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Vitamin D and Breast Cancer Incidence and Outcome

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Abstract: Based on preclinical studies and early clinical observations, an association between vitamin D status and breast cancer incidence and outcome has been proposed. Against this background, information on vitamin D and breast cancer was reviewed with focused attention on emerging clinical studies in this area. Prospective cohort studies do not associate 25-hydroxyvitamin D levels with breast cancer incidence. While case-control studies of this question are positive, they may be confounded by reverse causality as 25-hydroxyvitamin D levels are influenced by breast cancer presence and stage. Studies of 25-hydroxyvitamin D and subsequent breast cancer recurrence provide mixed results but strongest associations were seen in analyses uncontrolled for prognostic variables, cancer therapy, BMI and physical activity. The one full-scale randomized, placebo-controlled trial evaluating calcium (1000 mg elemental calcium per day) and vitamin D supplementation (400 IU D_3 per day) with 36,282 participants failed to demonstrate a supplement effect on lowering breast cancer incidence. Breast cancer patients not uncommonly have vitamin D deficiency but limited control populations in available reports preclude precise prevalence estimates. As breast cancer patients are at risk for bone loss and musculoskeletal complaints from cancer or associated therapies, monitoring 25-hydroxyvitamin D levels and vitamin D_3 supplementation in moderate dose (1,000-1,500 IU D_3 per day) can be recommended with expectation of mainly bone benefit. In women with breast cancer, future vitamin D supplementation studies need to be appropriately designed and powered to provide definitive assessments. However, a full-scale randomized trial evaluating the influence of vitamin D supplementation on breast cancer recurrence is likely not feasible.

Keywords: 25-hydroxyvitamin D, Breast cancer, Breast cancer incidence, Breast cancer prevention, Calcium, Case control studies, Cohort studies, Joint symptoms, Meta-analysis, Randomized clinical trials, Total mortality, Vitamin D.

INTRODUCTION

Information on the influence of vitamin D on breast cancer incidence and outcome is complex and controversial. Examination of the same body of clinical evidence has led to a range of conclusions and recommendations regarding public policy and practice in the clinic but also what direction future clinical research activity should take [1-3]. Nonetheless, there is agreement that a solid base of preclinical evidence supports the biological plausibility of a potential role for vitamin D to influence breast cancer [4-6]. A range of developments in the past year have clarified a number of important issues in this area and will be the focus of this presentation.

IDENTIFICATION OF STUDIES

Literature was searched for both observational studies and clinical trials assessing associations among vitamin D intake and 25 hydroxyvitamin D levels with breast cancer incidence and outcome. Factors associated with 25-hydroxyvitain D levels and vitamin D supplementation studies in breast cancer patients also were a focus. Pubmed and EMBASE databases were searched and symposium proceedings from the American Society of Clinical Oncology, The San Antonio Breast Cancer Symposium, and the European Society of Medical Oncology were reviewed and literature was searched through March 31, 2012. Titles and abstracts were reviewed and full articles were reviewed when relevant.

VITAMIN D INTAKE AND BREAST CANCER INCIDENCE

The association between vitamin D intake from both diet and supplements with breast cancer incidence has been addressed in 11 case-control studies [7-17] with mixed results and some [11, 14-16, 18, 19] find significant associations between higher vitamin D intake and lower breast cancer risk only in premenopausal women. Similarly, cohort studies also provide mixed results, [20-24] precluding definitive conclusions. Taken together, the available observational studies do not provide a compelling or consistent association between vitamin D intake and breast cancer incidence.

SOLAR EXPOSURE AND BREAST INCIDENCE

The relationship between sunlight exposure, UV radiation and breast cancer risk, a topic beyond the scope of the current review, is also controversial with mixed results. While some reviews suggest strong, consistent relationships between higher UV radiation exposure and lower breast cancer risk [26] other recent cohort study reports are negative [21, 22] and problems in using latitude as a proxy for UV radiation have been raised [22]. While a recent report [24] suggested higher dietary and supplement vitamin D intake were associated with lower breast cancer risk only in geographic regions with highest UV radiation exposure, no such effect was seen in the nested case-control study in the Women's Health Initiative randomized trial [26]. Again, white further studies can be supported, current evidence does not support a compelling association between higher UV radiation exposure and lower breast cancer risk.

25-HYDROXYVITAMIN D LEVELS AND BREAST CANCER INCIDENCE

25-hydroxyvitamin D is a generally accepted marker for vitamin D status [27]. There have been eight nested-case control studies examining the association between baseline 25-hydroxyvitamin D levels and subsequent breast cancer risk. Seven of these studies were nested in cohorts [28-34] and one in a randomized trial [30]. Of these, only the French E3N cohort identified a statistically significant association between lower 25-hydroxyvitamin D levels and higher breast cancer risk [31] (Table **1**).

In the Women's Health Initiative analyses, the initial odds ratio for the association of invasive breast cancer with serum 25-hydroxyvitamin D levels was 1.33 (95% confidence interval [CI]; 1.02-0.72, P-trend = 0.01) [26]. However, when body mass index (BMI) and physical activity were added to the model the

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Table 1. 25-Hydroxyvitamin D and Breast Cancer Incidence: Nested Case Control Studies in Cohorts and Randomized Trials

Cohort	Lead Author	Cohort (n)	Case Patients	Control Subjects	MR or OR	P-trend
Cancer Prevention Study-II Nutrition Cohort	McCullough	21,965	516	516	0.92	0.60
Malmo Diet and Cancer Study	Almquist	53,000	764	764	0.96	0.78
Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial	Freedman	38,660	1005	1005	0.96	0.81
Women's Health Initiative	Chlebowski	32,826	895	898	1.06	0.20
Nurses Health Study	Bertone-Johnson	32,826	701	724	1.37	0.06
French E3N Cohort	Engel	17,391	636	1272	1.37	0.02
Nurse's Health Study II	Eliasien	29,611	613	1218	1.20	0.32
NSABP P-1 (randomized)	Amir	19,388	231	856	1.06	0.76

association was strongly attenuated [26, 35], was no longer significant and approached the nul (OR=1.06, 95% CI 0.78-1.43). In addition, the 25-hydroxyvitamin D levels were statistically significantly associated with recreational physical activity and BMI (P<0.001 for both) [26]. As both low physical activity and high BMI are independent, strong predictors of increased breast cancer risk, [34, 35] analyses unable to accurately control for these parameters are subject to confounding. Reliable and comprehensive determination of physical activity levels can be problematic, especially for cohorts who entered patients in earlier times when reasonable assessment methodology was not available.

