1)	2.5	(4.4)	<.001
0	217	50.5	577	42.1	414	37.9	
>0-<7	179	41.6	653	47.7	542	49.6	
≥7	34	7.9	139	10.2	136	12.5	
Medical history							
Hypertension	237	55.1	613	44.8	405	37.1	<.001
Treated diabetes	34	7.9	90	6.6	44	4.0	<.001
Treated hyperlipidemia	61	14.2	157	11.5	113	10.3	.05
Statin use	42	9.8	118	8.6	93	8.5	.55
Coronary heart disease	18	4.2	34	2.5	18	1.6	.008
Myocardial infarction	8	1.9	26	1.9	15	1.4	.34
CABG/PTCA	12	2.8	18	1.3	8	0.7	.005
Stroke	5	1.2	26	1.9	10	0.9	.22
Peripheral artery disease	11	2.6	20	1.5	12	1.1	.07
Heart failure	3	0.7	5	0.4	5	0.5	.78
Any personal calcium supplement use	142	33.0	727	53.1	729	66.8	<.001
Any personal vitamin D supplement Use	106	24.7	643	47.0	675	61.8	<.001
Total vitamin D intake (IU/d), mean (SD)	234.2	(198.7)	369.7	(266.6)	454.6	(285.3)	<.001
<200	262	60.9	488	35.6	282	25.8	
200-<400	74	17.2	286	20.9	200	18.3	
400-<600	67	15.6	355	25.9	287	26.3	
≥600	27	6.3	240	17.5	323	29.6	
Hormone therapy trial arm							.21‖
Not randomized	201	46.7	708	51.7	589	53.9	
Active	104	24.2	314	22.9	252	23.1	
Placebo	125	29.1	347	25.3	251	23.0	
Dietary modification trial arm							.62‖
Not randomized	150	34.9	450	32.9	351	32.1	
Intervention	113	26.3	338	24.7	277	25.4	
Comparison	167	38.8	581	42.4	464	42.5	
CaD trial assignment							.58
Intervention	219	50.9	662	48.4	555	50.8	
Placebo	211	49.1	707	51.6	537	49.2	

* Race/ethnicity, education, and region by latitude, as well as variables missing at year 1 for individual participants, were defined at WHI baseline.
† *P* value for linear trend across quartiles from either a linear (continuous, ordinal variables) or logistic (dichotomous variables) regression model with the variable of interest as a function of trend over vitamin D category medians.
‡ *P* value compares white versus nonwhite participants.
§ *P* value compares current versus never/past smokers.
‖ *P* value compares active versus nonactive intervention.

Figure 3



Cumulative incidence of atrial fibrillation by serum 25(OH)D level (ng/mL).Serum 25(OH)D level is measured in ng/mL.Event totals are inverse probability weighted to the full CaD trial sample.

weighted this subset to the full CaD trial and observed no effect of assignment to CaD on risk of AF even after adjustment for baseline serum 25(OH)D levels (HR 0.96 for CaD vs placebo, 95% CI 0.71-1.28) (Table II).

In a sensitivity analysis requiring 1 inpatient or 2 outpatient AF diagnoses within 1 year, we captured fewer events (1,022 vs 1,453) but found that there was still no association between CaD randomized arm and incident AF (unweighted HR 0.99 for CaD vs placebo among all included participants, 95% CI 0.88-1.12). Similarly, our results remained unchanged when we conducted an as-treated analysis of the intervention's effect (unweighted HR 1.01 for CaD vs placebo among all participants, 95% CI 0.88-1.17) and also separately when we accounted for the competing risk of death in the development of incident AF (unweighted HR 1.02 for CaD vs placebo among all participants, 95% CI 0.92-1.13). Finally, in an additional sensitivity analysis, we followed all included participants beyond the end of the trial and still found no significant effect of the intervention over an average of 8.6 years (unweighted HR 1.03 for CaD vs placebo, 95% CI 0.96-1.09).

