Effect of 5 y of calcium plus vitamin D supplementation on change in circulating lipids: results from the Women's Health Initiative[1–4]

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ABSTRACT

Background: Dietary calcium and vitamin D intakes may be inversely associated with cardiovascular disease (CVD) risk, possibly because of their potential beneficial effects on circulating lipids. Clinical trials that have evaluated the effect of calcium supplementation on lipids are limited by a short follow-up, and data on vitamin D are scarce.

Objective: The objective was to evaluate the effect of a longer-term effect (over 5 y) of calcium and vitamin D (CaD) supplementation on changes in the concentrations of several lipids: LDL, HDL, non-HDL, total cholesterol, triglycerides, and lipoprotein(a) [Lp(a)].

Design: The study was conducted in 1259 postmenopausal women in the Calcium plus Vitamin D Trial (1 g elemental Ca as carbonate plus 400 IU vitamin D_3/d compared with placebo) of the Women's Health Initiative. Analyses were conducted by intention-to-treat. Repeated measurements on lipids during follow-up were analyzed by linear mixed-effects models.

Results: Overall, the change in lipids was relatively small [$\leq$5% except for Lp(a), which was 20–25%], and there was no significant difference in the mean change of any lipid variable between the active and placebo groups.

Conclusions: Our results indicate that CaD supplementation is not associated with lipid changes over 5 y. Existing and future CaD trials should consider evaluating this association for different doses of supplements. This study was registered at clinicaltrials.gov as NCT00000611. *Am J Clin Nutr* 2010;91:894–9.

INTRODUCTION

The role of diet in the prevention of cardiovascular disease (CVD) is well known (1). In this regard, existing research has focused mainly on macronutrients and dietary composition. Micronutrients, however, may also play a role in CVD prevention. Specifically, limited data suggest an inverse association between a relatively high dietary intake of calcium and vitamin D and risk of CVD (2–4). One potential mechanism that may explain this inverse association is an effect of these nutrients on the concentrations of circulating lipids—an established risk factor for CVD.

Several (5–11), but not all (12–15), randomized, placebo-controlled, clinical trials have suggested that supplementation with 1–2 g elemental Ca/d may lower total and LDL-cholesterol concentrations by ≈5% and increase HDL by 5%. However, all of these studies evaluated the effect of short-term ($\leq$1 y) calcium supplementation. In addition, these studies were also limited by relatively small sample sizes; the largest study thus far had only 223 participants. In terms of the effect of vitamin D supplementation on concentrations of lipids, the results from the 3 small studies conducted so far are inconsistent (16–18). Furthermore, only one study has evaluated the effect of the combined supplementation of calcium and vitamin D on the lipid profile, and it reported a null effect (15). Using data from the Women's Health Initiative (WHI), we conducted the current study to evaluate the long-term effect of calcium and vitamin D supplementation on circulating concentrations of lipid variables, including total cholesterol, LDL cholesterol, HDL cholesterol, non-HDL cholesterol, triglycerides, and lipoprotein(a) [Lp(a)].

SUBJECTS AND METHODS

The WHI consists of a set of clinical trials and a parallel observational study among ethnically diverse women from different parts of the United States, which was designed to address some of the major causes of morbidity and mortality in postmenopausal women. Details of the scientific rationale, eligibility

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requirements, and other aspects of the design of the WHI were published elsewhere (19). In brief, the WHI clinical trials (WHI-CT) were designed to evaluate the benefits and risks of dietary modification (DM), hormone therapy (HT), and supplementation with calcium and vitamin D (CaD) (19). Overall, a total of 68,132 women aged 50–79 y were randomly assigned into the DM and HT trials. At the first or second annual visits, eligible participants from these trials were invited to be randomly assigned further to CaD supplementation (n = 18,176) or placebo (n = 18,106) (20, 21). Women were eligible to participate in the CaD trial if they *1*) were postmenopausal volunteers of any race or ethnicity, *2*) were aged 50–79 y inclusive at the first screening, *3*) were likely to reside in the study area for ≥3 y after randomization, and *4*) provided written informed consent. The exclusion criteria for the CaD component included current daily use of ≥600 IU supplemental vitamin D or calcitriol, inadequate adherence to the DM component during the first or second year of follow-up, a history of renal calculi, hypercalcemia, current use of oral corticosteroids for >6 mo, or a previous osteoporosis-related fracture being treated with CaD. Women in the CaD trial were randomly assigned to take either 2 tablets for a total of 1 g elemental Ca (as calcium carbonate) and 400 IU vitamin D_3/d or 2 identical-looking placebo tablets.