In the case-control study nested in the randomized National Surgical Adjuvant Breast and Bowel Project (NSABP) P-1 primary breast cancer prevention trial, baseline 25-hydroxyvitamin D levels were not associated with subsequent breast cancer risk (OR 1.06, P=0.76) [30]. In addition, these NSABP investigators also conducted a meta-analyses separately evaluating studies of 25-hydroxyvitamin D and breast cancer where blood was collected before diagnosis (cohort studies) and studies where blood was collected after lower breast cancer diagnosis (case-control studies) [30]. Of the six case-control studies, [39-44] all were nominally statistically significant, associating lower 25-hydroxyvitamin D levels with higher breast cancer risk, (OR of 2.49). In contrast, of eight studies with blood collected before diagnosis, only one was statistically significant and the subtotal OR was 1.10. [30]. This observation followed an earlier meta-analysis by Yin and colleagues with a similar observation [44].

As 25-hydroxyvitamin D levels are higher in early compared to later stage breast cancers, [45] the clear dichotomy (interaction P=0.001) [30] in findings between the negative cohort studies and the positive case-control studies suggest reverse causality may explain the low 25-hydroxyvitamin D levels seen in the case-control reports. As more advanced stage [45] and poor prognosis breast cancers [46] have lower 25-hydroxyvitamin D levels, the presence of breast cancer itself, effects of cancer therapy or it's sequalae could be responsible for the low 25-hydroxyvitamin D levels seen. For example, both chemotherapy use [47] and low physical activity are associated with low 25-hydroxyvitamin D levels and physical activity is commonly decreased for some period following a breast cancer diagnosis, likely related to associated cancer therapies [36, 48].

Taken together, this emerging body of evidence challenges the concept that vitamin D status, as measured by 25-hydroxyvitamin D levels is associated with future breast cancer risk. While other explanations for the available evidence are possible, [49] further studies of this issue must carefully consider the timing of sample collection relative to the breast cancer diagnosis and be able to adjust for BMI and physical activity assessed using state of the art methodology.

Examining the available evidence, other agencies have come to similar conclusions regarding the relationship between vitamin D status and breast cancer incidence risk. The long awaited 2011 update on dietary requirements for calcium and vitamin D from the Institute of Medicine, basing their finding largely on clinical trial evidence, concluded that for cancer the evidence was "inconsistent, inconclusive as to causality and insufficient to inform nutritional requirements" [50, 51]. Most recently, an updated meta-analysis from the US Preventative Services Task Force found no statistically significant dose-response relationship for 25-hydroxyvitamin D levels and breast cancer risk [52].

25-HYDROXYVITAMIN D LEVELS AND BREAST CANCER RECURRENCE

An initial report from Goodwin and colleagues [54] prompted interest in the potential for 25-hydroxyvitamin D levels to influence breast cancer outcome. A cohort of 512 Canadian early stage, resected breast cancer patients had blood samples obtained prior to systemic therapy initiation. After a mean 5.6 years follow-up, those with deficient (< 15 nmol/L [6 ng/ml]) compared to sufficient (> 72 nmol/L [30 ng/ml]) 25 hydroxyvitamin D levels were at increased risk for distant recurrence risk and death (both, P<0.01) (Table **2**). However, the associations were attenuated and became non-significant after multivariate analyses adjusted for traditional prognostic factors [53]. Another Canadian study reported on 25-hydroxyvitamin D levels and recurrence in 667 postmenopausal women with early stage resected breast cancer within the context of a randomized clinical trial. In that setting, where breast cancer therapy was protocol defined, no significant association with event-free survival was seen (P=0.43) [54, 55]. Similarly, in a US population of early stage, resected breast cancer patients entered as a nested case-control study in a randomized nutrition trial within four years from diagnosis, no significant association with breast cancer recurrence or death was seen with baseline 25-hydroxyvitamin D levels [56].

Within the last year, however, three studies have reported positive associations. In a German population of 1295 breast cancer patients of all stages, after 5.8 years follow-up, those with the lowest (< 35 nmol/L [14.0 ng/ml]) vs highest (≥ 55 nmol/L [22 ng/ml]) 25-hydroxyvitamin D levels had a statistically significantly lower distant disease-free survival and lower overall survival [58] (Table **2**). In a relatively small cohort of 251 pre and postmenopausal breast cancer patients of all stages, deaths from breast cancer were significantly lower (HR 0.42, 95% CI 0.21-0.82) in those in the highest quartile (> 81 nmol/L [32 ng/ml]) vs lowest

Table 2. 25-Hydroxyvitamin D Concentration and Subsequent Breast Cancer Outcome in Patients with Resected Early Stage Disease

Lead Author	n	Menopausal Status	Category	Country	Systemic Therapy	Adjusted for BMI, PA	Follow-up (mean)	Study Outcome
Goodwin	512	Pre, Post	Early breast cancer, resected Cohort Pre and postmenopausal	Canada	Tamoxifen per clinical decision	Yes	11.6 yrs	Deficient (<50 nmol/L) vs sufficient (>72 nmol/L) 25(OH) D levels, Distant recurrence HR 1.71; 95% CI 1.02, 2.86 [1] Survival HR 1.60, 95% CI 0.96-2.64
Piura, Pritchard	667	Post	Early breast cancer, resected Cohort within a randomized clinical trial Postmenopausal	Canada	Tamoxifen x 5 yrs vs Tamoxifen x 5 yrs plus octreotide x 2 yrs (per protocol)		7.9 yrs	No significant association with event-free survival with 25(OH) D level (P=0.43)
Jacobs	1024	Pre, Post	Early breast cancer, resected entered within 4 yrs from diagnosis Nested case-control within a randomized clinical trial Pre and postmenopausal	USA	Varies		7.3 yrs	No significant association with breast cancer recurrence (local, regional, or distant) or death with 25(OH) D level
Vrieling	1294	Post	Stage I-IV Cohort	Germany	Varies		5.8 yrs (median)	Lowest tertile (< 35 nmol/L) vs highest (≥ 55 nmol/L) Distant disease free survival HR 2.09; 95% CI 1.29-3.41 Survival HR 1.55: 95% CI 1.00-2.39
Tretli	251	Pre, Post	Stage I-IV	Norway	Varies		NA	Highest quartile (> 81 nmol/L) vs lowest quartile (< 46 nmol/L) Death from breast cancer HR 0.42: 95% CI CI 0.21-0.82.

quartile (< 43 nmol/L [17 ng/ml]) [58]. In a Korean population of 310 early stage breast cancer patients, low 25-hydroxyvitamin D levels determined before definitive surgery were associated with significantly increased recurrence risk (P=0.002) for both luminal A and luminal B subtypes but not HER positive or triple negative tumors [59]. Importantly, the latter three positive studies did not adjust for established prognostic features, cancer therapy, BMI, or physical activity. As a result, their findings are open to some question.