Subgroup analyses

We examined whether an effect of CaD supplementation on risk of AF during the trial was present in any specified subgroups despite showing no benefit overall (Table III). When stratified by age or race, we found no significant association in these subgroups. We then tested whether CaD supplementation might show benefit for those with vitamin D deficiency or those who may be at greater risk for vitamin D deficiency. We found no statistically significant effect across subgroups of baseline 25(OH)D or in any subgroups defined by geographic latitude. We assessed whether there was any benefit in those with hypertension or diabetes, as these are AF risk factors associated with vitamin D deficiency,[5,6] and no significant association was found. Because participants were allowed to continue personal supplementation of vitamin D in addition to their assigned trial intervention, we examined whether background supplementation attenuated any possible treatment effect. However, even among patients with no background vitamin D supplement use, there was no effect of treatment assignment on risk of AF (HR 0.97, 95% CI 0.83-1.13) (Table III). There was also no difference in effect based on whether or not background personal calcium supplementation was used. For all subgroup analyses, we tested interaction between trial assignment and the subgroup variable of interest and found no significant interaction for any of these models (Table III).

Table V. Atrial fibrillation as a function of serum 25(OH)D level (ng/mL)

Outcome	<10 (n = 430)	10-<20 (n = 1369)	≥20 (n = 1092)	*P* value†
Atrial fibrillation				
Observed events	91	301	234	
Weighted events*	804	2992	2379	
Weighted events per 1000 person-years*	20.8	22.9	22.0	
Model 1, HR (95% CI)	0.96 (0.71-1.29)	1.02 (0.83-1.25)	1.00 (ref)	.93
Model 2, HR (95% CI)	0.96 (0.71-1.30)	0.99 (0.80-1.22)	1.00 (ref)	.82
Model 3, HR (95% CI)	0.95 (0.70-1.30)	0.96 (0.78-1.19)	1.00 (ref)	.68
Model 4, HR (95% CI)	0.89 (0.65-1.22)	0.94 (0.76-1.17)	1.00 (ref)	.45
Model 5, HR (95% CI)	0.92 (0.66-1.28)	0.96 (0.77-1.19)	1.00 (ref)	.59

All models are stratified by intervention assignment for the serum study source and the WHI Hormone, Dietary Modification, and Calcium / Vitamin D trial assignments.
Model 1: unadjusted.
Model 2: age.
Model 3: model 1 + race/ethnicity, education, BMI, physical activity, smoking, and alcohol.
Model 4: model 2 + hypertension, diabetes, coronary heart disease, and peripheral arterial disease.
Model 5: model 3 + vitamin D intake.
* Event totals and annualized rates are inverse probability weighted to the full CaD trial sample.
† *P* values from a separate model with atrial fibrillation as a function of linear trend across quartiles of serum 25(OH)D.

Baseline 25(OH)D levels and long-term incident AF

Finally, similar to existing observational studies, we tested whether baseline vitamin D status was prognostic of incident AF on long-term follow-up. For this, we examined those with baseline serum 25(OH)D measurements in both treatment and placebo arms and followed these participants beyond the end of the trial. Among other statistically significant differences at baseline, participants with deficient levels of 25(OH)D were more likely to be African American or Hispanic ($P<.001$), had a higher BMI ($P<.001$), had more medical comorbidities, and had lower total vitamin D intake ($P<.001$) (Table IV). The average long-term follow-up until incident AF or censorship for this subset of participants was 8.3 years. During long-term follow-up for this subset, there were 626 participants (21.7%) who developed incident AF at an average rate of 26.1 events per 1,000 person-years. Figure 3 displays the cumulative incidence of AF for categories of baseline serum 25(OH)D weighted to the full trial population, and there is no clear dose-response effect. After adjusting for age, race, education, BMI, physical activity, smoking, alcohol, hypertension, diabetes, coronary heart disease and peripheral artery disease, and baseline vitamin D intake, there was no significant association between baseline 25(OH)D serum levels and incident AF for the lowest 25(OH)D category versus the highest category (HR 0.92, 95% CI 0.66-1.28) (Table V). There was also no significant interaction between baseline serum 25(OH)D and race (white vs nonwhite, $P=.12$ by linear trend across categories of 25[OH]D).

Discussion

The interest in the relationship between vitamin D status and cardiovascular disease has been driven by numerous studies. Activated vitamin D has been shown to reduce levels of inflammatory cytokines,[7,22] suppress the renin-angiotensin-aldosterone system,[23] promote insulin sensitivity,[24] and exert antihypertrophic effects on myocardial tissue.[8] These findings provide a plausible basis for effects of vitamin D status on cardiovascular health, and a number of studies have associated vitamin D deficiency with hypertension,[5] diabetes,[6] heart failure,[25,26] and myocardial infarction.[27] Our study similarly found that participants with the lowest serum 25(OH)D levels at baseline had higher rates of hypertension, diabetes, and coronary artery disease.