The current analysis was conducted within the 6% subsample of the WHI-CT (n = 4571) women with repeated blood sample collections at baseline and years 1, 3, and 6 y and in whom a number of core analytes, including lipids, were measured at each time point. Of these women, 2478 participated in the CaD trial, of whom we excluded 1259 women who were in the active treatment arms of either the DM or the HT trial. We chose to exclude women in the active arms of the other trials to eliminate any potential bias due to the effect of the other interventions on lipid concentrations. The final sample size for the analysis was 1191 women (**Figure 1**). Because the CaD trial was initiated after 1 y of the other WHI-CT trials, the year 1 follow-up of the WHI-CT women was considered as the baseline for this analysis, and repeated measurements of lipids were available at years 2 and 5. Lipid measures were obtained from frozen stored (at −70°C) samples and assayed at Medical Research Laboratories Inc (Highland Heights, KY). The Friedewald formula was used to calculate the concentration of LDL cholesterol from the concentrations of total cholesterol, HDL cholesterol, and triglycerides for those women who had a triglyceride value <400 mg/dL.

The active and placebo groups were compared in terms of baseline (year 1 of the WHI-CT was baseline for the CaD trial) characteristics, including sociodemographic data (eg, age and race), anthropometric variables (BMI and waist circumference), and lifestyle factors (eg, smoking and physical activity) All analyses were conducted by using the intention-to-treat approach, ie, data from each study participant were analyzed as per her initial assignment to the active or the placebo arm.

We calculated mean changes in the concentration of each biomarker from baseline to a specified follow-up time (year 2 or 5) within the active and placebo arms. We also calculated the difference in mean change between the active and placebo groups at years 2 and 5 separately. Furthermore, using data on lipid biomarkers from both the follow-up visits together, we conducted an analysis with a linear mixed-effects model to evaluate change in lipid biomarker concentrations over time from baseline with a random intercept to account for correlations between repeated observations for the same woman. Time since randomization was included in the model as a categorical variable (year 2 compared with year 5). An interaction term between time since randomization and intervention group was also included to allow the effect of intervention to be different at year 2 and year 5. A Wald's test was used to assess whether the intervention effect was statistically different from the placebo. The baseline concentration of each lipid biomarker was also included as a covariate to account for the potential effect due to "regression to the mean" (22).

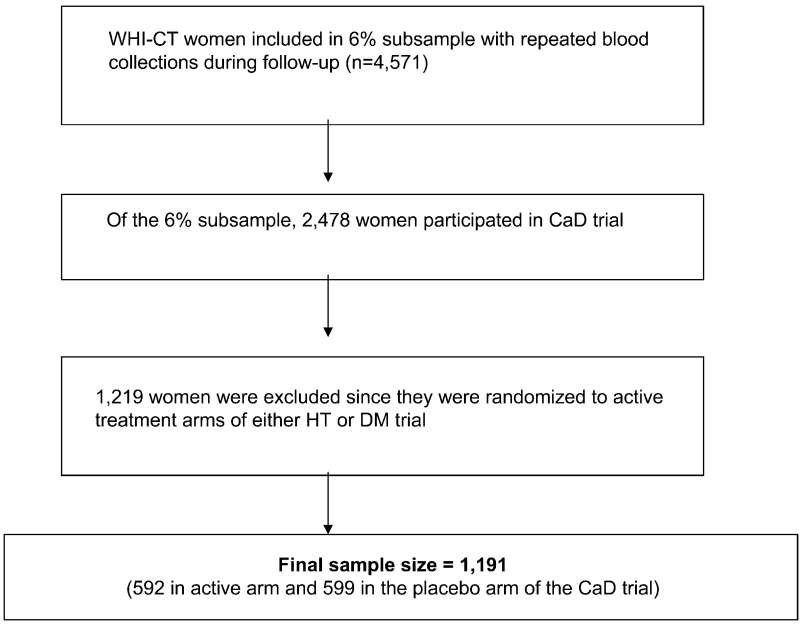

FIGURE 1. Study population and sample size of the current study. WHI-CT, Women's Health Initiative clinical trials; DM, dietary modification; HT, hormone therapy, CaD, Calcium plus Vitamin D.

Because weight change may contribute to the changes in lipids, we also evaluated the effect of additional adjustment for the corresponding change in weight between baseline and follow-up time. In a sensitivity analysis, we evaluated the effect of adjustment of average compliance over the follow-up as a covariate. We used likelihood-ratio tests to test for interactions. All statistical analyses were conducted by using SAS software (SAS Institute, Cary, NC), and 2-sided P values ≤ 0.05 were considered statistically significant. Missing data were handled by the complete case method by using observations without any missing data (23).

