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Vitamin D Supplementation and Cancer: Review of Randomized Controlled Trials

Matteo Lazzeroni[a], Davide Serrano[a], Stefan Pilz[b,c] and Sara Gandini[d,*]

[a]*Division of Cancer Prevention and Genetics, European Institute of Oncology, Milan, Italy;* [b]*Department of Epidemiology and Biostatistics, EMGO Institute for Health and Care Research, VU University Medical Center, Amsterdam, The Netherlands;* [c]*Department of Internal Medicine, Division of Endocrinology and Metabolism, Medical University of Graz, Graz, Austria;* [d]*Division of Epidemiology and Biostatistics, European Institute of Oncology, Milan, Italy*

Abstract: Data from experimental studies suggest that vitamin D receptor activation exerts anti-cancer effects on virtually all steps of carcinogenesis. Epidemiological data support an inverse association of vitamin D serum levels and vitamin D receptor polymorphisms with cancer incidence and mortality. Based on this promising rationale for use of vitamin D and its analogues in cancer prevention and treatment, several interventional studies have been initiated and partially published. Trials with vitamin D were mainly organized for the prevention of fracture in elderly people, usually in association with calcium supplements. Prevention studies with vitamin D have rarely been done in the context of vitamin D to evaluate a protective effect on cancer. Findings from prospective cohort studies on colorectal cancer risk and on mortality constitute pieces of evidence strong enough to consider that previous randomized controlled trials (RCTs) of vitamin D use and cancer may not have correctly addressed the question, and that new randomized trials should be organized. The reasons are due to several unsolved issues including selection of the effective dose, varying baseline levels of subjects before randomization, compliance with the intervention, contamination of the placebo group (i.e., intake of vitamin D supplements by subjects allocated to the placebo group) and unknown effective lag time between start of the intervention and disease onset. The present review summarizes the existing knowledge on vitamin D RCTs and cancer. In addition we also briefly describe the design of some ongoing trials on vitamin D supplementation and cancer.

Keywords: Vitamin D, Randomized Controlled Trials, Cancer Mortality, Cancer Incidence.

BACKGROUND

Vitamin D represents a group of fat-soluble prohormones, the two major forms of which are vitamin D2 (or ergocalciferol) and vitamin D3 (or cholecalciferol). Endogenous synthesis of vitamin D3 takes place in the skin under the influence of ultraviolet B (UVB) radiation. Exogenous vitamin D2 or D3 comes from dietary intake. The overall vitamin D intake is therefore the sum of cutaneous vitamin D and nutritional vitamin D. Only a few foods naturally contain appreciable amounts of vitamin D to make an impact on vitamin D serum level, either through the form of cholecalciferol derived from animal sources, or ergocalciferol from plant food.

Vitamin D is indeed more like a (pro-)hormone and not strictly a vitamin according to the classical criteria that an essential nutrient is a substance the body cannot synthesize in sufficient quantities itself. Also, vitamins are usually involved in biochemical reactions, while 1a,25-dihydroxyvitamin D (1,25(OH)2D or calcitriol) exerts its action *via* the vitamin-D Receptor (VDR). The 25-hydroxyvitamin D (25(OH)D), which is formed by hydroxylation of vitamin D in the liver, is biologically not active, and an additional hydroxylation in the kidney is necessary to produce the physiologically active vitamin D metabolite, 1,25(OH)2D.

After synthesis in the kidneys, calcitriol circulates as a hormone, regulating the concentration of calcium and phosphate in the bloodstream and promoting the healthy growth and remodeling of bone. Vitamin D prevents rickets in children and osteomalacia in adults, and, together with calcium, is recommended for the treatment of osteoporosis. Vitamin D also affects neuromuscular function and inflammation [1,2].

Since the 1980s calcitriol has been established also as an antiproliferative and pro differentiation agent, and as a pro apoptotic agent and an inhibitor of cell migration, which may imply an inhibitory effect on cancer [3].

In combination with vitamin D, calcium supplements have proven anti-fracture efficacy. There is, however, much controversy about the effect of calcium on the risk of cancer, with observational studies showing no effect, a protective effect or even an increased cancer risk [4,5].

A meta-analysis of published randomized trials conducted in frail elderly people who are at high risk of fall, summarized data on 57 311 participants, and 4777 deaths for any cause and showed a significant reduction of 7% in total mortality (RR=0.93, 95%CI: 0.87, 0.99) in subjects taking vitamin D [6]. Eighty-two percent of patients received vitamin D3 (cholecalciferol), the remaining vitamin D2 (ergocalciferol), either orally or by injection. Average daily doses ranged from 300 IU to 2,000 IU. Treatment ranged from daily to 4-monthly, and follow-up ranged from 6 months to 7 years. The main recommendation in light of the results from this study was the conduction of large population-based randomized trials of prolonged vitamin D3 treatment.

While further large randomized control trials (RCTs) are still needed to establish the effect of vitamin D supplementation on mortality a further Cochrane meta-analysis confirmed that vitamin D3 supplementation significantly decreases all-cause mortality [7].

A meta-analysis on 25(OH)D serum levels and cancer risk in epidemiological studies showed a 40% significant reduction in colorectal cancer risk comparing the highest levels versus the lowest level of 25(OH)D, with a significant dose-response effect. Among the studies included, the lowest values of 25(OH)D for the upper categories in average were 34 ng/ml and the upper levels of the lowest category was 18 ng/ml [8].

Current efforts to assess optimal serum concentrations of 25(OH)D generally focus on bone health in older white persons, and the common definition of the optimal level has been the concentration that maximally suppresses serum parathyroid hormone (PTH). However based on the analysis on cancer risk vitamin D deficiency should be considered as a 25(OH)D level of

*Address correspondence to this author at the Division of Epidemiology and Biostatistics, European Institute of Oncology, *Via* Ripamonti 435, 20141 Milan, Italy; Tel: 00390257489819; Fax: 00390257489922;
E-mail: sara.gandini@ieo.it

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less than 20 ng/ml (multiply by 2.496 to convert ng/ml to nmol/l) [9-12], and the total 25(OH)D serum levels, i.e. 25(OH)D2 plus 25(OH)D3, is what should be determined. By these standards significant parts of both the European and US populations are vitamin D deficient.

A meta-analysis of prospective observational studies suggests a nonlinear decrease in overall mortality risk as circulating 25(OH)D increases, with optimal concentrations around 30–35 ng/ml [13].

The experience accumulated in the last twenty years with chemoprevention and hormonal substances shows that no compound should be recommended for cancer chemoprevention if its efficacy and side effects have not been evaluated in large randomized trials. Laboratory data and observational studies should only be considered as indicative of potential for chemopreventive use.

While we still have to wait for further results of upcoming RCTs on vitamin D supplementation and cancer, we aim to provide an overview of existing knowledge on vitamin D RCTs and cancer. In addition we also briefly describe the design of some ongoing trials on vitamin D supplementation and cancer.

