Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial[1,2]

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ABSTRACT

Background: Numerous observational studies have found supplemental calcium and vitamin D to be associated with reduced risk of common cancers. However, interventional studies to test this effect are lacking.

Objective: The purpose of this analysis was to determine the efficacy of calcium alone and calcium plus vitamin D in reducing incident cancer risk of all types.

Design: This was a 4-y, population-based, double-blind, randomized placebo-controlled trial. The primary outcome was fracture incidence, and the principal secondary outcome was cancer incidence. The subjects were 1179 community-dwelling women randomly selected from the population of healthy postmenopausal women aged >55 y in a 9-county rural area of Nebraska centered at latitude 41.4°N. Subjects were randomly assigned to receive 1400–1500 mg supplemental calcium/d alone (Ca-only), supplemental calcium plus 1100 IU vitamin D_3/d (Ca + D), or placebo.

Results: When analyzed by intention to treat, cancer incidence was lower in the Ca + D women than in the placebo control subjects ($P <$ 0.03). With the use of logistic regression, the unadjusted relative risks (RR) of incident cancer in the Ca + D and Ca-only groups were 0.402 ($P = 0.01$) and 0.532 ($P = 0.06$), respectively. When analysis was confined to cancers diagnosed after the first 12 mo, RR for the Ca + D group fell to 0.232 (CI: 0.09, 0.60; $P < 0.005$) but did not change significantly for the Ca-only group. In multiple logistic regression models, both treatment and serum 25-hydroxyvitamin D concentrations were significant, independent predictors of cancer risk.

Conclusions: Improving calcium and vitamin D nutritional status substantially reduces all-cancer risk in postmenopausal women. This trial was registered at clinicaltrials.gov as NCT00352170. *Am J Clin Nutr* 2007;85:1586–91.

KEY WORDS Serum 25-hydroxyvitamin D, cancer, women, calcium and vitamin D_3 supplementation

INTRODUCTION

The relation of solar radiation to reduced cancer mortality in North America was identified >60 y ago (1). Garland and Garland (2) were the first to propose that vitamin D was responsible, specifically for the association with colon cancer. The inverse association between ambient solar radiation and cancer mortality rates has subsequently been described for cancers of the breast, rectum, ovary, prostate, stomach, bladder, esophagus, kidney, lung, pancreas, and uterus, as well as for non-Hodgkin lymphoma and multiple myeloma (3–10).

This seeming protection was presumed to be mediated by the effect of solar radiation on vitamin D status. Exploration of the connection between vitamin D nutriture and chronic disease in humans received a critical stimulus with the availability of a physiologically stable indicator of vitamin D status [serum 25-hydroxyvitamin D, or 25(OH)D] and the designation of 25(OH)D as the functional indicator of vitamin D status by the Institute of Medicine (11). These developments have facilitated a more precise definition of the relation between cancer risk and vitamin D status. The inverse association has now been established for incident colorectal cancer (12) and for prostate cancer (13), among others. Gorham et al (14), quantifying the inverse relation between serum 25(OH)D and risk of colorectal cancer, calculated a 50% reduction in cancer risk at serum 25(OH)D concentrations ≥80 nmol/L.

Giovannucci (15, 16) and Holick (17, 18) have each recently reviewed the now large body of evidence linking low vitamin D status to increased risk of cancer. Similar associations were earlier noted for high calcium intake and reduced cancer risk (19–21), most prominently for colorectal cancer, whereby a luminal effect of high calcium intake provided a plausible mechanism.

The human evidence to date linking cancer and vitamin D has been observational in character, although several of the many positive studies linking vitamin D and cancer have been prospective. We had the opportunity to examine the relation of these nutrients to cancer incidence in a 4-y, double-blind, placebo-controlled trial of calcium and vitamin D supplementation for which cancer was the principal secondary endpoint. The null hypothesis was that there would be no difference in all-cancer incidence between the 3 calcium and vitamin D treatment groups.