In a provocative report of a case-control study of breast cancer in African American and European American women, 25-hydroxyvitamin D levels were measured and associations between breast cancer risk and single nucleotide polymorphisms (SNPs) in VDR, CYP 24A1 and CYP 27B1 were evaluated by estrogen receptor status [60]. More African Americans had vitamin D deficiency (< 10 ng/ml) than East European Americans with lowest levels among those with highest African ancestry. Associations for SNPs differed by race and the authors suggested that such genetic variance could be related to the higher incidence of estrogen receptor negative breast cancers in African Americans. However, most of the associations were not significant after correction for multiple comparisons so validation is needed.

In summary, the results of 25-hydroxyvitamin D association with breast cancer recurrence are inconsistent but the question remains of interest. Future studies must provide multi-variable adjustment to incorporate the known important variables including BMI, physical activity, and breast cancer therapy which can influence both 25-hydroxyvitamin D levels and breast cancer outcome.

CALCIUM AND VITAMIN D SUPPLEMENTATION AND BREAST CANCER INCIDENCE IN RANDOMIZED TRIALS

In the only full scale study which has been conducted, the Women's Health Initiative investigators randomly assigned 36,282 postmenopausal women to 1,000 mg elemental calcium with 400 IU of vitamin D_3 daily or placebo in a trial where hip fracture was the primary outcome [61] and colorectal cancer [62] and invasive breast cancer were the secondary outcomes [26]. The incidence of invasive breast cancer was closely comparable in the two randomization groups (528 vs 546 breast cancers, respectively) (HR 0.96, 95% CI 0.85-1.09). For women in the lowest baseline vitamin D intake quartile, fewer breast cancers were seen in the supplement group (HR 0.79, 95% CI 0.65-0.97). However, there were more breast cancers in the supplement group than in the placebo group for those in the highest quartile of total vitamin D intake at baseline (HR 1.34, 95% CI 1.01-1.78) (P interaction = 0.003) [26]. As about 60% of participants took over 80% of their study pills, adherence is a study limitation and also that 25-hydroxyvitamin D levels were evaluated only in a subset of participants [26].

Against this background, Bolland and colleagues [63] performed additional analyses of the same data using a limited-access dataset obtained from the National Heart Lung and Blood Institute. In analyses similar to those published in the original WHI report, [26] calcium and vitamin D supplement use was examined in the 15,646 women who were not taking personal calcium or vitamin D supplement at randomization. In that setting, randomization to the calcium and vitamin D supplement group was associated with a significantly lower risk of invasive breast cancers while in women taking personal calcium and vitamin D supplements, the additional protocol calcium and vitamin D supplementation did not alter cancer risk. Based on these findings, Bolland and colleagues suggested that there was a low threshold for calcium or vitamin D for breast cancer benefit that was exceeded by the non-protocol use of such supplements [63].

In a Letter to the Editor in response, we suggested caution in reinterpreting the Women's Health Initiative calcium and vitamin D trial breast cancer results [64]. We indicated that we had originally reported similar findings but did not emphasize them because other

results were not consistent with an hypothesis that achieving a low threshold for supplemental calcium and vitamin D was sufficient to result in a breast cancer reduction. On the basis of such a hypothesis, women with the lowest baseline 25-hydroxyvitamin D levels would have the greatest breast cancer reduction with randomization to the supplement group. However, no such effect was seen. Also a low threshold hypothesis would predict that women not taking personal supplements and randomized to the placebo group would have a high breast cancer incidence since all others in the WHI trial would be above the presumptive vitamin D threshold and have a lower incidence. Again, no such effect was seen. In summary, the WHI published data are inconsistent with a low threshold effect resulting in breast cancer risk reduction for relatively low dose vitamin D supplementation with calcium.

Some have questioned the adequacy of the WHI vitamin D_3 dose of 400 IU per day. The dosage reflected the then current recommendations from the Institutes of Medicine [65]. However, as use of non-protocol vitamin D supplementation was permitted, total vitamin D intake incorporating diet plus non-protocol supplement plus protocol supplement was 773 IU (mean) per day in the supplement group compared to 367 IU (mean) per day in the placebo group, both after two years [26]. Based on a comprehensive analyses of a dose response randomized trial, it has recently been concluded that a vitamin D_3 total dosage of 600 IU per day would "meet the nutritional requirements of nearly all (97.5%) healthy persons" [66]. Thus, the WHI trial of calcium and vitamin D supplementation evaluated the influence of the currently recommended vitamin D intake.

Calcium plus vitamin D influence on cancer incidence has been evaluated in one other randomized clinical trial using a larger vitamin D dose but in a smaller study population. A total of 1,179 postmenopausal women were randomized to placebo, calcium alone (1,400-1,500 mg/d) or calcium plus 1,100 IU vitamin D_3 in a 1:2:2 ratio [67]. Of the 50 cancer cases seen, there were fewer cancers in the supplement compared to placebo group (2.9% vs 6.9%, $P<0.05$) but only 13 breast cancers were including precluding reliable interpretation.

25-HYDROXYVITAMIN D LEVELS IN BREAST CANCER PATIENTS

While studies consistently report a high prevalence of relatively low 25-hydroxyvitamin D levels in breast cancer patients, [68, 69] Cescon and colleagues [70] addressed the feasibility of conducting a randomized trial evaluating the influence of vitamin D on recurrence risk in early stage breast cancer patients. They examined 25-hydroxyvitamin D levels and vitamin D supplement use in a recently diagnosed cohort of breast cancer patients from Los Angeles and Toronto identified between March 2009 and January 2010 identified within two years of diagnosis.

The author's pre-specified feasibility criteria for conducting a vitamin D versus placebo randomized clinical trial were that > 30% of patients would have 1) deficient or insufficient vitamin D levels, 2) were taking < 1,000 IU vitamin D supplement per day and 3) were willing to participate in such a study. Of the 173 eligible patients, 84% were using vitamin D supplements with median dose per day of about 1300 IU. The rates of deficiency, insufficiency and adequacy of hydroxyvitamin D levels were about 4%, 22% and 73% [70]. The relatively high proportion of vitamin D supplement use suggest patients and/or clinicians have largely recognized the issue of vitamin D deficiency in the breast cancer setting. As only under 13% of study population met the pre-specified feasibility criteria, it appears that, at least in large urban centers in the North America, a randomized trial addressing the issue of vitamin D deficiency influence on breast cancer recurrence may not be practical.

VITAMIN D SUPPLEMENTATION AND 25-HYDROXYVITAMIN D LEVELS

The recent update of the Institute of Medicine report on calcium and vitamin D requirements with a recommendation based primarily on bone health outcomes as related to 25-hydroxyvitamin D levels concluded that levels of at least 50 nmol/L (20 ng/ml) would meet the needs of at least 97.5% of the population. In addition, the committee concluded that there was no compelling evidence that 25-hydroxyvitamin D levels above this were associated with greater benefit for bone health or other outcomes [50, 51].