SYSTEMATIC LITERATURE SEARCH AND STUDY RETRIEVAL

The search was carried out on clinical trials, and no language or time restrictions were applied. The literature to February 2012 was searched using the following databases: Pubmed, ISI Web of Science (Science Citation Index Expanded), Embase, and the Cochrane library. For intervention, the following keywords or corresponding MeSH terms were used: "vitamin D", "colecalciferol", "ergocalciferol". For study design, "clinical trial" and or corresponding MeSH terms were used as keywords. For the outcome the following keywords were used in searching: "cancer" and "neoplasm". A manual search was also done of references cited in the selected articles, and in selected reviews or books. Any abstract or article whose title or summary contained at least one intervention keyword and one method keyword, or one intervention keyword and one outcome keyword, was retrieved and read.

We reviewed all published independent trials evaluating the association of vitamin D and cancer incidence or mortality.

We also looked at the web site www.clinicaltrials.gov to find ongoing phase III trials on vitamin D intervention and cancer incidence or mortality and we focused the research on "recruiting" and "active" status.

We excluded trials that evaluated treatment with 1-alpha-hydroxy-vitamin D3 (alfacalcidol) or the physiologically active form of vitamin D, the 1,25(OH)2D (calcitriol), or other vitamin D analogues in patients with advanced cancer or chronic kidney disease. Calcitriol and other vitamin D analogues have seldom been tested for prevention purposes. The few small trials that used these compounds for fracture prevention reported a total of 20 deaths from all-causes [14].

RANDOMIZED TRIALS ON VITAMIN-D SUPPLEMENTS AND CANCER RISK

A randomized trial is the "gold standard" to establish a causal association. Trials with vitamin D have mainly been organized for the prevention of fracture in elderly people, usually in association with calcium supplements. Prevention studies with vitamin D have rarely been done in the context of vitamin D to evaluate a protective effect on cancer. The reasons are due to several unsolved issue including selection of the effective dose, varying baseline levels of subjects before randomization, compliance with the intervention, contamination of the placebo group (i.e., intake of vitamin D supplements by subjects allocated to the placebo group) and unknown effective lag time between start of the intervention and disease onset. Four double-blind, placebo-controlled randomized trials have examined the influence of vitamin D and calcium supplements on cancer risk [15-19].

Table **1** summarizes the published randomized trials on vitamin D and cancer risk. Results of vitamin D and calcium supplementation on diabetes [20], cardiovascular events [21], blood pressure [22], physical functioning [23] and risk of benign proliferative breast disease [24] were, by the majority, negative.

Table **2** presents data on vitamin D supplementation with cancer mortality derived from randomized controlled trials (RCTs).

Table **3** shows ongoing phase III clinical trials.

The UK Trial for the Prevention of Osteoporotic Fractures

In this trial by Trivedi *et al*. [16] the reduction of fracture risk was the primary objective and vitamin D supplementation was performed by a single dose of 100,000 IU every four months. Main finding was a significantly reduced risk of fracture, but no significant reduction in all-cancer risk was recorded (age-adjusted RR = 1.11; 95%CI: 0.86, 1.42). Baseline serum 25(OH)D levels were not measured. Blood samples collected during the trial showed that subjects in the intervention group had serum 25-hydroxyvitamin D level on average 40% higher than subjects in the control group. Means (standard deviation) of serum vitamin D concentrations in a subgroup of 238 participants were 74.3 (20.7) nmol/l vs. 53.4 (21.1) nmol/l in intervention and control group respectively $p < 0.001$; 75.6 (19) nmol/l vs. 61.0 (21.5) nmol/l in men, and 72.0 (22.5) nmol/l vs. 45.37 (17.6) nmol/l in women, $p < 0.001$ [16].

The Nebraska Trial

A 4-year, population-based, randomized placebo-controlled trial of vitamin D and calcium was conducted with the primary outcome of fracture incidence, and the principal secondary outcome of cancer incidence [15].

The subjects in the study were 1,179 community-dwelling women randomly selected from the population of healthy postmenopausal women aged 55. Subjects were randomly assigned to receive each day 1.4-1.5 g supplemental elementary calcium alone (Ca-only), supplemental calcium plus 1100 IU of vitamin D3 (Ca + D), or placebo. When analyzed by intention to treat, the authors found a remarkable 60% to 77% reduction in relative risk of cancer in the (Ca + D) women compared to the placebo group ($P < 0.03$). Authors concluded that improving calcium and vitamin D nutritional status substantially reduces all-cancer risk in postmenopausal women [15].

The intent-to-threat analysis comparing the (Ca + D) group with Ca-only (pooled with placebo) shows no significant decrease in cancer risk. In contrast, an intent-to-threat analysis of (Ca + D pooled with Ca-only) versus placebo shows a significant reduced cancer risk due to calcium supplements. The methodology and statistical analyses of this trial have been much criticized [25-27] and several authors point out that the design of the Nebraska trial was biased and its results were negative for vitamin D. The power of the study was too low to make definite conclusions on the effect of vitamin D and cancer risk. Furthermore, cancer incidence was unusually high in the placebo group, a bias that undermined the trial's findings [27].

The Women's Health Initiative Trial

Between 1993 and 1998, postmenopausal women 50 to 79 years of age were enrolled in the Women's Health Initiative (WHI) [28]. Main objectives of this randomized trial were to evaluate the risks and benefits of hormone therapy and dietary modification. The WHI Clinical Trial (CT) includes three overlapping components, each a randomized controlled comparison among women who were postmenopausal and 50 to 79 years of age at randomization. The dietary modification (DM) component randomly assigned 48,836

Table 1. Summary of Randomized Trials on Vitamin D and Cancer Incidence

First Author, Publication Year	Type of Randomization	Vitamin D Daily Dose (μg)*	Elementary Calcium Daily Dose (g)	Mean Trial Duration (Years)	No. In Intervention Group	No. In Control Group	Age at Inclusion	Endpoint	N° cases in Intervention Group	N° Cases in Control Group	Contrast Used for HR	HR (95% CI)**
Trivedi, 2003 [16]	Double-blind, placebo controlled	21	No calcium supplement	5	1345 men and women	1341 men and women	65-84	All Cancers Colorectal Cancer	144 28	130 27	D versus Placebo	1.11 (0.86-1.42) 1.02 (0.60-1.74)
Lappe, 2007 ***(Nebraska trial)*** [15]	Double-blind, placebo controlled Arms: -Ca+D -Ca -Placebo	27.5	1.5	4	446 women Vitamin D; 891 women Calcium	733 women vitamin D; 288 women calcium	> 55	All cancers	13 30	37 20	D+Ca versus Placebo	0.23 (0.09-0.60)§
Wactawski-Wende, 2006 (WHI) [18] **Chlebowski, 2008** [17] ***Update:*** **Brunner, 2011** [30] **Bolland, 2011** [31]	Double-blind, placebo controlled	10	1	7	18.176 women	18.106	50-79	All Cancers Colorectal Cancer Breast Cancer	1306 168 528	1333 154 546	D+Ca versus Placebo	0.98 (0.90-1.05) 0.86 (0.78, 0.96) ¥ 1.08 (0.86-1.34) 0.83 (0.60, 1.15) ¥ 0.96 (0.85, 1.09) 0.80 (0.66, 0.96) ¥
Avenell, 2011 (RECORD) [19]	Factorial-design trial. Arms: -D -Ca -D+Ca -Placebo.	20	1	2-4	3960 men and women	1332	70 years or older	All cancers			D or Ca+D versus Ca or placebo	1.07 (0.92-1.25)