RESULTS

The baseline characteristics of the study participants, by intervention group, are shown in **Table 1**. The treatment and placebo groups were similar with respect to their sociodemographic characteristics, lifestyle factors, anthropometric variables, medical history, and biochemical variables at baseline. The mean lipid values at baseline and at the end of years 2 and 5 and the associated mean changes by intervention group are shown in **Table 2**. Overall, the change in all the lipid factors was relatively small; for example, the mean decrease in LDL-cholesterol concentrations was 6.5 ± 33.0 mg/dL (−5.0%) in the intervention group and 6.3 ± 35.2 mg/dL (−4.7%) in the placebo group at the end of 5 y of the intervention. Similarly, the mean changes in HDL-cholesterol and triglyceride concentrations corresponded to a change of <5% in both trial arms at the end of the study. In general, compared with the change at the end of 2 y, the mean change at 5 y was of a larger magnitude and in the same direction. However, the concentrations of Lp(a) decreased at the end of 2 y (3.5% in the active arm and 7.9% in the placebo arm) but increased by 25.8% in the active arm and 17.3% in the placebo arm at the end of 5 y. Importantly, the differences in mean change between the active and placebo groups were not statistically significant at the end of year 2 or year 5 for any of the lipid variables. The lipid estimates (β coefficient) from the linear mixed-effects model with repeated measurements are shown in **Table 3**. These estimates represent the mean change in lipid variables between active and placebo groups at year 2 or year 5 after control for the baseline concentration of the lipid variable. We found no statistically significant differences in the changes of any lipid variables. Furthermore, the intervention effects did not vary over time (ie, the interaction was not significant). In addition, the adjustment for weight change during follow-up did not change the results.

The results for changes in lipids at the end of the study differed little in the sensitivity, analysis which excluded women who reported current use of postmenopausal hormones, women who were <60% compliant with the study medications, or women who self-administered calcium or vitamin D supplements. However, the statistical power for these analyses was limited.

TABLE 1
Baseline characteristics of the study population in the Calcium Plus Vitamin D Trial, by intervention group[1]

Characteristics	Active arm	Placebo arm	P value[2]
No. of subjects	592	599	
Age (y)	61.6 ± 6.8[3]	62.1 ± 7.1	0.27
Race [*n* (%)]			0.62
White	290 (49)	302 (50)	
Other	302 (51)	297 (50)	
Smoking status [*n* (%)]			0.25
Never smoked	298 (51)	329 (56)	
Past smoker	232 (40)	211 (36)	
Current smoker	53 (9)	48 (8)	
Missing	9 (1.5)	11 (1.8)	
Hormone use status [*n* (%)]			0.40
Never used	316 (53)	342 (57)	
Past user	110 (19)	107 (18)	
Current user	165 (28)	149 (25)	
BMI (kg/m^2)	29.7 ± 6.4	29.3 ± 6.1	0.83
Waist (cm)	89.3 ± 14.0	88.8 ± 13.8	0.64
Total METs per week	9.8 ± 11.9	10.4 ± 11.9	0.41
Hypertension [*n* (%)]	214 (36)	205 ± 35	0.55
Diabetes [*n* (%)]	46 (8)	33 ± 6	0.12
Glucose (mg/dL)	103.6 ± 32.4	103.5 ± 32.9	0.65
Insulin (μIU/mL)	12.7 ± 8.1	12.1 ± 7.7	0.60
Alcohol intake (g/d)	2.9 ± 6.9	3.6 ± 9.2	0.29
Total fat intake (% of energy)	35.7 ± 7.0	35.9 ± 6.6	0.85
Dietary calcium intake (mg)	707.7 ± 403.2	734.8 ± 424.4	0.29
Calcium supplement use [*n* (%)]	90 (15)	74 (13)	0.17
Vitamin D use [*n* (%)]	26 (4)	21 (4)	0.45

[1] METs, metabolic equivalent tasks.
[2] Chi-square test was used for categorical variables, and Kruskal-Wallis test was used for continuous variables.
[3] Mean ± SD (all such values).

DISCUSSION

To our knowledge, this was the first randomized controlled trial to evaluate the effect of CaD supplementation on lipid concentrations over a 5-y period. Our results indicate that CaD supplementation is not associated with a significant change in important lipid variables between postmenopausal women.