The Institute of Medicine reports emphasis the randomized clinical trial evidence. Some have taken exception to the Institute of Medicine findings [71, 72] and give more weight to evidence from ecological and case-control reports. Readers are invited to review those publications and make their own judgments.

The first comprehensive evaluation of dose-response evaluation to vitamin D supplementation in healthy postmenopausal women provides a framework for consideration of dosage in breast cancer patients. The Vitamin D Supplementation in Older Subjects (VIDOS) placebo-controlled, clinical trial randomized postmenopausal white women with vitamin D insufficiency (25-hydroxyvitamin D level $\leq$ 50 nmol/L [20 ng/ml]) and adequate calcium intake to placebo or vitamin D_3 doses ranging from 400-4,800 IU per day [66]. Based on results, a 600 IU dose was modeled to achieve a 25-hydroxyvitamin D level > 50 nmol/L [20 ng/ml], a level judged sufficient by the Institute of Medicine to meet vitamin D requirements [51]. Somewhat higher dosage was required for women with BMI > 25 kg/m^2.

While others report no increased risk of hypercalcemia with usual vitamin dose [73] in this study, some concern was raised regarding high dosages of vitamin D since between 3-9% of participants had hypercalcemia and 12-33% had hypercalcuria, [66], with hypercalcuria related to renal stone risk [74]. In addition, a recent comprehensive review of conventional dose vitamin D (< 1000 IU per day) studies found hypercalcemia, gastroenteritis symptoms and renal disease significantly increased [75]. In the WHI clinical trial, renal stones were significantly increased with the 400 IU per day dose, an increase of 68 cases over placebo in 18,176 treated women [61]. It is unclear how these comprehensive findings in healthy postmenopausal women relate to vitamin D dosage in breast cancer patients with vitamin D deficiency.

VITAMIN D SUPPLEMENTATION IN BREAST CANCER PATIENTS

In breast cancer patients, a series of uncontrolled studies have reported a relatively high frequency of low 25-hydroxyvitamin D levels [68, 69] but several of these reports based findings on criteria for sufficient levels of 25-hydroxyvitamin D substantially higher (75 nmol/L [30 ng/ml]) [68] and 80 nmol/L [32 ng/ml] [69] than the 50 nmol/L [20 ng/ml] level recommended by the Institute of Medicine [51]. Nonetheless, a substantial number of breast cancer patients have lower 25-hydroxyvitamin D levels then currently recommended. The absence of appropriate control populations make estimation of the actual prevalence of 25-hydroxyvitamin D deficiency in women with breast cancer relative to women without cancer but having similar age and characteristics difficult to determine. In one report, using a convenience control population, a higher prevalence of vitamin D insufficiency was seen in the controls compared to breast cancer patients who had completed cancer treatment [76].

The influence of vitamin D supplementation on 25-hydroxyvitamin D levels in breast cancer patients have been reported from retrospective observational studies and studies using both randomized and non-randomized designs (Table **3**). Crew and

Table 3. Effects of Vitamin D Supplementation on 25-Hydroxyvitamin D Levels and Musculoskeletal Symptoms in Breast Cancer Patients

Study	Author	N	Design	Population	Intervention:	Outcome:
Aromatase Inhibitor-Induced Musculoskeletal Symptom (AIMISS) Study	Rastelli	60	Randomized, double-blind, placebo controlled	Breast cancer patients with AIMISS	50,000 vitamin D capsules/wk for 8-16 weeks, then monthly for 4 months	Statistically significant reduction in AIMSS with vitamin D
	Amir	40	Prospective, single-center, single arm phase II study	Breast cancer patients with bone metastases receiving bisphosphonate	10,000 IU vitamin D_3 po qwk x 4 months	No significant palliative effect on bone pain
B-ABLE Aromatase Inhibitor Bone Loss	Prieto-Alhambra	222	Prospective cohort	Breast cancer patients initiating aromatase inhibitor therapy, ineligible for bisphosphonate	Calcium (1 g) plus vitamin D_3 (800 IU) per day and 16,000 IU q2wk of 25OH D < 30 ng/ml x 3 months	Higher 25-hydroxyvitamin D protected against bone loss (P=0.0001) and those with levels $\geq$ 40 ng/ml had less loss (P=0.005).
Columbia University	Crew	103	Single arm study	Stage I-III breast cancer participating in zoledronic intervention trial after chemotherapy	All prescribed calcium carbonate 1000 mg/d and vitamin D_3 400 IU/d	25-hydroxyvitamin D median level of 17 ng/ml at baseline increased to 19 ng/ml at 12 months Note: 20 ng/ml adequate per Institute of Medicine
University of Kansas	Khan	60	Single arm study	Breast cancer patients beginning letrozole began	A standard dose calcium and vitamin D x 4 weeks, if 25-hydroxyvitamin D $\leq$ 40 ng/ml begun on D_3 50,000 IU/wk x 12	25-hydroxyvitamin D levels > 40 ng/ml in all 41; those with levels > 66 ng/ml (median) had less disability from joint pain (52 vs 19%, P=0.026)
Rochester Medical Center	Peppone	224	Retrospective study	224 breast cancer patients Stage O-III	Vitamin D supplement based on baseline 25-hydroxyvitamin D levels either non, 1000 IU/d, or high dose $\geq$ 50,000 IU/d	Only high dose significantly increased 25-hydroxyvitamin D levels

colleagues noted that, with 400 IU vitamin D_3 with calcium per day in their clinic population, < 15% achieved 25-hydroxyvitamin D levels > 75 nmol/L [30 ng/ml] [68]. In a retrospective study of newly diagnosed breast cancer patients, administration of vitamin D_3, 8,000 IU daily increased mean 25-hydroxyvitamin D levels from 19.7 [49 nnol/L] to 37.6 [94 nmol/L], respectively, P<0.01 but many remained below 32 ng/ml [80 nmol/L] [77]. In a retrospective study of 224 early stage breast cancer patients, vitamin D supplementation was based on baseline 25-hydroxyvitamin D levels: $\geq$ 32 ng/ml, no supplement; 25-31 ng/ml, 1,000 IU per day; < 24 ng/ml, 60,000 IU per week; < 15 ng/ml, 100,000 IU per week. After 8-16 weeks, weekly high dose supplementation significantly increased 25-hydroxyvitamin D levels while daily 1,000 IU dose supplementation did not [78]. This study was performed as a component of usual clinical care and was not randomized, controlled or blinded and the 1,000 IU dose was recommended but not prescribed. Taken together, the data suggest, but do not currently conclusively support, an hypothesis that higher vitamin D dosage may be needed in early stage breast cancer patients with low 25-hydroxyvitamin D levels.