D: vitamin D; Ca: Calcium ¥In women who were not taking personal calcium or vitamin D supplements at baseline.*Dose equivalence of Vitamin D: 1 μg = 40 IU. §Including only free of cancer at 1 y; ** HR or RR: Hazard ratio or Relative Risk;

Table 2. Trials on Vitamin D Supplements and Cancer Mortality

First Author, Publication Year	Arms	Cancer Deaths	HR (95% CI)
Trivedi, 2003 [15]	-Vitamin D -Placebo	63	0.86 (0.61-1.20)
Wactawski-Wende, 2006 [18] **(WHI)** **Update: Brunner, 2011** [30]	-Vitamin D + calcium -Placebo	744	0.90 (0.77–1.05)
Avenell, 2011 [19] ***(RECORD)***	-Vitamin D -Calcium -Vit D+ Calcium -Placebo	329	*****0.85 (0.68-1.06)

* Vitamin D versus no Vitamin D

(target 48,000) eligible women to either a sustained low-fat eating pattern (40%) or self-selected dietary behavior (60%), with breast cancer and colorectal cancer as designated primary outcomes and coronary heart disease as a secondary outcome. The postmenopausal hormone therapy (PHT) component comprises two randomized, double-blind trials among 27,347 (target 27,500) women, with coronary heart disease (CHD) as the primary outcome, with hip and other fractures as secondary outcomes, and with breast cancer as a potential adverse outcome. At their one year anniversary from DM and/or PHT trial enrollment all women were further screened for possible randomization in the calcium and vitamin D (CaD) component, a randomized double-blind trial of 1000 mg elemental calcium plus 400 IU of vitamin D daily, vs. placebo. The WHI CaD trial was designed to determine whether calcium plus vitamin D supplementation would prevent hip fracture [29] (primary outcome) and colorectal cancer [18] (a designated secondary outcome). Between 1995 and 2000, 36,282 post-menopausal women were randomized to 400 IU of vitamin D per day plus 1 g of elementary calcium, or to placebo. After a mean of 7 years' follow-up the intervention did not alter the risk of colorectal, breast or overall cancer incidence. There was also no association with the stage of colorectal cancer. During the entire trial period there was no divergence in the cumulative incidence of colorectal cancer between the two groups [18].

Table 3. Summary of Ongoing Randomized Trials on Vitamin D and Cancer

Trial	Status	Study Population	Gender	Age Groups	Study Design	Endpoints	Sample	Treatment Groups	Time Frame
Vitamin D3 and cancer prevention in postmenopausal women (CAPS)	Active, not recruiting	Postmenopausal women	Female	≥ 55	Randomized Double Blind Placebo controlled (Phase III)	Event free survival (cancer)	2300	1) vitamin D3 (2000 IU/d) and calcium (1500 mg/d); 2) vitamin D3 placebo; 3) calcium placebo	5 years
High Dose vs. Standard Dose Vit D2 with Docetaxel in Met. Breast ca. (GORG 002)	Recruiting	Metastatic breast cancer	Female	≥ 18	Randomized Single blind (Phase III)	Time to progression is from the start of Docetaxel to disease progression. Overall survival	260	1)Docetaxel + High dose Vitamin D2; 2)Docetaxel + Standard dose Vitamin D2	1 year
Vitamin D Calcium Polyp Prevention Study	Active, not recruiting	Colorectal Cancer Polyps; Adenomas	Both	45 to 75	Randomized Double Blind Placebo controlled 2 x 2 factorial design (phase II/III)	Event free servival (Colorectal Cancer Polyps Adenomas)	2200	1) vitamin D (1000 IU/day), calcium carbonate (1200 mg elemental calcium/day); 2) placebo	1 to 5 years
Vitamin D for Stage II Melanoma (MelaViD)	Recruiting	Melanoma (resected stage II)	Both	18 to 75	Randomized, Double Blind Placebo controlled (Phase III)	Disease free (melanoma recurrence) and Overall survival	878	1)Vitamin D3 100000 IU every 50 days; 2) placebo	3 years of treatment and 2 years of follow-up.
Vitamin D and Longevity : Randomized Feasibility Study (VITAL)	Ongoing	General population	Both	<65 or >84	Randomized controlled trial, open or double-blind placebo control (Phase III Feasibility study)	Determination of the most cost-effective randomization and follow-up procedures for the main clinical trial.	1600	1) 100,000 IU monthly of oral vitamin D3; 2)Placebo.	2 years
The Effect of 25-OH-Vitamin-D3 Substitution in Patients With Malignant and Immune-hematologic Diseases (D-HEM)	Recruiting	Chronic Lymphoid Leukemia	Both	≥ 18	Randomized, Double Blind Placebo controlled (Phase III)	Overall Survival	300	1)180,000 IU monthly of oral vitamin D3 2)Placebo	Up to 5 years

After a review of initial findings, a nested case-control study organized within the WHI trial including colorectal cancer cases and 317 matched controls (matching on age, race, centre, and date of blood sample), showed that the risk of colorectal cancer increased with decreasing serum 25(OH)D levels at baseline. Results were suggestive of a possible interaction between baseline serum 25(OH)D levels and vitamin D supplement intake i.e. the impact of supplements on colorectal cancer risk increased with decreasing baseline levels, but the interaction term was not statistically significant (p=0.54). However, to fully examine such an interaction, this nested-case-control study should have included at least ten times more subjects.

Breast cancer incidence was also analyzed. Invasive breast cancer incidence was similar in the two groups (528 supplement vs. 546 placebo; hazard ratio = 0.96; 95% confidence interval = 0.85 to 1.09). In the nested case – control study, no effect of supplement group assignment on breast cancer risk was seen. Baseline 25-hydroxyvitamin D levels were modestly correlated with total vitamin D intake (diet and supplements) (r= 0.19, P < .001) and were higher among women with lower BMI and higher recreational physical activity (both P < .001). Baseline 25-hydroxyvitamin D levels were not associated with breast cancer risk in analyses that were adjusted for BMI and physical activity (P_{trend} = .20). However, in the intervention group, the mean size of invasive breast cancer was 1.54 cm (SD: 1.23) versus 1.71 cm (SD: 1.29) in the control group (P=0.05) [17].

