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Effects of Calcium and Vitamin D Supplementation on Blood Pressure and Serum Lipids and Carotenoids: A Randomized, Double-blind, Placebo-controlled, Clinical Trial

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Abstract

Purpose—To estimate the effects of calcium or/and vitamin D supplementation on blood pressure and serum lipid and carotenoid levels.

Methods—92 colorectal adenoma patients were randomized in a pilot, double-blind, placebo-controlled clinical trial of supplemental vitamin D₃ 800 IU and elemental calcium 2.0 g (as calcium carbonate) alone or in combination in divided doses twice daily with meals over six months.

Results—Relative to placebo, mean serum triglycerides decreased 30% ($P=0.10$) and 32% ($P=0.10$) in the calcium and calcium plus vitamin D₃ treatment groups, respectively. When the two calcium intervention groups were pooled and compared to the pooled non-calcium groups, the estimated supplemental calcium treatment effects were statistically significant for triglycerides ($P=0.04$). Similar, but non-statistically significant decreases (5–7%) were observed for serum total cholesterol levels. Mean systolic blood pressure increased 6% ($P=0.08$) in the calcium group; otherwise, there were no appreciable changes in systolic or diastolic blood pressures in any active treatment group. Mean serum total carotenoid levels decreased 14% ($P=0.07$) in the calcium and 9% ($P=0.10$) in the calcium plus vitamin D₃ groups.

Conclusions—Our results suggest that supplemental calcium alone or combined with vitamin D₃ but not vitamin D₃ alone may reduce serum lipids and lipophilic micronutrients.

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Keywords

Calcium; vitamin D; lipids; blood pressure; carotenoids

INTRODUCTION

Hyperlipidemia and hypertension are considered causal risk factors for cardiovascular disease (CVD) [1–3], a leading cause of death in the United States [3, 4]. It is suggested that calcium may have a beneficial effect on CVD risk through its influences on serum lipid concentrations [5, 6] and blood pressure [7]. However, there is also evidence that calcium supplementation or high intakes of calcium may increase risk for myocardial infarction [8] and mortality [9] despite possible beneficial effects on serum lipid levels. Several [5, 10–17], but not all [18–21], clinical trials of calcium and serum lipids reported that supplemental calcium lowered serum total and LDL-cholesterol and increased HDL-cholesterol levels. Findings from human studies of calcium and circulating triglycerides have been inconsistent, with most studies finding no beneficial effects of calcium supplementation [5, 10, 11, 13, 16–18, 20, 21]. In addition, calcium treatment was found to reduce blood pressure in rats [22], and a previous meta-analysis of randomized, placebo-controlled clinical trials suggested that calcium supplementation may lead to a small reduction in systolic blood pressure in humans [23, 24].

Vitamin D is closely associated with calcium metabolism [25] and may also affect lipid concentrations due to its effects on insulin sensitivity [26]. Previous small-scale studies of the effects of supplemental vitamin D on circulating lipids produced inconsistent results [27–29]. Recently, in a large (n=1,259), randomized clinical trial [21], Raipahak et al. reported 5% changes in levels of circulating lipids, including cholesterol and triglycerides, after daily supplementation of 1.0 g elemental calcium (as carbonate) plus 400 IU vitamin D₃ for 5 years. However, only the combined effects of calcium and vitamin D were assessed in the above study.

Fat intake may be important with respect to uptake of carotenoids, and previous research found an inverse association of carotenoids with CVD and cancer risk [30, 31]. Due to their lipophilic nature, the bioavailability of carotenoids is partially dependent on the presence of fat in the intestine [32], therefore, reduction of available fat for transport in the gut, caused by its interaction with calcium to form calcium-lipid complexes [6, 21], may lead to decreased carotenoid absorption and correspondingly lower serum concentrations.

Using data and stored blood samples from a pilot clinical trial [33], we examined the effects of calcium or/and vitamin D₃ on blood pressure (systolic and diastolic) and serum levels of lipids (total cholesterol and triglycerides) and carotenoids.