VITAMIN D AND JOINT AND BONE PAIN IN BREAST CANCER PATIENTS

While some studies have associated low 25-hydroxyvitamin D levels with exacerbation of joint symptoms, [27, 80] in a subset of 1911 participants in the WHI randomized clinical trial, calcium (1,000 mg) and vitamin D_3 (400 IU) supplementation daily did not influence joint pain or swelling when compared to placebo [80]. Aromatase inhibitor use, which profoundly lowers estrogen levels, not uncommonly increases musculoskeletal symptoms [81] and an interaction between aromatase inhibitor associated musculoskeletal complaints and low 25-hydroxyvitamin D levels has been proposed [82]. However, no such association was seen in subgroup analyses in the large Arimidex, Tamoxifen Alone or Together (ATAC) Trial [83].

Other studies have evaluated the influence of vitamin D supplementation on clinical endpoints in breast cancer patients. In a single arm study of 40 breast cancer patients receiving bisphosphonate who had bone metastases, vitamin D_3 (10,000 IU) and calcium (1,000 mg) daily were provided for four months but no significant improvement in global pain scale was seen [84]. Sixty postmenopausal breast cancer patients beginning aromatase inhibitors with baseline 25-hydroxyvitamin D levels < 40 ng/ml [10 nmol/L] received 50,000 IU D_3 supplementation weekly for 12 weeks. The intervention increased 25-hydroxyvitamin D levels and women with levels > 66 ng/ml [16 nmol/L] reported "no disability from joint pain" less commonly than did women with lower levels [85]. In a similar population of 60 postmenopausal breast cancer patients who were on aromatase inhibitors, using a double-blind, placebo-controlled randomized phase II study design, supplementation with higher dose vitamin D_2 50,000 IU capsules weekly for either 8 or 16 weeks and then monthly to complete five months was compared to placebo. While musculoskeletal pain was initially improved in the supplementation groups, the effect was subsequently attenuated [86].

In a larger study, vitamin D threshold to prevent both aromatase-inhibitor arthralgia [87] and bone loss [88] was examined in 223 women who were ineligible for bisphosphonate. All received calcium (1 g) and oral vitamin D_3 800 IU daily plus 16,000 IU every 2 weeks for baseline 25-hydroxyvitamin D < 30 ng/ml [7.5 nmol/L]. Those who reached levels $\geq$ 40 ng/ml [10 nmol/L] had significantly attenuated joint pain (P=0.02) [87] reduced bone loss (P=0.005) [87] compared to those with 25-hydroxyvitamin D levels < 30 ng/ml [7.5 nmol/L]. The absence of bone targeted intervention like bisphosphonates complicates interpretation.

Taken together, the findings are suggestive of potential joint symptom benefit with higher dose vitamin D use but larger studies with clear hypothesis and study designs are needed to confirm an association between some target 25-hydroxyvitamin D level and aromatase inhibitor-associated joint pain.

FACTORS INFLUENCING 25-HYDROXYVITAMIN D LEVELS

Interpretation of associations between 25-hydroxyvitamin D levels and clinical outcome are not straight forward since several factors such as BMI and physical activity are associated not only with 25-hydroxyvitamin D levels but also clinical outcomes of interest such as breast cancer. In addition, a substantial proportion of the difference in 25-hydroxyvitamin D levels between individuals is currently not understood.

In a cohort consortium of 4,723 pooled samples, statistically significant positive correlates of 25-hydroxyvitamin D level included male sex, summer sample, physical activity and multivitamin use. Significant negative correlates included BMI, winter and spring samples, diabetes, sedentary behavior, smoking, and Black race/ethnicity [89]. Considering the differences in 25-hydroxyvitamin D levels between individuals, in 3,055 postmenopausal women participating in the WHI clinical trial, a multi-variate predictive model could account for only 21% of the differences [90]. This finding suggests, as has other reports, [91, 92] that a large portion of differences in 25-hydroxyvitamin D seen between individuals are likely genetically determined. Such findings raise legitimate questions regarding an expectation that strategies involving high dose vitamin D supplementation to increase 25-hydroxyvitamin D levels will, in fact, impact target diseases of interest.

VITAMIN D SUPPLEMENTATION AND TOTAL MORALITY IN RANDOMIZED TRIALS

Based on the potential influence of low vitamin D status on common life-threatening conditions, meta-analyses have examined risk of death from any cause in participants evaluating vitamin D supplementation (ergocalciferol [vitamin D_2] or cholecalciferol [vitamin D_3]). In a Cochrane Database Systematic Review, Bjelakovic and colleagues [93] identified 50 randomized trials with 94,148 participants (including 36,282 from the WHI trial) [94]. Vitamin D_3 was associated with a modest but statistically significant decrease in all cause mortality in studies described as including "predominately elderly women" [93]. Autier and Gandini [95] included a meta-analyses of nine larger trials (all entering > 582 participants) with a total of 57,311 randomized with 5.7 years median follow-up. An 8% lower total mortality was seen in the vitamin D supplement group ($P<0.05$) with the average vitamin D dosage of 528 IU per day. Finally, Zittermann and colleagues [96] in another meta-analysis for found lowest mortality 25-hydroxyvitamin D levels from 30 to 35 ng/ml [7.5-8.7 nmol/L].

These results are of interest and will be pursued in an ongoing full-scale randomized trial evaluating supplemental vitamin D_3 2,000 IU per day plus omega 3 fatty acids (1,00 mg/day) vs placebo in 20,000 otherwise healthy men and women [97] and a similar study is ongoing in the United Kingdom [98]. While awaiting results from such efforts, caution is indicated as reliable information on long term safety of vitamin D supplementation is unclear with a solitary, unconfined analysis suggesting a U shaped curve for lower mortality at moderate 25-hydroxyvitamin D levels and higher mortality at both high and low 25-hydroxyvitamin D levels [99].

RECOMMENDATIONS FOR THE CLINIC

Randomized clinical trial evidence indicates that vitamin D supplementation (in dose of about 400-800 IU per day), together with calcium decreases fracture risk in women at higher fracture risk. As many early stage breast cancer patients are at fracture risk based on age and effects of cancer therapy (such as oophorectomy, chemotherapy associated amenorrhea, and aromatase inhibitor use), monitoring of 25-hydroxyvitamin D levels and use of vitamin D_3 supplementation (1,000-1,500 IU per day) plus calcium following clinical recommendations [100] in those at fracture risk can be endorsed. Current evidence does not support use of high dose vitamin D supplementation to some target level above 50 nmol/L [20 ng/ml] with an expectation of reducing breast cancer recurrence risk or symptoms.