SUBJECTS AND METHODS

Participants

The participants have been described in detail in an article describing their vitamin D status (22). Briefly, participants were recruited as a population-based sample from a 9-county, largely rural area in eastern Nebraska (latitude 41.4°N), with the use of random telephone dialing of all listed telephones in the counties

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concerned. Inclusion criteria consisted mainly of age >55 y, absence of known cancers, and both mental and physical status sufficiently good to permit 4-y participation. Sample size was determined by power calculation based on estimated fracture risk. During the course of 1 y, 1180 women meeting inclusion criteria were enrolled. One woman was excluded after enrollment when she disclosed a history of hypoparathyroidism after thyroidectomy and reported having taken 50 000 IU vitamin D daily for the past 25 y. Mean ($\pm$SD) age at enrollment was 66.7 $\pm$ 7.3 y, body mass index (BMI; in kg/m^2) was 29.0 $\pm$ 5.7, and baseline serum 25(OH)D was 71.8 $\pm$ 20.3 nmol/L. Relations of vitamin D status, serum parathyroid hormone (PTH), and calcium intake in the members of this cohort are described in a separate publication (22). Although there were no ethnic inclusion criteria, all participants were white in ancestry. All were free of known cancer, both on entry into trial and for $\geq$10 y before entry. Forty-six percent ($n = 543$) received estrogen on prescription from their personal physicians for $\geq$6 mo during the study. The 3 treatment groups did not differ significantly in estrogen use. The project had been approved by Creighton University's Institutional Review Board, and all participants gave written consent. Study visits were conducted at a research site established in a community hospital in the rural area.

Design

The 1180 participants were randomly assigned to 1 of 3 interventions: *1)* placebo, consisting of both a vitamin D placebo and a brand-specific calcium placebo; *2)* calcium, consisting of either calcium citrate (1400 mg Ca/d) or calcium carbonate (1500 mg Ca/d) plus a vitamin D placebo (designated Ca-only group); and *3)* calcium plus vitamin D, consisting of calcium (as above) plus 1000 IU (25 μg) cholecalciferol (vitamin D$_3$)/d (designated Ca + D group). The study statistician generated the randomization sequence with the use of a computer-generated permuted blocks ($n = 5$) randomization scheme, and the study nurses enrolled the subjects and assigned them to groups. By design, the 2 active treatment groups were each allocated $\approx$40% of the cohort, and the placebo group 20%. The calcium supplements were provided by Mission Pharmacal (San Antonio, TX) and GlaxoSmithKline (Parsippany, NJ) in forms previously shown in our laboratory to exhibit identical bioavailability (23); the vitamin D (labeled to contain 1000 IU) was obtained from Tishcon Corporation (Westbury, NY). Supplements were given to the subjects by the project nurses at each 6-mo visit. Vitamin D was reordered annually, and a sample of each lot was analyzed by Tai Chen of Boston University at the beginning and end of each year to assure potency of at least 1000 IU. Mean content on analysis was 1100 IU.

Of 1180 women enrolled, 1024 (86.8%) completed the 4 y of study. Most of the losses ($n = 92$) occurred within the first year. Compliance with study medication (both active and placebo) was assessed at 6-mo intervals by bottle weight. Mean adherence (defined as taking $\geq$80% of assigned doses) was 85.7% for the vitamin D component of the combined regimen and 74.4% for the calcium component.

Laboratory methods

Various analytes related to calcium and vitamin D physiology were measured on serum samples obtained at baseline and annually thereafter. Measurement of 25(OH)D was done by radioimmunoassay after extraction with the use of the IDS kit (Fountain Hills, AZ). Our laboratory participates in the international quality assessment scheme for 25(OH)D assays (24), and during the course of the study our findings on test samples were regularly close to the international mean.

Outcome measures

The primary design endpoints of the study concerned skeletal status and the calcium economy. These outcomes will be described elsewhere. Here, we present data related solely to a secondary endpoint, incident cancers. Health status was assessed at 6-mo intervals during the course of the study. When a participant reported that a diagnosis of cancer had been made in the interval between assessments, the medical record was examined to confirm that diagnosis and to establish the primary site. The date of diagnosis was used to time the occurrence of cancer in subsequent analyses.