In 2011 Brunner and colleagues [30] examined the treatment effect on incidence and mortality for all invasive cancers. After 7.0 years, 1,306 invasive cancers were diagnosed in the treatment and 1,333 in the placebo group [hazard ratio (HR) = 0.98; 95%CI: 0.90, 1.05]. Mortality did not differ between the supplement and the placebo group [HR = 0.90; 95%CI: 0.77, 1.05]. By contrast, supplementation lowered cancer risk in the WHI healthy diet trial arm and in women without a first degree relative with cancer.

A further analysis of WHI CaD took into account that more than half of the participants were taking personal, non protocol calcium or vitamin D supplements at randomization. Personal calcium supplements of up to 1 g/d and personal vitamin D supplements of up to 600 IU/d (and later 1000 IU/d) were permitted in WHI CaD. A reanalysis of the publicly available limited-access WHI clinical trials database showed that the use of personal

calcium or vitamin D supplements at randomization significantly influenced the effect of CaD on cancer. In the entire WHI cohort, significant interactions were found between allocation to CaD and personal calcium and/or vitamin D supplement use for total, breast, and colorectal cancers. In the 43% of WHI CaD participants who were not taking personal calcium or vitamin D supplements at randomization, CaD decreased the risk of total, breast, and colorectal cancers by 14–20%. In contrast, in WHI CaD participants taking personal calcium or vitamin D supplements at randomization, allocation to CaD did not significantly alter cancer risk [31]. On the basis of data from women not taking personal calcium or vitamin D at randomization, the treatment of 1000 women with CaD for 5 y would prevent 5 breast cancers, 1 colorectal cancer, and 8 total cancers. The analyses of women taking personal calcium or vitamin D supplements, which compared higher doses of CaD with lower doses of CaD, suggest that higher doses of CaD do not further decrease cancer incidence compared with lower doses of CaD. These results suggest that there may be a threshold effect, rather than a dose-dependent effect of CaD on these endpoints.

In subgroup analyses, women with history of non melanoma skin cancer (NMSC) assigned to CaD had a reduced risk of melanoma by 57% versus those receiving placebo (HR=0.43; 95%CI: 0.21, 0.90; P interaction=0.04). This latter effect was not observed in women without history of NMSC. Additionally, CaD supplementation tended to reduce risk of melanoma in a subgroup with higher BMI, lower baseline vitamin D intake and lower ultraviolet exposure [32].

Some considerations on this study are mandatory:

-Insufficient trial duration [33,34]: time between vitamin D action and change in colorectal cancer occurrence could be longer than 7 years. However, in the last years of the WHI trial, there was no indication in the data for an eventual start of a reduction in colorectal cancer incidence. Data even showed that at the end of the trial colorectal cancer incidence was slightly higher in women supplemented with vitamin D and calcium than in women receiving placebo.

-Low compliance to supplementation. Throughout the entire trial duration, only 50 to 60% of women took 80% of the scheduled supplementation regimen [18]. In this context it should be noted that in analyses restricted to women adhering to the regimen found hip fracture risk was reduced by 29% (95%CI: -48%, -3%) whereas there was no significant effect on hip fracture in the entire WHI study population [29].

-Insufficient doses of vitamin D supplements might be another limitation of the WHI trial but we can only speculate about this issue since baseline and in-study measurements of serum 25(OH)D D levels were not available for the whole study population. Lack of this information also hampers examinations of whether vitamin D supplements would be beneficial in subjects with low vitamin D status at baseline.

-Another possible explanation for the null results is that the study design allowed off-protocol calcium and/or vitamin D supplementation; women were allowed to take up to 600 IU of vitamin D daily initially and up to 1,000 IU daily from 1999 onward. It is therefore conceivable that some women in the placebo group were taking more vitamin D compared to women in the treatment group. This might have limited the ability to detect significant treatment effects by vitamin D supplementation. This notion is further supported by the fact that in WHI CaD the use of personal calcium or vitamin D supplements at randomization significantly influenced the effect of CaD on the risk of cancer. In 15,646 women (43%) who were not taking personal calcium or vitamin D supplements at randomization, CaD significantly decreased the risk of total, breast, and invasive breast cancers by 14–20% and non significantly reduced the risk of colorectal cancer by 17%. In women taking personal calcium or vitamin D supplements, CaD did not alter cancer risk [31].

-Many interactions seem to exist between vitamin D and other substances, for instance, menopause hormone therapy (MHT) and calcium. The WHI trial was initially designed for testing the impact of MHT on various health conditions. So, the trial organized with vitamin D and calcium supplementation randomized women some of whom were already assigned to taking active MHT and others already assigned to taking the MHT placebo. Reanalysis of the WHI trial results found that concurrent active MHT led to increased colorectal cancer risk associated with calcium plus vitamin-D supplementation (HR=1.50, 95%CI: 0.96, 2.33) while placebo MHT led to decreased colorectal cancer risk associated with calcium plus vitamin-D supplementation (HR= 0.71, 95%CI: 0.46, 1.09; P-value for-estrogen-interaction = 0.02) [35]. Consistent interaction was also found by reported estrogen use (p interaction = 0.04). These results suggest that biological interactions between vitamin D, calcium and estrogens at the cellular level may have reduced the potential beneficial influence of vitamin D and calcium supplementation in the prevention of colorectal cancer.

-Calcium is another compound of possible interaction. For instance, randomized trials have shown that calcium supplements in the order of 1.2 to 2.0 g of elemental calcium per day during 3 or 4 years may decrease the recurrence of colonic adenoma [36]. This protective effect on polyps and also on colorectal cancer was more pronounced when serum 25(OH)D levels were high [37,38]. We do not know whether 25-hydroxyvitamin D levels obtained in the intervention group in the WHI trial were high enough to influence the effect of calcium.

The RECORD Trial

Avenell A. *et al.* published the results on cancer incidence and mortality of the RECORD Trial, a randomized placebo-controlled trial of vitamin D3 and/or calcium. The RECORD Trial was a pragmatic, factorial-designed trial for the secondary prevention of fragility fractures in 5292 old people (85% women), aged at least 70 yr, with previous low-trauma fracture, from 21 orthopedic centers in the United Kingdom [19].

Participants were randomly allocated to daily vitamin D3 (800 IU), calcium (1000 mg), both, or placebo for 24–62 months, with a follow-up of 3 yr after intervention. Main outcome measures included all-cause mortality, vascular disease mortality, cancer mortality, and cancer incidence. In a post hoc statistical analysis adjusting for compliance, thus with fewer participants, trends for reduced mortality with vitamin D and increased mortality with calcium were observed, although all results remain non significant. In conclusion, daily vitamin D or calcium supplementation did not affect mortality, vascular disease, cancer mortality, or cancer incidence [19].