METHODS

Participant Population and Protocol

We used data and stored serum samples from a previous pilot, randomized, double-blind, placebo-controlled, 2 × 2 factorial design, 6-month clinical trial to test the effects of calcium

and/or vitamin D on biomarkers of risk for colorectal cancer [33]. In brief, 92 participants (30 to 75 years of age) in general good health; capable of informed consent; with at least one pathology-confirmed adenomatous colorectal polyp within the past 36 months; no contraindications to calcium or vitamin D supplementation or rectal biopsy procedures; no habits, medical conditions, or medication usage that would otherwise interfere with study interpretation; and had taken at least 80% of their study pills during the 30-day placebo run-in trial, were recruited from the patient population attending the Digestive Diseases Clinic of Emory University. Individuals were eligible for the study regardless of history of hypertension, hypercholesterolemia, or the use of anti-hypertensive or cholesterol-lowering medications. The Institutional Review Boards of Emory University and the University of Hawaii approved the current study protocol.

At baseline, eligible participants were randomly assigned to the following four treatment groups: a placebo control group (placebo), a 2.0 g elemental calcium (1.0 g twice daily as calcium carbonate) group (calcium), an 800 IU vitamin D₃ (400 IU twice daily) group (vitamin D₃), and a 2.0 g elemental calcium plus 800 IU vitamin D₃ group (calcium plus vitamin D₃). The corresponding supplement and placebo pills were identical in size, appearance, and taste. The placebo was free of calcium, magnesium, vitamin D, and chelating agents. All study pills were taken twice daily with meals. The treatment period was six months, and participants attended follow-up visits at two and six months after randomization and were contacted by telephone between the second and final follow-up visits. Pill-taking adherence was assessed by interview, questionnaire, and pill count. Participants were instructed to maintain their usual diet and not take any nutritional supplements (except for those provided by the study) upon study enrollment. Blood was collected at baseline (randomization) and at study completion (6-month follow-up) and diet was assessed with a semi-quantitative food frequency questionnaire [34]. Participants were not required to fast for their visits.

Protocol for Measuring Blood Pressure

Blood pressures were measured at baseline and 6 months of follow-up by certified technicians following a standard protocol before blood draws or any other procedures. Briefly, arm circumference was measured, the appropriate size cuff was chosen, and then the participant remained quietly seated with both feet on the floor for 5 minutes. The correct size cuff was wrapped on the arm with the center of the bladder over the brachial artery. The time and then the pulse at the radial artery for 30 seconds were recorded. Using a calibrated standard aneroid sphygmomanometer, the cuff was then inflated to determine the pressure at which the radial pulse was obliterated, then deflated. The peak inflation level was determined as the pulse obliteration pressure plus 30 mm Hg. After the participant raised his or her arm for 5 seconds followed by a 30-second rest, the cuff was rapidly inflated to the peak inflation level, held constant for 5 seconds with the bell of the stethoscope placed on the brachial artery, then the cuff was slowly deflated at 2 mm Hg per second and the first and the fifth blood pressure phases were recorded. The cuff was completely deflated, then, after the participant raised his or her arm for five seconds and after another 30 seconds at rest, the blood pressure measurement was repeated. The average of the two recordings was taken as the blood pressure of the person on that visit.

Sample Analyses

Serum levels of total cholesterol and triglycerides were measured based on a latex particle enhanced immunoturbidimetric method using a Cobas MiraPlus clinical autoanalyser (Roche Diagnostics, Ltd., Rotkreutz, Switzerland) and an assay kit from Point Scientific, Inc. (Lincoln Park, MI).

Serum carotenoid (α -carotene, β -carotene, α -cryptoxanthin, β -cryptoxanthin, lutein/zeaxanthin, lycopene, and total carotenoids) levels were analyzed by HPLC with pre-column electrochemical oxidation and post-column UV detection, as previously described in detail [35]. Assays were regularly validated for carotenoids through inclusion of external standards in each analysis batch and by participation in quality assurance programs organized by the US National Institute of Standards and Technology (NIST, Gaithersburg, MD) every year since 1994 [36].

Statistical Analysis

Treatment groups (placebo, calcium, vitamin D₃, calcium plus D₃) were assessed for comparability of characteristics at baseline and at final follow-up by ANOVA for continuous variables and Fisher's exact test for categorical variables.