Statistical analysis

Statistical analysis was by group assignment (ie, intention-to-treat) and by serum 25(OH)D concentration. For this analysis, serum 25(OH)D values obtained at baseline and at 12 mo were used to characterize the basal vitamin D status of the cohort and its response to treatment. Survival analysis was used to plot and evaluate differences in cancer incidence; however, because proportional hazards would be predicted to change for both interventions during the course of treatment, Cox modeling, which assumes a constant hazard ratio (25), was not used. Instead, various logistic regression models were developed to estimate relative risk of being in the cancer group at the conclusion of the trial and to explore the determinants of observed rates of cancer incidence. Analysis was performed by using SPSS for WINDOWS (version 13.0; SPSS, Chicago, IL).

RESULTS

Fifty women developed nonskin cancer during the course of the study, 13 in the first year and 37 thereafter. Cancer sites by treatment assignment are shown in **Table 1**. For all cancers combined, both the Ca-only and the Ca + D groups had rates less than that of the placebo-treated women (intention-to-treat; chi square = 7.3 with 2 df; $P < 0.03$). Survival free of cancer is shown as a Kaplan-Meier plot for the 3 contrast groups in **Figure 1**. As is visually evident, the Ca-only and Ca + D groups followed similar time courses, differing after approximately 1 y from the placebo group. In comparison to the placebo group, the relative risk (RR) of developing cancer at study end was 0.402 (CI: 0.20, 0.82; $P = 0.013$) for the Ca + D group and 0.532 (CI: 0.27, 1.03; $P = 0.063$) for the Ca-only group.

We repeated the survival analysis for the group free of cancer at 1 y (**Figure 2**; Table 1), on the hypothesis that cancers diagnosed early in the study would have been present, although unrecognized, on entry. The total number of incident cancers fell to 37, but the RR for the Ca + D group by simple logistic regression dropped to 0.232 (CI: 0.09, 0.60; $P < 0.005$). However, for the Ca-only group, RR was essentially unchanged at 0.587 (CI: 0.29, 1.21; $P = 0.147$).

Logistic regression models were developed with the use of intervention, baseline 25(OH)D, 12-mo 25(OH)D (as a measure of vitamin D treatment response), BMI, and age. Both treatment assignment and either 12-mo 25(OH)D or baseline 25(OH)D concentration were significant, independent determinants of cancer risk [$P < 0.002$ and $P < 0.03$, respectively, for the 2 serum

TABLE 1
Cancers by primary site and by treatment assignment

| | Years 1–4 | | | Years 2–4 | | |
Site	Placebo ($n = 288$)	Calcium only ($n = 445$)	Vitamin D plus calcium ($n = 446$)	Placebo ($n = 266$)	Calcium only ($n = 416$)	Vitamin D plus calcium ($n = 403$)
Breast(*n*)	8	6	5	7	6	4
Colon (*n*)	2	0	1	2	0	0
Lung (*n*)	3	3	1	3	2	1
Lymph, leukemia, myeloma (*n*)	4	4	2	4	4	2
Uterus (*n*)	0	2	1	0	1	0
Other (*n*)	3	2	3	2	2	1
Total[1]	20 (6.9)	17 (3.8)	13 (2.9)	18 (6.8)	15 (3.6)	8 (2.0)

[1] *n;* percentages of the total randomly assigned in each group who developed cancer in parentheses.

25(OH)D values]. In models testing both treatment and 12-mo 25(OH)D concentration, only the latter variable was a significant predictor ($R^2 = 0.037$); however, when baseline 25(OH)D was used instead, both it and the fact of treatment were significant predictors ($R^2 = 0.055$). Neither age nor BMI was a significant predictor.

To quantify the size of the vitamin D effect, we used a simple logistic regression by using cancer as the outcome variable and baseline 25(OH)D concentration as the predictor variable. The RR of cancer per unit concentration of serum 25(OH)D was 0.983 (CI: 0.968, 0.997; $P < 0.01$). Because the unit for 25(OH)D is 1 nmol/L, this RR translates to a predicted 35% reduced risk of cancer for every 25 nmol/L (10 ng/mL) increase in serum 25(OH)D.