Trials and Cancer Mortality

Three randomized trials presented estimates for cancer mortality and results suggest a non significant decreased risk for cancer mortality (Table **2**). However this was not the main endpoint for none of them and the issue of low statistical power has to be considered for all of them.

No other randomized trial on vitamin D supplementation and any health condition (e.g., fracture risk) reported relevant details on cancer mortality.

Other Ongoing Trials

Further trials are ongoing to assess the preventive and therapeutic effect of vitamin D.

CAPS Trial: Clinical Trial of Vitamin D3 to Reduce Cancer Risk in Postmenopausal Women

The purpose of this study is to determine whether vitamin D3 and calcium supplementation decreases the risk of developing

cancer. The estimated enrollment is 2300 women. Subjects will be randomized to one of two treatment groups: 1) vitamin D3 (2000 IU/d) and calcium (1500 mg/d), or 2) vitamin D3 placebo and calcium placebo, and they will be followed for four years. The principal aim is to determine the effect of supplementation with vitamin D3 and calcium on incidence of all types of cancer combined and to determine in a nested-case control study the association of serum 25(OH)D collected at randomization and at the end of year one of the study with risk of cancer over four years. Other aims include the effect of supplementation with calcium and vitamin D3 on incidence of specific cancers (breast, lung, colon, myeloma, leukemia, and lymphoma) and on incidence of other disorders, specifically hypertension, cardiovascular disease, osteoarthritis, colonic adenomas, diabetes, upper respiratory infections, fractures, and falls. This study is ongoing, but not recruiting participants. Last Updated on November 3, 2011.

GORG - 002 Randomized Phase III Trial to Determine the Effectiveness of High Dose Versus Standard Dose of Vitamin D2 (Ergocalciferol) Given With Docetaxel in Patients With Metastatic Breast Cancer

This is a randomized phase III trial to determine the effectiveness of High dose versus Standard dose of Vitamin D2 (Ergocalciferol) given with Docetaxel in 260 patients with metastatic breast cancer. Primary endpoint is time to progression from the start of Docetaxel to disease progression. Secondary endpoint is overall survival, defined as the time from start of Docetaxel to death due to any cause. This study is currently recruiting participants. Last Updated on December 11, 2011.

Vitamin D/Calcium Polyp Prevention Study

This study is a phase II/III double-blind, placebo-controlled trial of vitamin D and/or calcium supplementation for the prevention of large bowel adenomas. Estimated enrollment is 2200 subjects. They will be recruited from 10 Study Centers in North America. Main inclusion criterion is one large bowel adenoma removed in the 4 months prior to study entry and no remaining polyps in the bowel after complete colonoscopic examination. Participants will be randomized in a modified 2 x 2 factorial design to vitamin D (1000 IU/day), calcium carbonate (1200 mg elemental calcium/day), both agents, or placebo only. The primary endpoint is the incidence of colorectal adenomas and colorectal cancers. This study is ongoing, but not recruiting participants. Last Updated on October 19, 2011.

MelaViD: A Trial on Vitamin D Supplementation for Resected Stage II Melanoma Patients

The purpose of this phase III, randomized, double blind trial is to assess the effect of vitamin D supplementation on recurrence in resected stage II melanoma patients. Patients (878) will be randomly assigned to vitamin D3 100,000 IU every 50 days or placebo for 3 years. The primary endpoint is disease free survival and overall survival. Secondary endpoints include: the evaluation at baseline of Vitamin D receptors and 25(OH)D by Breslow thickness; change in time of 25(OH)D serum level by Vitamin D receptor and other genes involved in vitamin D metabolisms; percentages of patients at desired levels of 25(OH)D (30 ng/ml) during 1 year; toxicity; compliance. This study is currently recruiting participants. Last Updated on August 2, 2011.

Vitamin D and Longevity (VITAL) Trial: Randomized Feasibility Study

A 2-year feasibility study on 1,600 patients (800 taking vitamin D and 800 controls) was proposed as an essential first step to develop cost-effective recruitment and follow-up procedures and monitor vitamin D levels in treated and control subjects before the beginning of the main trial. In this phase III feasibility trial participants will receive either: 1) 100,000 IU monthly (average 3300 IU/day) of oral vitamin D3 or double-blind placebo control (800 participants) 2) 100,000 IU monthly (average 3300 IU/day) of oral vitamin D3 or open control (800 participants). The principal aims are to demonstrate feasibility in a representative range of GP practices and to determine the most cost-effective randomization and follow-up procedures for the main study.

The Rationale and design of the main trial, the VITamin D and OmegA-3 TriaL (VITAL), have recently been published [39]. This is a large randomized, double-blind, placebo-controlled, 2x2 factorial trial of vitamin D (in the form of vitamin D3 [cholecalciferol], 2000 IU/day) and marine omega-3 fatty acid (Omacor® fish oil, eicosapentaenoic acid [EPA]+docosahexaenoic acid [DHA], 1 g/day) supplements in the primary prevention of cancer and cardio vascular disease among a multi-ethnic population of 20,000 U.S. men aged $\geq$ 50 and women aged $\geq$ 55. The mean treatment period will be 5 years. Baseline blood samples will be collected in at least 16,000 participants, with follow-up blood collection in about 6000 participants. Yearly follow-up questionnaires will assess treatment compliance (plasma biomarker measures will also assess compliance in a random sample of participants), use of non-study drugs or supplements, occurrence of endpoints, and cancer and vascular risk factors. Self-reported endpoints will be confirmed by medical record review by physicians blinded to treatment assignment, and deaths will be ascertained through national registries and other sources [39].

Phase III, Controlled, Double-blind, Randomized Study of 25-OH-Vitamin-D3 Substitution in Patients With Malignant and Immune-hematologic Diseases(D-HEM)

In this study the investigators examine the role of the adequate vitamin D substitution in the improvement of the outcomes of haematologic disorders. The issue is whether the normalization of vitamin D status in patient with vitamin D inadequacy is able to improve the prognosis and survival. Patient (300) with 25-OH-Vitamin-D3 level between 10 and 30 ng/mL will be randomly assigned to Vitamin D3 180,000 IU monthly or placebo. The primary endpoint is overall survival. This study is currently recruiting participants. Last Updated on February 17, 2012.

DISCUSSION

All randomized trials found that mortality from cancer was reduced, although not significantly. Lappe *et al.* in 2007 also reported a reduced risk of cancer on calcium and vitamin D compared with placebo group. The WHI trial found that vitamin D was significantly associated to a reduced cancer risk in subgroup analyses restricted to participants without concomitant intake of calcium and/or vitamin D [17,18].