CONCLUSIONS

In summary, prospective cohort studies do not associate high 25-hydroxyvitamin D levels with low breast cancer incidence. The universally positive case-control studies of the same question may well be confounded by reverse causality as the presence of breast cancer or sequalae of associated therapy lower 25-hydroxyvitamin D levels. Studies of 25-hydroxyvitamin D and subsequent breast cancer recurrence provide mixed results with strongest associations of low 25-hydroxyvitamin D levels with high risk of recurrence seen with analyses uncontrolled for prognostic variables, cancer therapy, BMI and physical activity. BMI and physical activity, associated with both 25-hydroxyvitamin D levels and breast cancer risk, if not carefully controlled, are potential confounders of observational study reports. The prevalence of relatively low vitamin D levels seems somewhat greater in early stage breast cancer patients but adequate control population information is lacking. The only full-scale randomized clinical trial, conducted in the Women's Health Initiative, did not demonstrate a lower breast cancer incidence in postmenopausal women randomized to 1,000 mg of calcium and 400 IU vitamin D_3 daily. Influence of higher dose vitamin D on secondary endpoints in breast cancer patients has been evaluated predominately in relatively small studies using randomized as well as non-randomized study designs precluding definitive conclusions. Stronger evidence regarding vitamin D and 25-hydroxyvitamin D associations with other clinical endpoints including overall mortality warrant further evaluation. Breast cancer patients with early stage disease are at risk for bone loss, so monitoring of 25-hydroxyvitamin D and appropriate vitamin D supplement use seems reasonable. Current evidence does not support use of high dose vitamin D regimens in anticipation of benefit for breast cancer recurrence or breast cancer survival.

CONFLICT OF INTEREST

The author confirms that this article content has no conflict of interest.

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REFERENCES

[1] Chlebowski, R.T. Vitamin D and breast cancer: interpreting current evidence. *Breast Cancer Res.*, **2011**, *13*(4), 217.

[2] Garland, C.F.; Gorham, E.D.; Mohr, S.B.; Garland, F.C. Vitamin D and cancer prevention: global perspective. *Ann. Epidemiol.*, **2009**, *19*(7), 468-483.

[3] Shao, T.; Klein, P.; Grossbard, M.L. Vitamin D and breast cancer. *Oncologist*, **2012**, *17*(1), 36-45.

[4] Haussler, M.R.; Haussler, C.A.; Bartik, L.; Whitfield, G.K.; Hsieh, J.C.; Slater, S.; Jurutka, P.W. Vitamin D receptor: molecular signaling and actions of nutritional ligands in disease prevention. *Nutr. Rev.*, **2008**, *66*(10 Suppl 2), S98-112.

[5] Matthews, D.; LaPorta, E.; Zinser, G.M.; Narvaez, C.J.; Welsh, J. Genomic vitamin D signaling in breast cancer: insights from animal models and human cells. *J. Steriod. Biochem. Mol. Biol.*, **2010**, *121*(1-2), 362-367.

[6] Krishnan, A.V.; Feldman, D. Mechanisms of the anti-cancer and anti-inflammatory actions of vitamin D. *Annu. Rev. Pharmacol. Toxicol.*, **2011**, *51*, 311-336.

[7] Fleet, J.C.; Desmet, M.; Johnson, R.; Li, Y. Vitamin D and cancer: a review of molecular mechanisms. *Biochem. J.;* **2012**, *441*(1), 61-76.

[8] Simard, A.; Vobecky, J.; Vobecky, J.S. Vitamin D deficiency and cancer of the breast: an unprovocative ecological hypothesis. *Can. J. Public Health*, **1991**, *82*, 300-303.

[9] Potischman, N.; Swanson, C.A.; Coates, R.J.; Gammon, M.D.; Brogan, D.R.; Curtin, J.; Brinton, L.A. Intake of food groups and associated micronutrients in relation to risk of early-stage breast cancer. *Int. J. Cancer,* **1999**, *82*(3), 315-321.

[10] Levi, F; Pasche, C.; Lucchini, F.; La Vecchia, C. Dietary intake of selected micronutrients and breast cancer risk. *Int. J. Cancer,* **2001**, *91*(2), 260-263.

[11] Shin, M.H.; Holmes, M.D.; Hankinson, S.E.; Wu, K.; Colditz, G.A.; Willett, W.C. Intake of dairy products, calcium, and vitamin D and risk of breast cancer. *J. Natl. Cancer Inst.,* **2002**, *94*(17), 1301-1311.

[12] Abbas, S.; Linseisen, J.; Chang-Chaude, J. Dietary vitamin D and calcium intake and premenopausal breast cancer risk in a German case-control study. *Nutr. Cancer,* **2007**, *59*(1), 54-61.

[13] Rossi, M.; McLaughlin, J.K.; Lagiou, P.; Bosetti, C.; Talamini, R.; Lipworth, L.; Giacosa, A.; Montella, M.; Franceschi, S.; Negri, E.; La Vecchia, C. Vitamin D intake and breast cancer risk: a case-control study in Italy. *Ann. Oncol.,* **2009**, *20*(2), 374-378.

[14] Anderson, L.N.; Cotterchio, M.; Vieth, R.; Knight, J.A. Vitamin D and calcium intakes and breast cancer risk in pre and postmenopausal women. *Am. J. Clin. Nutr.*, **2010**, *91*(6), 1699-707.

[15] Knight, J.A.; Lesosky, M.; Barnett, H.; Raboud, J.M.; Veith, R. Vitamin D and reduced risk of breast cancer: A population-based case-control study. *Cancer Epidemiol. Biomarkers Prev.*, **2007**, *16*(3), 422-9.

[16] Kawase, T.; Matsuo, K.; Suzuki, T.; Hirose, T.; Hirose, D.; Hosono, S.; Watanabe, M.; Inagaki, M.; Iwata, H.; Tanaka, H.; Tajima, K. Association between vitamin D and calcium intake and breast cancer risk according to menopausal status and receptor status in Japan. *Cancer Sci.,* **2010**, *101*(5), 1234-1240.

[17] Lee, M.S.; Huang, Y.C.; Wahlqvist, M.L.; Wu, T.Y.; Chou, Y.C.; Wu, M.H.; Yu, J.C.; Sun, C.A. Vitamin D decreases risk of breast cancer in premenopausal women of normal weight in subtropical Taiwan. *J. Epidemiol.*, **2011**, *21*(2), 87-94.

[18] Blackmore, K.M.; Lesosky, M.; Barnett, H.; Raboud, J.M.; Vieth, R.; Knight, J.A. Vitamin D from dietary intake and sunlight exposure and the risk of hormone-receptor-defined breast cancer. *Am. J. Epidemiol.*, **2008**, *168*(8), 915-24.