The effect of treatment on vitamin D status was reflected in the induced change in serum 25(OH)D. Baseline and 12-mo values for serum 25(OH)D by treatment assignment are presented in **Table 2**. The 1100 IU vitamin D/d dose produced an elevation in serum 25(OH)D in the Ca + D group of 23.9 ± 17.8 nmol/L, whereas the placebo and Ca-only groups had no significant change (either biological or statistical). Within the Ca + D group, the rise in serum 25(OH)D was directly related to recorded compliance ($P < 0.01$; data not shown). As expected, PTH fell from baseline to 1 y in both the Ca-only and the Ca + D groups ($\bar{x}$ changes ± 1 SEM: −2.61 ± 0.70 and −5.26 ± 0.66, respectively; $P < 0.001$ for both). All intergroup comparisons were statistically significant. Because baseline PTH averaged 37 pg/mL, these changes represent declines of 7% and 14%, respectively.

During the course of study, there were no serious supplement-related adverse events. Five subjects were diagnosed with renal calculi: 1 subject in the placebo group, 1 subject in the Ca + D group, and 3 subjects in the Ca-only group. These incidences did not differ significantly by group. No patterns of adverse events were seen among the 3 groups.

DISCUSSION

The current study is, to our knowledge, the first randomized controlled trial that involved a vitamin D intervention sufficient to raise serum 25(OH)D >80 nmol/L and reported a cancer
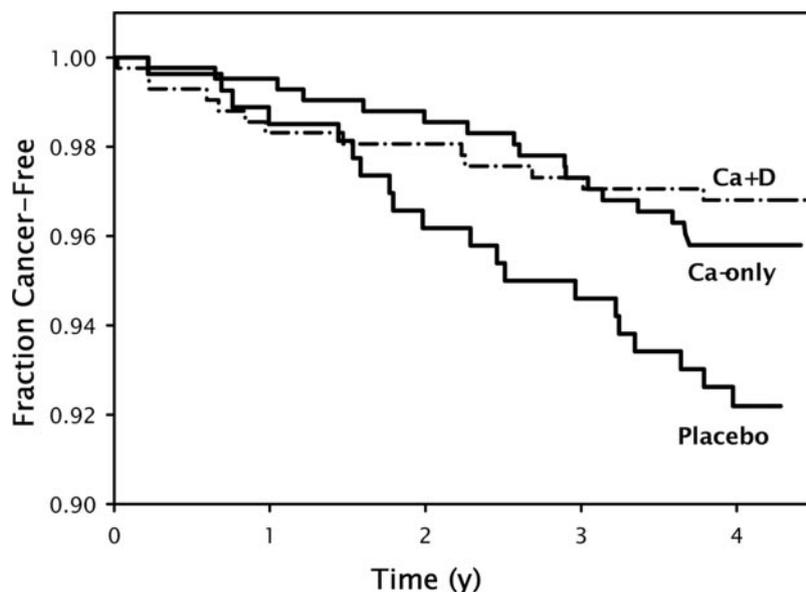
FIGURE 1. Kaplan-Meier survival curves (ie, free of cancer) for the 3 treatment groups randomly assigned in the entire cohort of 1179 women. Sample sizes are 288 for the placebo group, 445 for the calcium-only (Ca-only) group, and 446 for the calcium plus vitamin D (Ca + D) group. The survival at the end of study for the Ca + D group is significantly higher than that for placebo, by logistic regression. (Copyright Robert P Heaney, 2006. Used with permission.)
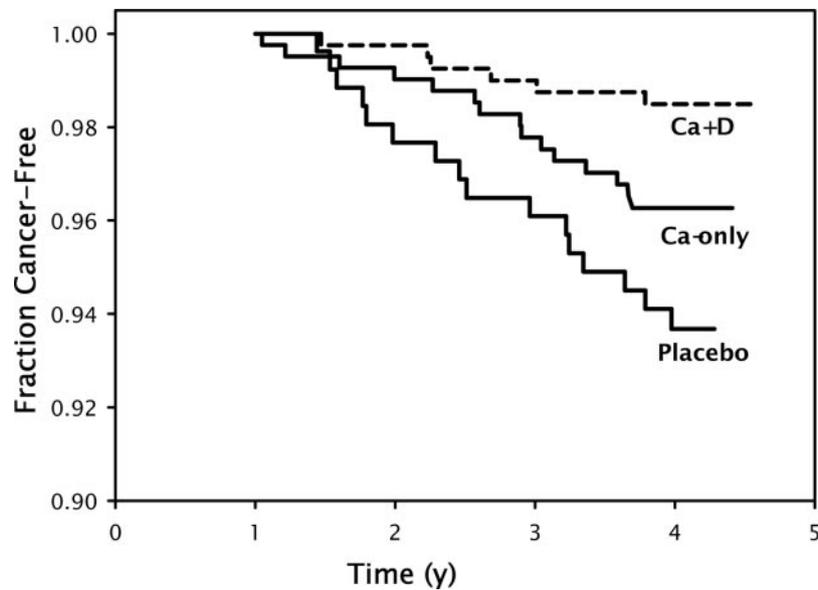
FIGURE 2. Kaplan-Meier survival curves (ie, free of cancer) for the 3 treatment groups randomly assigned in the cohort of women who were free of cancer at 1 y of intervention ($n = 1085$). Sample sizes are 266 for the placebo group, 416 for the calcium-only (Ca-only) group, and 403 for the calcium plus vitamin D (Ca + D) group. The survival at the end of study for the Ca + D group is significantly higher than that for the placebo group, by logistic regression. (Copyright Robert P Heaney, 2006. Used with permission.)