Primary analyses were based on assigned treatment at the time of randomization regardless of adherence status (intent-to-treat analysis). Outcome variable means were calculated for each treatment group for the baseline and six-month follow-up visits. A repeated-measures linear mixed-effects model was used to evaluate the absolute treatment effects by assessing the differences in the outcome variables from baseline to the 6-month follow-up visit between participants in each active treatment group and the placebo group. The model included the intercept, time effect (baseline and 6-month follow-up), and interactions between treatment groups and time (the absolute treatment effect). The model for total cholesterol also included a treatment group * cholesterol-lowering medication use (yes/no) interaction term. Analyses for total cholesterol were also stratified on cholesterol-lowering medication use.

Since the treatment groups were balanced on all other measured potentially influential factors at baseline, no adjustment was made for these covariates in the primary intent-to-treat analyses. However, the analyses for blood pressure were repeated adjusting for a history of having hypertension (yes/no) and taking anti-hypertension medication (yes/no) and there were no material changes in the results. To provide perspective on the magnitude of the treatment effects, we also calculated proportional treatment effects, defined as: absolute treatment effect/treatment group baseline $\times$ 100% (e.g., a proportional effect of -30% would mean an estimated 30% decrease in the active treatment group relative to the placebo group). SAS software (SAS Institute, Cary, NC) was used for analyses. All tests were 2-sided, and $P < 0.05$ was considered statistically significant.

RESULTS

The four treatment groups did not differ significantly on participant characteristics and dietary intakes measured at baseline (Table 1) or at the end of the study (data not shown)

except for the higher proportion of individuals taking cholesterol-lowering medications in the calcium plus vitamin D₃ intervention group compared to the placebo group. The mean age of participants was 60.5 ± 7.9 (SD) years and 64% of participants were men, 71% were white, 12% were current smokers, 82% were overweight or obese, 48% had history of hypertension, and 45% had a history of hypercholesterolemia.

On average, at least 80% of pills were taken by 93% of participants at the first follow-up visit and 84% at the final follow up visit. No treatment related complications were observed. Seven people (8%) were lost to follow-up due to perceived drug intolerance (n=2), unwillingness to continue participation (n=3), physician's advice (n=1), or death (n=1). Dropouts included one participant from the vitamin D₃ intervention group and two from each of the other three treatment groups. The average adherence to visit was 92% and did not significantly differ across the four treatment groups.

Serum total cholesterol, triglycerides, and blood pressure (systolic and diastolic) levels are summarized in Table 2. There were no substantial or statistically significant differences in these parameters among the four treatment groups at baseline. Relative to placebo, serum levels of triglycerides decreased by 30.1% (P=0.10) and 32.1% (P=0.10) in the calcium and calcium plus vitamin D₃ groups, respectively. Both calcium intervention groups also had small, non-statistically significant decreases in serum total cholesterol levels relative to placebo (calcium, 5.3%, P=0.31; calcium plus vitamin D₃, 6.8%, P=0.19). Supplemental vitamin D₃ alone did not appear to affect circulating lipid concentrations.

There was no evidence that either vitamin D or calcium treatment had any beneficial effect on systolic blood pressure; indeed, there was a non-statistically significant increase in systolic blood pressure (6.1%, P=0.08) among individuals who received calcium supplements compared to placebo. Likewise, no estimated overall treatment effects were observed for diastolic blood pressure.

Serum carotenoid levels at baseline and at six-month follow-up are summarized in Table 3. Total carotenoid levels were observed to decrease 13.5% in the calcium group (P=0.07) and 9.1% in the calcium plus vitamin D group (P=0.10) relative to the placebo group. Serum concentrations of individual carotenoids decreased approximately 2 – 17% in both of the calcium intervention groups relative to placebo, and the finding for lycopene was borderline statistically significant (–17%, P=0.08) in the calcium alone group. Serum β -carotene and lycopene decreased 15.6% (P=0.40) and 19.6% (P=0.07), respectively, in the supplemental vitamin D₃ alone group compared to placebo.