Regarding the association between vitamin D status and AF, several case-control studies have shown an increased risk of AF in patients with vitamin D deficiency.[9-11] These studies however are limited by their small size and inability to establish a temporal relationship between 25 (OH)D level and onset of new AF. An additional limitation in the study by Demir et al[11] is that their predictive models were not adjusted for comorbid conditions that may confound the association. In contrast to these studies, large cohort studies on this topic have shown that baseline serum 25(OH)D does not predict risk of AF,[12-14] and our findings support these conclusions as well. The largest cohort study to date is by Alonso et al on 12,303 participants of the Atherosclerosis Risk in Communities study.[14] The authors reported an association between vitamin D deficiency and increased risk of AF when adjusted by age, sex, and race, but this was no longer significant after full adjustment for several clinical covariates. Interestingly, they did find a significant interaction between vitamin D deficiency and age, where younger patients (<58 years old) with the vitamin deficiency had a higher risk of AF. Because the ascertainment of outcomes in our study relies mainly on entry into Medicare at age 65 years, we were unable to validate effects of CaD on incident AF in a younger cohort.

To our knowledge, we present the first analysis of a randomized trial of daily vitamin D supplementation using risk of AF as the outcome. Although previous studies have aimed to determine whether low levels of 25(OH)D lead to AF, we directly tested the causal effect of a relevant intervention without the inherent limitations of observational studies. Our results show that vitamin D in combination with calcium administration does not affect rates of AF in this population. We also found no significant effect across subgroups defined by baseline 25(OH)D, but our estimate of the effect in those with severe vitamin D deficiency [25(OH)D < 10 ng/mL] was imprecise because of a small sample size in this subgroup.

One limitation is that we cannot distinguish the independent effect of vitamin D supplementation from the effect of calcium supplementation in this study. Increased risk of cardiovascular events, particularly myocardial infarction, has been reported with calcium supplementation (with or without vitamin D) of ≥500 mg/d.[28-31] It is possible that calcium increases the risk of AF, as it may for other cardiovascular events, and that this detriment may counterbalance any potential benefit of vitamin D. However, the original WHI CaD trial[16] was intended to study fracture prevention, and calcium in combination with vitamin D is known to increase bone mineral density; therefore, both supplements were used in combination.[32] Another limitation of our study is regarding its generalizability. Because our study participants are an older population of all women, we may not be able to extrapolate these results to other demographic groups. A minor additional limitation is that the assay used for 25(OH)D measurement in this study is not considered to be the current gold standard, but we attempted to minimize measurement misclassification bias by adjusting for the laboratory sources.

It is also important to note that the doses of vitamin D3 assigned by this trial (400 IU/d) are well below what would be recommended for repletion of vitamin deficiency, which may partly explain the nonsignificant treatment effects obseved.[33] However, other studies suggest that there may simply be little to no cardioprotective effect of vitamin D even at high-dose supplementation. In 1 study, a single dose of 50,000 IU of vitamin D reduced the risk of postoperative AF after coronary artery bypass graft surgery in a subgroup of patients with vitamin D deficiency, but there was no significant effect overall.[34] In the Vitamin D Assessment Study, there was no association of high-dose monthly vitamin D supplementation with the secondary outcome of risk of arrhythmias, but the authors did not examine AF specifically, and the arrhythmia outcome was not tested for interaction with vitamin deficiency.[35] In the Vitamin D and Omega-3 Trial, participants were randomized to 2,000 IU/d of vitamin D3, with or without marine n-3 fatty acids.[15] Although the Vitamin D and Omega-3 Trial did not have any arrhythmia outcome, their dosing frequency was identical to ours, and they found no reduction in incidence of cardiovascular events even among the subgroup of participants with deficient levels of serum 25(OH)D.

In summary, CaD supplementation did not affect the overall incidence of AF in this study, and similarly, there were no statistically significant effects seen in the subgroups. Furthermore, although vitamin D deficiency was associated with baseline cardiovascular risk factors, baseline 25(OH)D serum level did not predict long-term risk of AF. There currently is insufficient evidence to support use of vitamin D supplementation for the prevention of AF.

Conflict of interest

There are no conflicts of interest.

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