However we cannot draw definite conclusions on cancer risk or mortality since these endpoints could not be investigated by these randomized trials on vitamin D supplements as they were neither designed nor sufficiently powered for that objective. Furthermore the review was limited by the absence of reporting of all cancer events or causes of mortality by all trials.

Vitamin D Dose

The recommendation for this nutrient, so far, is mostly based on its role in bone health, and the American Institute of Medicine (IOM) has set the daily recommended dose as 600 to 800 IU. The doses used in all trials are ordinary doses of vitamin D supplements as for the prevention of fractures [14,40,41] and we do not know the exact dose that could be effective for cancer risk or mortality. However we know that daily vitamin D doses in the range of 10 to 20 μg (400-800 IU) per day were able to significantly

decrease all-cause mortality by 7% [6]. The negative results, or the unknown results, on specific causes of death diseases, but positive results on all-cause mortality support the notion that apparently "low doses" of vitamin D nevertheless have significant physiological impact.

The likelihood of the mortality reduction associated with the use of 10 to 20 μg (400-800 IU) per day of vitamin D supplements is supported by recent observations that patients with chronic kidney disease receiving vitamin D supplements have better overall survival [42]. The biological mechanisms underlying the gain in life expectancy remain obscure but are probably not (mainly) mediated by a reduction in cancer risk.

Vitamin D and Calcium

The WHI trial studied CaD, so it is not possible to determine whether the observed effects on cancer were due to calcium, vitamin D, or the combination of agents.

Few data from randomized controlled trials—other than WHI—have assessed the effect of calcium or vitamin D, individually or in combination, on cancer outcomes.

Trivedi *et al*. reported no significant effects of 4 monthly doses of 100,000 IU vitamin D on cancer incidence or mortality in 2686 people followed for 5 years [16]. Lappe *et al*. [15] reported a 60% reduction in cancer incidence with CaD (P = 0.01) and a 47% reduction with calcium alone (P = 0.06) in 1179 women followed for 4 years, although the apparent reductions might largely be due to an unexpectedly high incidence of cancer in the placebo group.

Thus, data from existing randomized controlled trials do not allow a definitive answer to this issue, but raise the possibility that combination therapy is required for cancer prevention.

Vitamin D Serum Level

Individual vitamin D status as measured by serum 25(OH)D level may be rather a cancer risk marker than a risk factor: low vitamin D status would reflect an individual's propensity to develop a cancer. This propensity would be associated with lifestyle, e.g., obesity, smoking, low physical activity. If the risk marker hypothesis is real, then serum 25(OH) D level would be a predictor of cancer risk. If the risk factor hypothesis is true, then supplementation with vitamin D should reduce cancer occurrence. The failure of randomized trials to show a decreased incidence of cancer favors the risk marker hypothesis. The discovery by the nested case-control study organized within the WHI trial that women developing a colorectal cancer had lower serum vitamin D at baseline than women who did not develop this cancer further supports the risk marker hypothesis.

CONCLUSION

Available data from published RCTs are, in our opinion, not sufficient to draw final conclusions on the effect of vitamin D on cancer.

Several issues were raised on the validity of the results. First of all, RCTs included study participants irrespective of their 25(OH)D level and may thus have failed to detect significant treatment effects in vitamin D deficient individuals.

Previous randomized trials of vitamin D use and cancer may not have correctly addressed the impact of vitamin D supplementation on cancer outcomes, and therefore new randomized trials should be organized. Given that there are several randomized trials ongoing we can expect that future trial results will shed more light on the role of vitamin D in cancer prevention.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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REFERENCES

[1] Ross, A.C.; Manson, J.E.; Abrams, S.A.; Aloia, J.F.; Brannon, P.M.; Clinton, S.K.; Durazo-Arvizu, R.A.; Gallagher, J.C.; Gallo, R.L.; Jones, G.; Kovacs, C.S.; Mayne, S.T.; Rosen, C.J.; Shapses, S.A. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J. Clin. Endocrinol. Metab.*, **2011**, *96*(1), 53-58.

[2] Holick, M.F. Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. *Am. J. Clin. Nutr.*, **2004**, *79*(3), 362-371.

[3] Deeb, K.K.; Trump, D.L.; Johnson, C.S. Vitamin D signalling pathways in cancer: potential for anticancer therapeutics. *Nat. Rev. Cancer*, **2007**, *7*(9), 684-700.

[4] Park, Y.; Leitzmann, M.F.; Subar, A.F.; Hollenbeck, A.; Schatzkin, A. Dairy food, calcium, and risk of cancer in the NIH-AARP Diet and Health Study. *Arch. Intern. Med.*, **2009**, *169*(4), 391-401.

[5] Body, J.J.; Bergmann, P.; Boonen, S.; Devogelaer, J.P.; Gielen, E.; Goemaere, S.; Kaufman, J.M.; Rozenberg, S.; Reginster, J.Y. Extraskeletal benefits and risks of calcium, vitamin D and anti-osteoporosis medications. *Osteoporos. Int.*, **2012**, *23 Suppl 1:*1-23.

[6] Autier, P. and Gandini, S. Vitamin D supplementation and total mortality: a meta-analysis of randomized controlled trials. *Arch. Intern. Med.*, **2007**, *167*(16), 1730-1737.

[7] Bjelakovic, G.; Gluud, L.L.; Nikolova, D.; Whitfield, K.; Wetterslev, J.; Simonetti, R.G.; Bjelakovic, M.; Gluud, C. Vitamin D supplementation for prevention of mortality in adults. *Cochrane. Database. Syst. Rev.*, **2011**, (7):CD007470.

[8] Gandini, S.; Boniol, M.; Haukka, J.; Byrnes, G.; Cox, B.; Sneyd, M.J.; Mullie, P.; Autier, P. Meta-analysis of observational studies of serum 25-hydroxyvitamin D levels and colorectal, breast and prostate cancer and colorectal adenoma. *Int. J. Cancer*, **2011**, *128*(6), 1414-1424.

[9] Holick, M.F. and Chen, T.C. Vitamin D deficiency: a worldwide problem with health consequences. *Am. J. Clin. Nutr.*, **2008**, *87*(4), 1080S-1086S.

[10] Holick, M.F. Vitamin D deficiency. *N. Engl. J. Med.*, **2007**, *357*(3), 266-281.

[11] Gorham, E.D.; Garland, C.F.; Garland, F.C.; Grant, W.B.; Mohr, S.B.; Lipkin, M.; Newmark, H.L.; Giovannucci, E.; Wei, M.; Holick, M.F. Vitamin D and prevention of colorectal cancer. *J. Steroid Biochem. Mol. Biol.*, **2005**, *97*(1-2), 179-194.

[12] Bischoff-Ferrari, H.A.; Giovannucci, E.; Willett, W.C.; Dietrich, T.; wson-Hughes, B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *Am. J. Clin. Nutr.*, **2006**, *84*(1), 18-28.