Since the estimated effects of supplemental vitamin D₃ observed in the current study were minimal, we pooled the calcium and calcium plus vitamin D₃ treatment groups (pooled calcium group) and compared them to the pooled placebo and vitamin D₃ alone treatment groups (pooled non-calcium group) (Table 4). In the pooled calcium group relative to the pooled non-calcium group, serum triglyceride levels statistically significantly decreased by 28% (P=0.04), serum total cholesterol decreased 6% (P=0.09), and total carotenoids decreased 8% (P=0.06).

Estimated treatment effects on serum total cholesterol levels stratified by cholesterol-lowering medication use are presented in Table 5. Among participants who were not taking a cholesterol-lowering medication, there was a 7.7% decrease ($P=0.09$) in cholesterol concentrations in the pooled calcium supplementation group relative to the pooled non-calcium group, whereas no changes were observed among the medication users (0.3% decrease, $P=0.96$) ($P_{\text{interaction}}=0.10$).

DISCUSSION

The results from this pilot, randomized, double-blind, placebo-controlled clinical trial suggest that daily supplementation with 2.0 g elemental calcium (as calcium carbonate) alone or in combination with 800 IU vitamin D₃ for 6 months may decrease serum triglyceride levels by approximately 30%. Although only small, non-statistically significant decreases (5 – 7%) in circulating total cholesterol levels were observed in both calcium intervention groups, the findings were stronger (–7.7%, $P=0.09$) among persons who were not taking cholesterol-lowering medications, suggesting that calcium supplementation may lower total cholesterol levels among persons who are not taking cholesterol-lowering medications, but have no effect among those already taking such drugs. It has been suggested that a high calcium intake may reduce lipid absorption by forming indigestible calcium-lipid complexes [6]. Also, calcium binds bile acids in the gut and increases their excretion, further contributing to decreased fat absorption [6, 19]. Previous animal studies also suggest that calcium supplementation may have beneficial effects on circulating lipid profiles [37–41].

A few early, small, clinical trials suggested that supplemental calcium may reduce serum cholesterol; however, these studies had major design limitations [10–14]. Subsequently, two randomized, placebo-controlled trials with larger sample sizes ($n=193$ – 223) and longer follow-up (6 – 12 months) [5, 19] reported no substantial effects of calcium on serum total cholesterol levels; however, the study by Reid et al. [5] found statistically significant increases in HDL and the HDL/LDL ratio after calcium supplementation. Similarly, no substantial changes (– 5%) were observed in a large, recent, long-term (5-year follow-up) clinical trial ($n=1,259$) that assessed the joint effect of calcium and vitamin D₃ on serum lipid levels. The 5 – 7% decreases in total cholesterol observed in our study were comparable to those found in most of the previously reported clinical trials [5, 13–15, 17, 19, 21].

Our results that suggested that calcium supplementation may moderately lower ($\approx 30\%$) serum triglyceride levels are in contrast to the majority of published clinical trials that found no beneficial effects of calcium on triglyceride levels [5, 11, 13, 16–18, 21]. This could be attributable to factors such as calcium dose, length of follow-up, study population, and chance. In the current study, a 2.0 g elemental calcium/day dose was used, which was twice that used in the two large clinical trials [5, 21] in which no substantial changes in serum triglyceride levels were observed for supplemental calcium alone or in combination with vitamin D.

One of the steps in the absorption process of carotenoids that may affect their bioavailability involves the incorporation of carotenoids into micelles and the presence of intestinal fat, which is thought to be crucial to micelle formation [32]. As stated above, calcium and lipids bind to one another in the gut, each interfering with the other's absorption [6, 21]. Thus, the possible formation of intestinal calcium-lipid complexes may explain in part the observed decreases in circulating carotenoids in both calcium intervention groups since serum lipid levels also decreased after six months of calcium supplementation. While the reduction in fat absorption may be beneficial, a reduction in carotenoid level may offset certain health benefits associated with calcium. However, the relative importance of calcium's potential lipid-and carotenoid-lowering effects is unknown, and there is other evidence suggesting that calcium supplements may increase risk of myocardial infarction [8] and mortality [9]. Therefore, caution is needed in deciding whether or not to take large daily doses of calcium supplements.