outcome. Our findings of decreased all-cancer risk with improved vitamin D status are consistent with a large and still growing body of epidemiologic and observational data showing that cancer risk, cancer mortality, or both are inversely associated with solar exposure, vitamin D status, or both (1–10, 12–18, 26). Our conclusion that the observed effect was not simply a chance association is strengthened both by the observed, substantial improvement in RR when cancers occurring early in the trial were excluded and by the highly significant predictive effect of both the baseline and the 1-y serum 25(OH)D values in addition to the intervention itself.

The only other randomized trial of vitamin D and cancer of which we are aware was the Women's Health Initiative (WHI), which used a much lower dose of vitamin D (400 IU) and a sample of women with substantially lower baseline vitamin D status [median serum 25(OH)D: 42 nmol/L] and with much poorer treatment adherence (27). The WHI reported no significant effect of the vitamin D intervention on colorectal cancer incidence but did note a highly significant inverse relation between baseline 25(OH)D and incident cancer risk (27, 28), just as we report here for all cancers.

Although the raw data suggested a marginal protective effect for the Ca-only intervention, per-protocol analysis based on compliance did not improve the RR for the Ca-only group, nor

did removal of first year cancers. Thus, we are uncertain whether the marginal calcium effect represents a chance occurrence. The results of many calcium trials have been reported, but few have had cancer endpoints or reported cancer outcomes. Exceptions include a trial that used calcium carbonate in persons at risk of colon polyps (29). Not only was polyp recurrence reduced significantly, but, in a secondary analysis, prostate cancer risk was also found to be reduced by approximately half (30). High calcium intakes are generally considered to be protective for colon cancer (19, 20), at least in part by virtue of their intraluminal binding of cancer promoters in the digestive residue (19). Only 3 of our 50 cancers were colorectal; 2 of those were in the placebo group. How calcium might have been operating in our study is unclear, but its effect, if real, can be plausibly connected to vitamin D status. High calcium intakes reduce circulating concentrations of calcitriol, which, in turn, is known to shorten the half-time for serum 25(OH)D (31)—ie, higher calcitriol concentrations result in greater metabolic consumption and degradation of 25(OH)D, effectively lowering vitamin D status. Such a mechanism, effectively equivalent to a lower vitamin D dose, could explain the weaker effect found for the Ca-only group. It has also been proposed that calcium intake may alter cell-to-cell adhesion, because calcium is an integral component of the structures responsible for intercellular adhesion (18). It is uncertain, however, whether this mechanism would be applicable within the physiologic range of serum calcium concentrations.

Strengths of the present study include that it was population based, that it had a low drop-out rate ($<3.5\%/y$) and a high level of treatment adherence, and that it used a vitamin D dose sufficient to raise serum 25(OH)D by a biologically meaningful amount. This latter feature may be a principal point of difference from most other investigations, both randomized trials and observational studies. In the WHI, the achieved vitamin D dose, taking compliance into consideration, would have been $\approx$200 IU/d (27), sufficient to elevate serum 25(OH)D from a median of

TABLE 2
Baseline and 12-mo serum 25-hydroxyvitamin D values by treatment assignment[1]

	Baseline	12 mo	Change
		nmol/L	
Placebo	72.1 ± 20.7	71.1 ± 19.8	−0.23 ± 14.7
Calcium only	71.6 ± 20.5	71.0 ± 20.3	−0.74 ± 13.0
Calcium plus D	71.8 ± 20.0	96.0 ± 21.4	23.9 ± 17.8

[1] All values are $\bar{x}$ ± SD.