[19] Lin, J.; Manson, J.E.; Lee, I.M.; Cook, N.R.; Buring, J.E.; Zhang, S.M. Intakes of calcium and vitamin D and breast cancer risk in women. *Arch. Intern. Med.*, **2007**, *167*(10), 1050-1059.

[20] Robien, K.; Cutlen, G.J.; Lazovich, D. Vitamin D intake and breast cancer risk postmenopausal women: the Iowa Women's Health study. *Cancer Causes Control*, **2007**, *18*(7), 775-782.

[21] Kuper, H.; Yang, L.; Sandin, S.; Lof, M.; Adam, H.O.; Weiderpass, E. Prospective study of solar exposure, dietary vitamin D intake, and risk of breast cancer among middle-aged women. *Cancer Epidemiol. Biomarkers Prev.*, **2009**, *18*(9), 2558-2561.

[22] Edvardsen, K.; Veierod, M.B.; Brustad, M.; Braaten, T.; Engelsen, O.; Lund, E. Vitamin D effective solar UV radiation, dietary vitamin D and breast cancer risk. *Int. J. Cancer,* **2011**, *128*(6), 1425-1433

[23] Gonzalez, C.A.; Riboli, E. Diet and cancer prevention: Contributions from the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *Eur. J. Cancer*, **2010**, *46*(14), 2555-2562.

[24] Engel, P.; Fagherazzi, G.; Mesrine, S.; Boutron-Ruault, M.C.; Clavel-Chapelon, F. Joint effects of dietary vitamin D and sun exposure on breast cancer risk: results from the French E3N cohort. *Cancer Epidemiol. Biomarkers Prev.*, **2011**, *20*(1), 187-198.

[25] Grant, W.B.; Garland, C.F. The association of solar ultraviolet B (UVB) with reducing risk of cancer: multifactorial ecologic analysis of geographic variation in age-adjusted cancer mortality rates. Anticancer Res., **2006**, *26*(4A), 2687-2699.

[26] Chlebowski, R.T.; Johnson, K.C.; Kooperberg, C.; Petttinger, 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.; Women's Health Initiative Investigators. Calcium plus vitamin D supplementation and the risk of breast cancer. *J. Natl. Cancer Inst.,* **2008**, *100*(2), 1581-1591.

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

[28] Freedman, D.M.; Chang, S.C.; Falk, R.T.; Purdue, M.P.; Huang, W.Y.; McCarty, C.A.; Hollis, B.W.; Graubard, B.I.; Berg, C.D.; Ziegler, R.G. Serum levels of vitamin D metabolites and breast cancer risk in the prostate, lung, colorectal, and ovarian cancer screening trial. *Cancer Epidemiol. Biomarkers Prev.,* **2008**, *17*(4), 889-894.

[29] Bertone-Johnson, E.R.; Chen, W.Y.; Holick, M.F.; Hollis, B.W.; Colditz, G.A.; Willett, W.C.; Hankinson, S.E. Plasma 25-hydroxyvitamin D and 1, 25-dihydroxyvitamin D and risk of breast cancer. *Cancer Epidemiol. Biomarkers Prev.*, **2005**, *14*(8): 1991-1997.

[30] Amir, E.; Cecchini, R.S.; Ganz, P.A.; Costantino, J.P.; Beddows, S.; Hood, N.; Goodwin, P.J. 25-hydroxyvitamin D, obesity, and associated variables as predictors of breast cancer risk and tamoxifen benefit in NSABP-P1. *Breast Cancer Res Treat,* **2012**, Mar 14. [Epub ahead of print].

[31] McCullough, M.L.; Stevens, V.L.; Patel, R.; Jacobs, E.J.; Bain, E.B.; Horst, R.L.; Gapstur, S.M.; Thun, M.J.; Calle, E.E. Serum 25-hydroxyvitamin D concentrations and postmenopausal breast cancer risk: a nested case control study in the Cancer Prevention Study-II Nutrition Cohort. *Breast Cancer Res.*, **2009**, *11*(4), R64.

[32] Almquist, M.; Bondeson, A.G.; Bondeson, L.; Malm, J.; Manjer, J. Serum levels of vitamin D, PTH and calcium and breast cancer risk – a prospective nested case-control study. *Int. J. Cancer,* **2010**, *127*(9), 2159-2168.

[33] Engel, P.; Fagherazzi, G.; Boutten, A.; Dupré, T.; Mesrine, S.; Boutron-Ruault, M.C.; Clavel-Chapelon, F. Serum 25(OH) vitamin D and risk of breast cancer: a nested case-control study from the French E3N cohort. *Cancer Epidemiol. Biomarkers Prev.,* **2010**, *19*(9), 2341-2350.

[34] Eliassen, A.H.; Spiegelman, D.; Hollis, B.W.; Horst, R.L.; Willett, W.C.; Hankinson, S.E. Plasma 25-hydroxyvitamin D and risk of breast cancer in the Nurse's Health Study II. *Breast Cancer Res.*, **2011**, *13*(11), R50.

[35] Neuhouser, M.L.; Manson, J.E.; Millen, A.; Pettinger, M.; Margolis, K.; Jacobs, E.T.; Shikany, J.M.; Vitolins, M.;Adams-Campbell, L.; Liu, S.;, Leblanc, E.; Johnson, K.C.; Wactawski-Wende, J. The influence of health and lifestyle characteristics on the relation of serum 25-hydroxyvitamin D with risk of colorectal and breast cancer in postmenopausal women. *Am. J. Epidemiol.*, **2012**, *175*(7), 673-684.

[36] Chlebowski, R.T.; Ligibel, J.A. Lifestyle issues in breast cancer survivors, in Harris JR, Lippman ME, Morrow M, *et al.,* (eds): Diseases of the Breast (ed 3). Philadelphia, PA, Lippincott Williams and Wilkins, **2009**.

[37] Irwin, M.L.; McTiernan, A.; Manson, J.; Thomson, C.A.; Sternfeld, B.; Stefanick, M.L.; Wactawski-Wende, J.; Craft, L.; Lane, D.; Martin, L.W.; Chlebowski, R. Physical activity and survival in women diagnosed with breast cancer. Results from the Women's Health Initiative. *Cancer Prev. Res (Phila)*, **2011**, *4*(4), 522-529.

[38] Abbas, S.; Linseisen, J.; Slanger, T.; Kropp, S.; Mutschelknauss, E.J.; Flesch-Janys, D.; Chang-Claude, J. Serum 25-hydroxyvitamn D and risk of postmenopausal breast cancer: results of a large case-control study. *Carcinogenesis*, **2008**, *29*(1), 93-99.