There are plausible mechanisms of action for a proposed effect of calcium on blood pressure in normal individuals as well as for individuals at high risk of developing hypertension [42–44]; however, none of these mechanisms has been clearly established. Overall, in our intention-to-treat analyses there was no support for either vitamin D or calcium treatment having any beneficial effects on systolic and diastolic blood pressures. The 6% non-statistically significant increase in systolic blood pressure observed in the calcium group may have been due to chance since we did not observe a similar finding in the calcium plus vitamin D₃ group; however, future studies are necessary to investigate this more fully.

The strengths of the current study included the randomized, double-blind, placebo-controlled trial design; high protocol adherence by the study participants; and that it was the first trial to evaluate both the independent and combined effects of calcium and vitamin D₃ on blood pressure and serum lipids and lipophilic micronutrients. Limitations included the relatively small sample size and relatively short follow-up period. In addition, we did not measure circulating HDL- and LDL-cholesterol levels. However, the findings from this pilot study are novel, biologically plausible, and have potentially important clinical and public health implications.

In conclusion, the results from the current study suggest that daily supplementation with 2.0 g of elemental calcium alone or in combination with 800 IU of vitamin D₃ for 6 months may reduce serum triglyceride and, possibly, total cholesterol levels, but that it may decrease serum carotenoid levels, and thus support further investigation in a larger trial to establish the value or risk of calcium supplementation. Our findings provide little or no support for the hypothesis that supplemental calcium or vitamin D, alone or combined, can lower blood pressure.

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Table 1

Selected baseline characteristics and dietary intakes of study participants (n = 92) *

Characteristics	Treatment Group				P [†]
	Placebo (n = 23)	Calcium (n = 23)	Vitamin D ₃ (n = 23)	Calcium + Vitamin D ₃ (n = 23)	
Demographics					
Age (y)	58.5 ± 8.2	61.9 ± 8.2	60.2 ± 8.1	62.1 ± 7.5	0.39
Men (%)	70	70	70	70	1.00
White (%)	74	83	65	61	0.39
College graduate (%)	65	61	57	44	0.53
Medical history					
History of hypertension (%)	57	35	39	61	0.21
History of hypercholesterolemia (%)	30	48	39	61	0.19
Taking anti-hypertensive Medication (%)	57	32	35	55	0.20
Taking cholesterol-lowering medication (%)	18	36	30	59	0.03
Habits					
Current smoke (%)	13	9	13	13	0.96
Taking multivitamin (%)	30	30	26	39	0.86
Anthropometrics					
Body mass index (kg/m ²)	30.6 ± 7.2	29.4 ± 5.5	28.9 ± 5.6	31.6 ± 6.0	0.44
Waist to hip ratio	0.93 ± 0.08	0.92 ± 0.09	0.92 ± 0.10	0.98 ± 0.11	0.17
Mean dietary intakes					
Total calcium (mg/d) [‡]	562 ± 273	681 ± 268	693 ± 426	560 ± 247	0.31
Total vitamin D (IU/d) [‡]	132 ± 49	193 ± 99	177 ± 123	150 ± 88	0.14
Total energy (kcal/d)	1596 ± 528	1788 ± 691	1848 ± 821	1845 ± 752	0.59
Total fat (g/d)	67 ± 32	72 ± 35	70 ± 32	74 ± 28	0.59
Saturated fat (g/d)	20 ± 9	22 ± 10	22 ± 12	22 ± 8	0.89
Cholesterol (mg/d)	302 ± 174	302 ± 191	261 ± 161	272 ± 113	0.76
Fiber (g/d)	15 ± 7	17 ± 9	18 ± 9	17 ± 11	0.56
Alcohol (g/d)	9 ± 14	11 ± 15	14 ± 18	10 ± 20	0.84

* Data are given as means ± SD unless otherwise specified.

[†] By Fisher's exact χ^2 test for categorical variables or ANOVA for continuous variables.

[‡] Diet only (intakes exclusive of supplements).