42 nmol/L to only 47 nmol/L (32). This is in striking contrast to the much higher 25(OH)D values in our treated women at both baseline and 1 y (Table 2). The principal weakness of our study was that, at the time the study was designed (1996), cancer was not a primary outcome variable. However, given the large body of observational data suggesting a causal linkage for vitamin D with a variety of cancers, it is logical to look for a cancer outcome in a study such as this. Further, the randomization, the blinding, and the high treatment adherence and completion rate in the present study make it hard to imagine how extraneous factors could have "pushed" cancers into the placebo group.

The mechanism by which vitamin D status may alter cancer development is still being delineated, but what is now known can be summarized briefly as follows. At least 200 human genes contain vitamin D response elements (33); many of these genes encode for proteins important in the regulation of cell proliferation, differentiation, and apoptosis. When vitamin D status is suboptimal, these activities are impaired. For example, mice rendered vitamin D deficient exhibit enhanced cancer development and cancer growth (34), as do vitamin D receptor knockout mice (35).

Evidence further indicates that the concentration of 25(OH)D in the extracellular fluid is the critical determinant of the ability of proliferating cells to regulate their response to various stimuli. A possibly generalizable illustration of how this relation operates was provided in a recent report by Liu et al (36), which characterized the innate immune response to a microbial stimulus. The first genes expressed in monocytes exposed to a *Mycobacterium* antigen were the genes for the vitamin D receptor and vitamin D 1-α-hydroxylase. In the absence of either calcitriol or 25(OH)D in the medium, nothing further happened, but as 25(OH)D was added to the system, the response increased in a dose-related manner and consisted in expression of the genes for both a microbicidal peptide (cathelicidin) and vitamin D-24-hydroxylase. Although this example relates specifically to the immune response, it illustrates the critical role serum 25(OH)D concentrations may play in enabling various cellular responses.

Because the coexpression of the 24-hydroxylase results in immediate inactivation of the intracellularly synthesized calcitriol, vitamin D functions as a rapid on-off switch for various cell responses. What is perhaps most notable in the study by Liu et al (36) was that human monocytes, in human serum, exhibited biologically important differences in microbicidal response within the range of serum 25(OH)D values commonly found in free-living populations.

Since the discovery of calcitriol in 1971 (37), the predominant focus of the medical community has been on the circulating concentration of this, the active hormonal form of the vitamin, and until recently little attention was given to serum 25(OH)D except as an indicator of vitamin D status. Nevertheless, it has been a nearly universal experience in adult medicine that serum calcitriol concentrations are poorly correlated with various vitamin D-related effects, whereas serum 25(OH)D is generally reported to be a better predictor of such effects as even calcium absorption (the canonical function of vitamin D) (38, 39). This paradox is at least partially explained by distinguishing between the autocrine and endocrine functions of vitamin D. The endocrine function (principally the regulation of active calcium transport across the intestinal mucosa) does depend on circulating calcitriol. However, from a variety of animal models and cell biologic systems, it appears that healthy serum concentrations of calcitriol are not sufficient to support the cell regulatory and immune effects that together comprise the autocrine components of the vitamin D system (35). The needed quantities of calcitriol are synthesized intracellularly from 25(OH)D, tissue by tissue. However, the 1-α-hydroxylase expressed in most tissues operates well below its Michaelis constant, which means that the amount of calcitriol that a cell can produce for itself in response to various stimuli is dependent on the serum concentration of 25(OH)D. Several of the effects of vitamin D in cancer model systems require concentrations of calcitriol substantially higher than can be achieved physiologically in intact humans (35).

We found that improving vitamin D nutritional status substantially reduced all-cancer risk in postmenopausal women. Furthermore, baseline and treatment-induced serum 25(OH)D concentrations were themselves strong predictors of cancer risk. These findings highlight the importance of promoting optimum vitamin D status and underscore the value of achieving and maintaining a high serum 25(OH)D concentration.

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