[13] Zittermann, A.; Iodice, S.; Pilz, S.; Grant, W.B.; Bagnardi, V.; Gandini, S. Vitamin D deficiency and mortality risk in the general population: a meta-analysis of prospective cohort studies. *Am. J. Clin. Nutr.*, **2012**, *95*(1), 91-100.

[14] Avenell, A.; Gillespie, W.J.; Gillespie, L.D.; O'Connell, D.L. Vitamin D and vitamin D analogues for preventing fractures associated with involutional and post-menopausal osteoporosis. *Cochrane. Database. Syst. Rev.*, **2005**, (3):CD000227.

[15] Lappe, J.M.; Travers-Gustafson, D.; Davies, K.M.; Recker, R.R.; Heaney, R.P. Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial. *Am. J. Clin. Nutr.*, **2007**, *85*(6), 1586-1591.

[16] Trivedi, D.P.; Doll, R.; Khaw, K.T. Effect of four monthly oral vitamin D3 (cholecalciferol) supplementation on fractures and mortality in men and women living in the community: randomised double blind controlled trial. *BMJ*, **2003**, *326*(7387), 469.

[17] Chlebowski, R.T.; Johnson, K.C.; Kooperberg, C.; Pettinger, M.; Wactawski-Wende, J.; Rohan, T.; Rossouw, J.; Lane, D.; O'Sullivan, M.J.; Yasmeen, S.; Hiatt, R.A.; Shikany, J.M.; Vitolins, M.; Khandekar, J.; Hubbell, F.A. Calcium plus vitamin D supplementation and the risk of breast cancer. *J. Natl. Cancer Inst.*, **2008**, *100*(22), 1581-1591.

[18] Wactawski-Wende, J.; Kotchen, J.M.; Anderson, G.L.; Assaf, A.R.; Brunner, R.L.; O'Sullivan, M.J.; Margolis, K.L.; Ockene, J.K.; Phillips, L.; Pottern, L.; Prentice, R.L.; Robbins, J.; Rohan, T.E.; Sarto, G.E.; Sharma, S.; Stefanick, M.L.; Van, H.L.; Wallace, R.B.;

Whitlock, E.; Bassford, T.; Beresford, S.A.; Black, H.R.; Bonds, D.E.; Brzyski, R.G.; Caan, B.; Chlebowski, R.T.; Cochrane, B.; Garland, C.; Gass, M.; Hays, J.; Heiss, G.; Hendrix, S.L.; Howard, B.V.; Hsia, J.; Hubbell, F.A.; Jackson, R.D.; Johnson, K.C.; Judd, H.; Kooperberg, C.L.; Kuller, L.H.; LaCroix, A.Z.; Lane, D.S.; Langer, R.D.; Lasser, N.L.; Lewis, C.E.; Limacher, M.C.; Manson, J.E. Calcium plus vitamin D supplementation and the risk of colorectal cancer. *N. Engl. J Med.*, **2006**, *354*(7), 684-696.

[19] Avenell, A.; Maclennan, G.S.; Jenkinson, D.J.; McPherson, G.C.; McDonald, A.M.; Pant, P.R.; Grant, A.M.; Campbell, M.K.; Anderson, F.H.; Cooper, C.; Francis, R.M.; Gillespie, W.J.; Robinson, C.M.; Torgerson, D.J.; Wallace, W.A. Long-Term Follow-Up for Mortality and Cancer in a Randomized Placebo-Controlled Trial of Vitamin D3 and/or Calcium (RECORD Trial). *J.Clin. Endocrinol. Metab*, **2012**, *97*(2), 614-622.

[20] de, B., I; Tinker, L.F.; Connelly, S.; Curb, J.D.; Howard, B.V.; Kestenbaum, B.; Larson, J.C.; Manson, J.E.; Margolis, K.L.; Siscovick, D.S.; Weiss, N.S. Calcium plus vitamin D supplementation and the risk of incident diabetes in the Women's Health Initiative. *Diabetes Care*, **2008**, *31*(4), 701-707.

[21] Wang, T.J.; Pencina, M.J.; Booth, S.L.; Jacques, P.F.; Ingelsson, E.; Lanier, K.; Benjamin, E.J.; D'Agostino, R.B.; Wolf, M.; Vasan, R.S. Vitamin D deficiency and risk of cardiovascular disease. *Circulation*, **2008**, *117*(4), 503-511.

[22] Margolis, K.L.; Ray, R.M.; Van, H.L.; Manson, J.E.; Allison, M.A.; Black, H.R.; Beresford, S.A.; Connelly, S.A.; Curb, J.D.; Grimm, R.H., Jr.; Kotchen, T.A.; Kuller, L.H.; Wassertheil-Smoller, S.; Thomson, C.A.; Torner, J.C. Effect of calcium and vitamin D supplementation on blood pressure: the Women's Health Initiative Randomized Trial. *Hypertension*, **2008**, *52*(5), 847-855.

[23] Brunner, R.L.; Cochrane, B.; Jackson, R.D.; Larson, J.; Lewis, C.; Limacher, M.; Rosal, M.; Shumaker, S.; Wallace, R. Calcium, vitamin D supplementation, and physical function in the Women's Health Initiative. *J. Am. Diet. Assoc.*, **2008**, *108*(9), 1472-1479.

[24] Rohan, T.E.; Negassa, A.; Chlebowski, R.T.; Ceria-Ulep, C.D.; Cochrane, B.B.; Lane, D.S.; Ginsberg, M.; Wassertheil-Smoller, S.; Page, D.L. A randomized controlled trial of calcium plus vitamin D supplementation and risk of benign proliferative breast disease. *Breast Cancer Res. Treat.*, **2009**, *116*(2), 339-350.

[25] Sood, M.M. and Sood, A.R. Dietary vitamin D and decreases in cancer rates: Canada as the national experiment. *Am. J. Clin. Nutr.*, **2007**, *86*(5), 1549-1550.

[26] Ojha, R.P.; Felini, M.J.; Fischbach, L.A. Vitamin D for cancer prevention: valid assertion or premature anointment? *Am. J. Clin. Nutr.*, **2007**, *86*(6), 1804-1805.

[27] Schabas, R. Artifact in the control group undermines the conclusions of a vitamin D and cancer study. *Am. J. Clin. Nutr.*, **2008**, *87*(3), 792-794.

[28] Anderson, G.L.; Manson, J.; Wallace, R.; Lund, B.; Hall, D.; Davis, S.; Shumaker, S.; Wang, C.Y.; Stein, E.; Prentice, R.L. Implementation of the Women's Health Initiative study design. *Ann. Epidemiol.*, **2003**, *13*(9 Suppl), S5-17.