Table 2

Serum levels of total cholesterol and triglycerides, and systolic and diastolic blood pressures (BP) at baseline and 6-month follow up

Treatment Group	Baseline			6-mo follow-up			Absolute Rx effect*			Proportional Rx effect [†] (%)
	N	Mean ± SE	P [‡]	N	Mean ± SE	P [‡]	N	Mean ± SE	P [‡]	
Cholesterol (mg/dL)										
Placebo	23	225.5 ± 9.5		21	217.7 ± 9.9		21	0		-
Calcium	23	235.5 ± 9.8	0.46	21	215.3 ± 10.5	0.94	21	-12.5 ± 12.2	0.31	-5.3
Vitamin D ₃	23	227.7 ± 9.5	0.87	22	219.6 ± 9.9	0.94	22	-0.4 ± 11.7	0.97	-0.2
Calcium + Vitamin D ₃	23	229.3 ± 9.8	0.78	21	206.1 ± 9.9	0.45	21	-15.5 ± 12.2	0.19	-6.8
Triglycerides (mg/dL)										
Placebo	23	112.9 ± 17.5		21	120.0 ± 18.3		21	0		-
Calcium	23	104.5 ± 17.9	0.82	21	80.0 ± 19.5	0.15	21	-31.5 ± 24.4	0.10	-30.1
Vitamin D ₃	23	93.3 ± 17.5	0.27	22	93.9 ± 18.3	0.17	22	-6.5 ± 23.5	0.68	-7.0
Calcium + Vitamin D ₃	23	98.0 ± 17.9	0.69	21	73.6 ± 18.2	0.11	21	-31.5 ± 23.4	0.10	-32.1
Systolic BP (mmHg)										
Placebo	23	123.3 ± 3.2		22	118.9 ± 3.2		21	0		-
Calcium	23	125.1 ± 3.3	0.69	23	128.3 ± 3.2	0.05	20	7.6 ± 4.3	0.08	6.1
Vitamin D ₃	22	125.9 ± 3.3	0.58	23	125.2 ± 3.2	0.19	22	3.7 ± 4.3	0.39	2.9
Calcium + Vitamin D ₃	23	131.5 ± 3.3	0.07	22	128.1 ± 3.2	0.05	21	1.0 ± 4.3	0.81	0.8
Diastolic BP (mmHg)										
Placebo	23	80.5 ± 2.2		22	80.7 ± 2.3		21	0		-
Calcium	23	79.1 ± 2.2	0.66	23	78.9 ± 2.3	0.58	20	-0.4 ± 2.5	0.86	-0.5
Vitamin D ₃	22	76.5 ± 2.3	0.21	23	76.5 ± 2.3	0.19	22	-0.2 ± 2.5	0.93	-0.3
Calcium + Vitamin D ₃	23	81.3 ± 2.2	0.82	22	78.5 ± 2.3	0.49	21	-3.0 ± 2.5	0.23	-3.7

^{*} Absolute treatment effect = [(treatment group follow-up) - (treatment group baseline)] - [(placebo group follow-up) - (placebo group baseline)].

[†] Proportional treatment effect = [(absolute treatment effect / treatment group baseline) x 100%].

[‡] P value for difference between each active treatment group and placebo group from repeated measures MIXED model. P values for triglycerides were calculated based on log-transformed values.