Only a few studies of the effects of dietary calcium and vitamin D on the risk of CVD in humans have been conducted. Although some of these studies have suggested an inverse association of calcium and vitamin D with CVD risk, the overall results have been inconsistent (2–4, 24–26). Analysis of the CaD trial of the WHI (26), in which our current study was conducted, reported a null effect for the association between CaD and risk of developing CVD. One mechanism by which these nutrients might potentially exert a beneficial effect on CVD risk in humans is through an effect on circulating lipids (27, 28). Several animal experimental studies have suggested that calcium supplementation has beneficial effects on circulating concentrations of lipoproteins (29–32). Mechanistically, a high calcium intake may inhibit the absorption of dietary fatty acids by promoting the formation of indigestible calcium-lipid complexes in the gastrointestinal tract (8, 33). In addition, calcium supplementation may also affect circulating lipid concentrations by its effect on concentrations of parathyroid hormones and vitamin D, which also regulate adipocyte activity (34, 35). Although the exact mechanism by which vitamin D affects lipid concentrations is not known, it is possibly related to its potential benefit with respect to insulin sensitivity (36).

Bhattacharrya et al (5) conducted the first randomized clinical trial to evaluate the association between calcium intake and concentration of circulating cholesterol. In this study of 11 healthy men, which was conducted in 1969, investigators reported a 5% greater decrease in total cholesterol concentrations

TABLE 2
Means and mean changes (in mg/dL) in lipid variables in the Calcium Plus Vitamin D Trial, by intervention group

	Total cholesterol		LDL cholesterol		HDL cholesterol		Non-HDL cholesterol		Triglycerides		Lipoprotein(a)	
	n	Mean ± SE	*n*	Mean ± SE	*n*	Mean ± SE	*n*	Mean ± SE	*n*	Mean ± SE	*n*	Mean ± SE
Mean[1]												
Active arm												
Baseline	569	217.2 ± 1.6	565	130.3 ± 1.4	569	57.6 ± 0.6	569	159.6 ± 1.6	569	148.8 ± 3.8	569	25.9 ± 1.0
Year 2	507	213.4 ± 1.6	500	127.3 ± 1.5	506	56.7 ± 0.7	506	156.8 ± 1.7	507	149.8 ± 3.5	507	25.1 ± 1.1
Year 5	472	208.8 ± 1.7	466	123.5 ± 1.6	470	56.4 ± 0.6	470	152.6 ± 1.7	472	148.4 ± 3.5	472	32.5 ± 1.2
Placebo arm												
Baseline	569	222.7 ± 1.6	554	133.9 ± 1.6	569	58.6 ± 0.7	569	164.2 ± 1.7	569	151.4 ± 3.6	569	27.7 ± 1.2
Year 2	513	218.5 ± 1.7	505	131.6 ± 1.6	510	57.6 ± 0.7	510	161.0 ± 1.7	513	148.4 ± 3.2	513	26.1 ± 1.1
Year 5	477	213.1 ± 1.8	473	127.1 ± 1.6	477	57.4 ± 0.7	477	155.7 ± 1.8	477	145.8 ± 3.5	477	31.8 ± 1.2
Mean change[2]												
Active arm												
Year 2: baseline	488	−2.6 ± 1.3	479	−1.8 ± 1.1	487	−1.2 ± 0.4	487	−1.4 ± 1.3	488	1.5 ± 3.3	488	−0.9 ± 0.6
Year 5: baseline	458	−8.5 ± 1.7	450	−6.5 ± 1.6	456	−1.4 ± 0.4	456	−6.8 ± 1.7	458	−2.4 ± 3.5	458	6.7 ± 0.7
Placebo arm												
Year 2: baseline	494	−3.4 ± 1.3	478	−1.3 ± 1.3	491	−1.4 ± 0.4	491	−2.1 ± 1.3	494	−3.7 ± 2.5	494	−2.2 ± 0.7
Year 5: baseline	458	−8.7 ± 1.8	442	−6.3 ± 1.7	458	−1.6 ± 0.4	458	−7.1 ± 1.8	458	−3.2 ± 3.7	458	4.8 ± 0.7
Difference in mean change between the 2 arms[3]												
Year 2	982	0.8 ± 1.9	987	−0.5 ± 1.7	978	0.2 ± 0.5	978	0.7 ± 1.9	982	5.2 ± 4.1	938	1.3 ± 0.9
Year 5	916	0.2 ± 2.4	892	−0.2 ± 2.3	914	0.1 ± 0.6	914	0.3 ± 2.5	916	0.8 ± 5.1	889	1.8 ± 1.0

[1] Means at year 2 or year 5 were not significantly different ($P > 0.05$) from baseline, and no significant difference between treatment arms was observed for the lipids.
[2] Mean change at year 2 and year 5 was not different from zero, and no significant difference between treatment arms was observed.
[3] Difference in mean change was calculated as the mean change in the active arm minus the mean change in the placebo arm.