[29] Jackson, R.D.; LaCroix, A.Z.; Gass, M.; Wallace, R.B.; Robbins, J.; Lewis, C.E.; Bassford, T.; Beresford, S.A.; Black, H.R.; Blanchette, P.; Bonds, D.E.; Brunner, R.L.; Brzyski, R.G.; Caan, B.; Cauley, J.A.; Chlebowski, R.T.; Cummings, S.R.; Granek, I.; Hays, J.; Heiss, G.; Hendrix, S.L.; Howard, B.V.; Hsia, J.; Hubbell, F.A.; Johnson, K.C.; Judd, H.; Kotchen, J.M.; Kuller, L.H.; Langer, R.D.; Lasser, N.L.; Limacher, M.C.; Ludlam, S.; Manson, J.E.; Margolis, K.L.; McGowan, J.; Ockene, J.K.; O'Sullivan, M.J.; Phillips, L.; Prentice, R.L.; Sarto, G.E.; Stefanick, M.L.; Van, H.L.; Wactawski-Wende, J.; Whitlock, E.; Anderson, G.L.; Assaf, A.R.; Barad, D. Calcium plus vitamin D supplementation and the risk of fractures. *N. Engl. J. Med.*, **2006**, *354*(7), 669-683.

[30] Brunner, R.L.; Wactawski-Wende, J.; Caan, B.J.; Cochrane, B.B.; Chlebowski, R.T.; Gass, M.L.; Jacobs, E.T.; LaCroix, A.Z.; Lane, D.; Larson, J.; Margolis, K.L.; Millen, A.E.; Sarto, G.E.; Vitolins, M.Z.; Wallace, R.B. The effect of calcium plus vitamin D on risk for invasive cancer: results of the Women's Health Initiative (WHI) calcium plus vitamin D randomized clinical trial. *Nutr. Cancer*, **2011**, *63*(6), 827-841.

[31] Bolland, M.J.; Grey, A.; Gamble, G.D.; Reid, I.R. Calcium and vitamin D supplements and health outcomes: a reanalysis of the Women's Health Initiative (WHI) limited-access data set. *Am. J. Clin. Nutr.*, **2011**, *94*(4), 1144-1149.

[32] Tang, J.Y.; Fu, T.; Leblanc, E.; Manson, J.E.; Feldman, D.; Linos, E.; Vitolins, M.Z.; Zeitouni, N.C.; Larson, J.; Stefanick, M.L. Calcium plus vitamin D supplementation and the risk of nonmelanoma and melanoma skin cancer: post hoc analyses of the women's health initiative randomized controlled trial. *J. Clin. Oncol.*, **2011**, *29*(22), 3078-3084.

[33] Holick, M.F. Calcium plus vitamin D and the risk of colorectal cancer. *N. Engl. J. Med.*, **2006**, *354*(21), 2287-2288.

[34] Giovannucci, E.; Liu, Y.; Rimm, E.B.; Hollis, B.W.; Fuchs, C.S.; Stampfer, M.J.; Willett, W.C. Prospective study of predictors of vitamin D status and cancer incidence and mortality in men. *J. Natl. Cancer Inst.*, **2006**, *98*(7), 451-459.

[35] Ding, E.L.; Mehta, S.; Fawzi, W.W.; Giovannucci, E.L. Interaction of estrogen therapy with calcium and vitamin D supplementation on colorectal cancer risk: reanalysis of Women's Health Initiative randomized trial. *Int. J. Cancer*, **2008**, *122*(8), 1690-1694.

[36] Weingarten, M.A.; Zalmanovici, A.; Yaphe, J. Dietary calcium supplementation for preventing colorectal cancer and adenomatous polyps. *Cochrane. Database. Syst. Rev.*, **2008**, (1):CD003548.

[37] Grau, M.V.; Baron, J.A.; Sandler, R.S.; Haile, R.W.; Beach, M.L.; Church, T.R.; Heber, D. Vitamin D, calcium supplementation, and colorectal adenomas: results of a randomized trial. *J. Natl. Cancer Inst.*, **2003**, *95*(23), 1765-1771.

[38] Jenab, M.; Bueno-de-Mesquita, H.B.; Ferrari, P.; van Duijnhoven, F.J.; Norat, T.; Pischon, T.; Jansen, E.H.; Slimani, N.; Byrnes, G.; Rinaldi, S.; Tjonneland, A.; Olsen, A.; Overvad, K.; Boutron-Ruault, M.C.; Clavel-Chapelon, F.; Morois, S.; Kaaks, R.; Linseisen, J.; Boeing, H.; Bergmann, M.M.; Trichopoulou, A.; Misirli, G.; Trichopoulos, D.; Berrino, F.; Vineis, P.; Panico, S.; Palli, D.; Tumino, R.; Ros, M.M.; van Gils, C.H.; Peeters, P.H.; Brustad, M.; Lund, E.; Tormo, M.J.; Ardanaz, E.; Rodriguez, L.; Sanchez, M.J.; Dorronsoro, M.; Gonzalez, C.A.; Hallmans, G.; Palmqvist, R.; Roddam, A.; Key, T.J.; Khaw, K.T.; Autier, P.; Hainaut, P.; Riboli, E. Association between pre-diagnostic circulating vitamin D concentration and risk of colorectal cancer in European populations:a nested case-control study. *BMJ*, **2010**, 340:b5500. doi: 10.1136/bmj.b5500

[39] Manson, J.E.; Bassuk, S.S.; Lee, I.M.; Cook, N.R.; Albert, M.A.; Gordon, D.; Zaharris, E.; Macfadyen, J.G.; Danielson, E.; Lin, J.; Zhang, S.M.; Buring, J.E. The VITamin D and OmegA-3 TriaL (VITAL): rationale and design of a large randomized controlled trial of vitamin D and marine omega-3 fatty acid supplements for the primary prevention of cancer and cardiovascular disease. *Contemp. Clin. Trials*, **2012**, *33*(1), 159-171.

[40] Cranney, A.; Horsley, T.; O'Donnell, S.; Weiler, H.; Puil, L.; Ooi, D.; Atkinson, S.; Ward, L.; Moher, D.; Hanley, D.; Fang, M.; Yazdi, F.; Garritty, C.; Sampson, M.; Barrowman, N.; Tsertsvadze, A.; Mamaladze, V. Effectiveness and safety of vitamin D in relation to bone health. *Evid. Rep. Technol. Assess. (Full. Rep.)*, **2007**, (158):1-235.

[41] Tang, B.M. Does calcium supplementation really cause more hip fractures? *Osteoporos. Int.*, **2009**, *20*(5), 833-834.

[42] Lishmanov, A.; Dorairajan, S.; Pak, Y.; Chaudhary, K.; Chockalingam, A. Treatment of 25-OH Vitamin D Deficiency in Older Men With Chronic Kidney Disease Stages 3 and 4 Is Associated With Reduction in Cardiovascular Events. *Am. J. Ther.*, **2011**, [Epub ahead of print] PubMed PMID: 22185755.

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