Table 3

Serum levels of carotenoids at baseline and 6-month follow up

Treatment Group	Baseline			6-mo follow-up			Absolute Rx effect*			Proportional Rx effect† (%)
	N	Mean ± SE	p‡	N	Mean ± SE	p‡	N	Mean ± SE	p‡	
α-Carotene (ng/mL)										
Placebo	23	40.1 ± 5.8		21	39.7 ± 6.0		21	0		-
Calcium	23	53.2 ± 6.0	0.12	21	45.5 ± 6.4	0.52	21	-7.4 ± 6.8	0.27	-13.9
Vitamin D ₃	23	39.6 ± 5.8	0.95	22	39.3 ± 6.0	0.96	22	0.1 ± 6.4	0.98	-0.2
Calcium + Vitamin D ₃	23	43.7 ± 6.0	0.67	21	36.9 ± 6.0	0.74	21	-6.4 ± 6.4	0.32	-14.6
β-Carotene (ng/mL)										
Placebo	23	176.3 ± 48.5		21	190.8 ± 49.3		21	0		-
Calcium	23	220.7 ± 50.0	0.52	21	202.4 ± 51.3	0.87	21	-32.9 ± 38.7	0.40	-14.9
Vitamin D ₃	23	204.6 ± 48.7	0.68	22	187.3 ± 49.3	0.96	22	-31.9 ± 37.6	0.40	-15.6
Calcium + Vitamin D ₃	23	302.9 ± 49.5	0.07	21	267.5 ± 49.8	0.28	21	-49.9 ± 36.8	0.18	-16.5
α-Cryptoxanthin (ng/mL)										
Placebo	23	40.8 ± 3.4		22	39.3 ± 3.4		21	0		-
Calcium	23	47.3 ± 3.4	0.18	23	45.0 ± 3.6	0.25	20	-0.7 ± 2.9	0.81	-1.5
Vitamin D ₃	22	46.8 ± 3.4	0.21	23	44.4 ± 3.4	0.30	22	-0.9 ± 2.8	0.74	-1.9
Calcium + Vitamin D ₃	23	49.1 ± 3.4	0.09	22	46.2 ± 3.5	0.16	21	-1.4 ± 2.7	0.62	-2.9
β-Cryptoxanthin (ng/mL)										
Placebo	23	156.5 ± 29.6		22	162.7 ± 30.3		21	0		-
Calcium	23	181.2 ± 30.0	0.56	23	158.7 ± 31.8	0.92	20	-28.8 ± 31.8	0.37	-15.9
Vitamin D ₃	22	194.8 ± 29.4	0.36	23	222.8 ± 30.3	0.16	22	21.8 ± 30.6	0.48	11.2
Calcium + Vitamin D ₃	23	194.9 ± 30.1	0.36	22	185.8 ± 30.3	0.59	21	-15.3 ± 30.3	0.62	-7.9
Lutein/zeaxanthin (ng/mL)										
Placebo	23	474.2 ± 40.8		21	449.6 ± 42.1		21	0		-
Calcium	23	550.4 ± 41.7	0.20	21	496.3 ± 44.3	0.44	21	-29.5 ± 44.9	0.51	-5.4
Vitamin D	23	553.9 ± 40.8	0.17	22	541.8 ± 42.1	0.13	22	12.5 ± 43.2	0.77	2.3
Calcium + Vitamin D ₃	23	548.0 ± 41.7	0.21	21	483.4 ± 42.1	0.57	21	-40.8 ± 42.8	0.35	-7.4
Lycopene (ng/mL)										

Treatment Group	Baseline			6-mo follow-up			Absolute Rx effect [*]			Proportional Rx effect [†] (%)
	N	Mean ± SE	p [‡]	N	Mean ± SE	p [‡]	N	Mean ± SE	p [‡]	
Placebo	23	313.0 ± 27.9		21	321.8 ± 29.2		21	0		-
Calcium	23	395.8 ± 28.5	0.04	21	335.5 ± 31.2	0.75	21	-69.0 ± 39.8	0.08	-17.4
Vitamin D ₃	23	357.2 ± 27.9	0.27	22	295.5 ± 29.2	0.53	22	-70.0 ± 38.4	0.07	-19.6
Calcium + Vitamin D ₃	23	383.2 ± 28.5	0.08	21	351.5 ± 29.0	0.47	21	-40.0 ± 38.1	0.29	-10.4
Total carotenoids (ng/mL)										
Placebo	23	1,296 ± 123		21	1,290 ± 125		21	0		-
Calcium	23	1,583 ± 126	0.11	21	1,402 ± 130	0.54	21	-175 ± 95	0.07	-13.5
Vitamin D ₃	23	1,536 ± 123	0.17	22	1,461 ± 125	0.34	22	-69 ± 92	0.45	-4.5
Calcium + Vitamin D ₃	23	1,646 ± 124	0.05	21	1,491 ± 127	0.26	21	-149 ± 91	0.10	-9.1

^{*} Absolute treatment effect = [(treatment group follow-up) - (treatment group baseline)] - [(placebo group follow-up) - (placebo group baseline)].