among those supplemented with 2 g/d of calcium carbonate or gluconate for 2 wk compared with those in the placebo group. However, lipid subfractions were not evaluated. Several randomized clinical trials conducted after this initial study, however, reported inconsistent results (7, 8, 10, 12–15, 37–39). The sample size across these clinical trials ranged from 13 to >200 participants, and follow-up time ranged from 4 wk to 1 y. Of the 10 placebo-controlled trials conducted so far, 4 found no significant change in serum cholesterol concentrations in the calcium supplementation arm compared with placebo (12–14, 37). The largest and the longest trial that evaluated this association was conducted in New Zealand, among 223 healthy postmenopausal women, in a study primarily designed to investigate the effect on bone fractures. The results from this study indicated that women who were randomly assigned to receive 1 g elemental Ca (as calcium citrate) per day had a significantly greater decrease in LDL (6%) and increase in HDL (7%) compared with the placebo group at the end of 1 y (38). The change in triglyceride concentrations, however, was similar in the 2 intervention groups.

Women randomly assigned to the active arm of the CaD trial in the WHI also received 400 IU vitamin D_3/d in addition to calcium carbonate. Only a few studies have evaluated the effects of vitamin D supplementation on concentrations of circulating lipids. In a Finnish randomized trial of 464 postmenopausal women, supplementation with 300 IU vitamin D_3/d had detrimental effects on the lipid profile, resulting in an increase in LDL-cholesterol and a decrease in HDL-cholesterol concentrations after 3 y of follow-up (16). However, a few other studies found no significant change in lipids in the intervention arm compared with placebo (17, 18), which may possibly be due to a short follow-up time (range: 6 wk to 1 y). Only one small clinical trial has previously evaluated the effect of combined supplementation with calcium and vitamin D, as in our current study. In this clinical trial of 39 healthy postmenopausal women, supplementation with 1 g elemental Ca as citrate along with 800 IU vitamin D_3 was not associated with any significant difference in lipid concentrations compared with placebo after a 3-mo period (15), which is consistent with our results.

The strengths of the present study included its randomized design, large sample size, long follow-up time, and availability of repeated lipid measurements. A few limitations of this study, however, also warrant consideration. The study intervention

TABLE 3
Difference in mean changes (in mg/dL) in lipid variables between the active and placebo arms of the Calcium Plus Vitamin D Trial[1]

	Year 2	Year 5
Total cholesterol	−1.31 ± 1.90	−1.67 ± 1.96
LDL	−1.68 ± 1.77	−1.51 ± 1.82
HDL	−0.01 ± 0.52	−0.05 ± 0.54
Non-HDL cholesterol	−1.15 ± 1.88	−1.30 ± 1.93
Triglyceride	3.69 ± 3.65	1.43 ± 3.77
Lipoprotein(a)	0.76 ± 0.90	1.79 ± 0.92

[1] All values are βs ± SEs. The β coefficient represents the mean change in lipid variables between the active and placebo arms at year 2 or year 5 after control for baseline lipid concentrations.

included combined supplementation with calcium and vitamin D, so that we were not able to evaluate the effect of calcium and vitamin D supplementation alone. Furthermore, given that, in contrast with calcium, vitamin D supplementation may result in an increase in LDL-cholesterol and a decrease in HDL-cholesterol concentrations (16), the null association observed in this study may have resulted from the potentially opposing effects of calcium and vitamin D on lipids. In addition, we cannot rule out the possibility that an effect of calcium or vitamin D on lipids may occur at doses higher than that used in the present study. Finally, compliance with treatment may have been an additional limitation. During year 1, 60% of the participants took $\geq$80% of their study medication, and this percentage remained stable during follow-up, with small differences between groups. In addition, $\geq$70% took $\geq$50% of their study medication. An additional limitation of the study was the use of over-the-counter prescriptions of calcium and vitamin D supplements. However, our results were similar to those of a sensitivity analysis that excluded noncompliers and those who self-administered calcium and vitamin D supplements.

In conclusion, this large clinical trial, the first study to evaluate the association of calcium plus vitamin D supplementation on changes in circulating lipids over 5 y, found no significant effects of CaD supplementation on changes in lipid concentrations. Existing and/or future trials of calcium and vitamin D with adequate sample size, long-term follow-up, and repeated blood collections should also consider evaluating the effects of different doses of calcium and vitamin D supplements, both separately and together, on circulating lipid concentrations.

The authors' responsibilities were as follows—SNR and TER: study concept and design; and SNR, XX, SW-S, and TER: data analysis. All authors contributed to the writing and editing of the manuscript. The authors thank Dan Wang for her assistance with the data analysis. No conflicts of interest were reported.

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