[†] Proportional treatment effect = [(absolute treatment effect / treatment group baseline) x 100%].

[‡] P value for difference between each active treatment group and placebo group from repeated measures MIXED model.

Table 4

Serum levels of total cholesterol, triglycerides, and total carotenoids for pooled calcium and pooled non-calcium intervention groups*

Treatment Group	Baseline			6-mo follow-up			Absolute Rx effect [†]			Proportional Rx effect [‡]	
	N	Mean ± SE	P [§]	N	Mean ± SE	P [§]	N	Mean ± SE	P [§]		(%)
Cholesterol (mg/dL)											
Non-calcium	46	226.6 ± 6.7		43	218.7 ± 7.0		43	0			-
Calcium	46	232.4 ± 6.8	0.55	42	210.4 ± 7.1	0.41	42	-14.0 ± 8.3	0.09		-6.0
Triglycerides (mg/dL)											
Non-calcium	46	103.1 ± 12.3		43	107.0 ± 12.9		43	0			-
Calcium	46	101.3 ± 12.6	0.74	42	76.7 ± 13.2	0.22	42	-28.4 ± 16.7	0.04		-28.0
Total carotenoids (ng/mL)											
Non-calcium	46	1,416 ± 87		43	1,375 ± 88		43	0			-
Calcium	46	1,614 ± 89	0.69	42	1,448 ± 90	0.05	42	-125 ± 65	0.06		-7.7

* Pooled calcium supplementation group (calcium) included participants from calcium alone and calcium plus vitamin D groups. Pooled non-calcium supplementation group (non-calcium) included participants from placebo and vitamin D alone groups.

[†] Absolute treatment effect = [(pooled calcium group follow-up) - (pooled calcium group baseline)] - [(pooled non-calcium group follow-up) - (pooled non-calcium group baseline)].

[‡] Proportional treatment effect = (absolute treatment effect / pooled calcium group baseline) x 100%.

[§] P value for difference between pooled calcium group and pooled non-calcium group from repeated measures MIXED model. P values for triglycerides were calculated based on log-transformed values.

Table 5

Serum levels of total cholesterol for pooled calcium and pooled non-calcium intervention groups, stratified by cholesterol-lowering medication use*

Treatment Group	Baseline			6-mo follow-up			Absolute Rx effect [†]		Proportional Rx effect [‡]	
	N	Mean ± SE	P [§]	N	Mean ± SE	P [§]	N	Mean ± SE	P [§]	(%)
<u>Taking cholesterol-lowering medications</u>										
Cholesterol (mg/dL)										
Non-calcium	11	231.6 ± 12.6		9	211.1 ± 13.4		9	0		-
Calcium	21	215.6 ± 9.1	0.31	17	194.5 ± 9.7	0.32	17	-0.73 ± 13.9	0.96	-0.3
<u>Not taking cholesterol-lowering medications</u>										
Cholesterol (mg/dL)										
Non-calcium	35	225.0 ± 7.7		31	220.7 ± 8.0		31	0		-
Calcium	23	247.7 ± 9.5	0.07	21	224.5 ± 9.8	0.77	21	-19.0 ± 10.9	0.09	-7.7
P for interaction [¶] = 0.10										

* Pooled calcium supplementation group (calcium) included participants from calcium alone and calcium plus vitamin D groups. Pooled non-calcium supplementation group (non-calcium) included participants from placebo and vitamin D alone groups.

[†] Absolute treatment effect = [(pooled calcium group follow-up) – (pooled calcium group baseline)] – [(pooled non-calcium group follow-up) – (pooled non-calcium group baseline)].

[‡] Proportional treatment effect = (absolute treatment effect / pooled calcium group baseline) x 100%.

[§] P value for difference between pooled calcium group and pooled non-calcium group from repeated measures MIXED model.

[¶] Interaction between treatment (calcium/non-calcium) and cholesterol-lowering medication use (yes/no).