[39] Abbas, S.; Chang-Claude, J.; Linseisen, J. Plasma 25-hydroxyvitamin D and premenopausal breast cancer risk in a German case-control study. *Int. J. Cancer*, **2009**, *124*(1), 250-255.

[40] Crew, K.D.; Gammon, M.D.; Steck, S.E.; Hershman, D.L.; Cremers, S.; Dworakowski, E.; Shane, E.; Terry, M.B.; Desai, M.; Teitelbaum, S.L.; Neugut, A.I.; Santella, R.M.; Association between plasma-25-hydroxyvitamin D and breast cancer risk. *Cancer Prev. Res. (Phila Pa)*, **2009**, *2*(6), 598-604.

[41] Lowe, L.C.; Guy, M.; Mansi, J.L.; Peckitt, C.; Bliss, J.; Wilson, R.G.; Colston, K.W. Plasma 25-hydroxyvitamin D concentrations, vitamin D receptor genotype and breast cancer risk in a UK Caucasian population. *Eur. J. Cancer,* **2005**, *41*(8), 1164-1196.

[42] Rejnmark, L.; Tietze, A.; Vestergaard, P.; Buhl, L.; Lehbrink, M.; Heickendorff, L.; Mosekilde, L. Reduced prediagnostic 25-hydroxyvitamin D levels in women with breast cancer: a nested case-control study. *Cancer Epidemiol. Biomark. Prev.*, **2009**, *18*(10), 2655-2660.

[43] Yao, S.; Sucheston, L.E.; Millen, A.E.; Johnson, C.S.; Trump, D.L.; Nesline, M.K.; Davis, W.; Hong, C.C.; McCann, S.E.; Hwang, H.; Kulkarni, S.; Edge, S.B.; O'Connor, T.L.; Ambrosone, C.B. Pretreatment serum concentrations of 25-hydroxyvitamin D and breast cancer prognostic characteristics: a case-control and a case-series study. *PLoS One*, **2011**, *6*(2), 17251-17252.

[44] Yin, L.; Grandi, N.; Rawm, E.; Haug, U.; Arndt, V.; Brenner, H. Meta-analysis: serum vitamin D and breast cancer risk. *Eur. J. Cancer*, **2010**, *46*(12), 2196-2205.

[45] Palmieri, C.; MacGregor, T.; Girgis, S.; Vigushin, D. Serum 25-hydroxyvitamin D levels in early and advanced breast cancer. *J. Clin. Pathol.*, **2006**, *59*(12), 1334-1136.

[46] Peppone, L.J.; Rickles, A.S.; Janelsins, M.C.; Insalaco, M.R.; Skinner, K.A. The association between breast cancer prognostic indicators and serum 25-oh vitamin d levels. *Ann. Surg. Oncol.*, **2012**, Mar 24. [Epub ahead of print].

[47] Fakih, M.G. Chemotherapy is linked to severe vitamin D deficiency in patients with colorectal cancer. *Int. J. Colorectal. Dis.*, **2009**, *24*(2), 219-224.

[48] Irwin, M.L.; McTiernan, A.; Bernstein, L.; Gilliland, F.D.; Baumgartner, R.; Baumgartner, K.;, Ballard-Barbash, R. Physical activity levels among breast cancer survivors. *Med. Sci. Sports Exerc.*, **2004**, *36*(9), 1484-1491.

[49] Grant, W.B. Effect of interval between serum draw and follow-up period on relative risk of cancer incidence with respect to 25-hydroxyvitamin D level. Implications for meta-analyses and setting vitamin D guidelines. *Dermatoendocrinol*, **2011**, *3*(3), 199-204.

[50] Institute of Medicine. Dietary reference intakes for calcium and vitamin D. Washington DC: The National Academies Press **2011**.

[51] Ross, C.A.; 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.

[52] Chung, M.; Lee, J.; Terasawa, T.; Lau, J.; Trikalinos, T.A. Vitamin D with or without calcium supplementation for prevention of cancer and fractures: an updated meta-analysis for the U.S. Preventive Services Task Force. *Ann. Intern. Med.*, **2011**, *155*(12), 827-838.

[53] Goodwin, P.J.; Ennis, M.; Pritchard, K.I.; Koo, J.; Hood, N. Prognostic effects of 25-hydroxyvitamin D levels in early breast cancer. *J. Clin. Oncol.*, **2009**, *27*(23), 2757-3763.

[54] Piura, E.; Chapman, J.W.; Lipton, A.; Zhu, L.; Leitzel, K.; Wilson, C.F.; Pritchard, K.I.; Shepherd, L.; Pollak, M.N. Serum 1-OH vitamin D and prognosis of postmenopausal breast cancer patients: NCIC-CTG MA14 trial [abstract]. *J. Clin. Oncol.*, **2009**, *27*, 15s.

[55] Pritchard, K.I.; Shepherd, L.E.; Chapman, L.A.W.; Norris, B.D.; Cantin, J.; Goss, P.E.; Dent, S.F.; Walde, D.; Vandenberg, T.A.; Findlay, B.; O'Reilly, S.E.; Wilson, C.F.; Han, L.; Piura, E.; Whelan, T.J.; Pollak, M.N. Randomized trial of tamoxifen versus combined tamoxifen and octreotide LAR therapy in the adjuvant treatment of early stage breast cancer in postmenopausal women: NCIC CTG MA 14. *J. Clin. Oncol.*, **2011**, *29*(29), 3869-3876.

[56] Jacobs, E.T.; Thomson, C.A.; Flat, S.W.; Al-Delaimy, W.K.; Hibler, E.A.; Jones, L.A.; Leroy, E.C.; Newman, V.A.; Parker, B.A.; Rock, C.L.; Pierce, J.P. Vitamin D and breast cancer recurrence in the Women's Healthy Eating and Living (WHEL) study. *Am. J. Clin. Nutr.*, **2011**, *93*(1), 108-117.

[57] Vrieling, A.; Hein, R.; Abbas, S.; Schneeweiss, A.; Flesch-Janys, D.; Chang-Claude, J. Serum 25-hydroxyvitamin D and postmenopausal breast cancer survival: a prospective patient cohort study. *Breast Cancer Res.*, **2011**, *13*(4), R74.

[58] Tretli, S.; Schwartz, G.G.; Torjesen, P.A.; Robsahm, T.E. Serum levels of 25-hydroxyvitamin D and survival in Norwegian patients with cancer f breast, colon, lung and lymphoma: a population-based study. *Cancer Causes Control*, **2012**, *23*(2), 363-370.

[59] Kim, H.J.; Lee, Y.M.; Ko, B.S.; Lee, J.W.; Yu, J.H.; Son, B.H.; Gong, G.Y.; Kim, S.B.; Ahn, S.H. Vitamin D deficiency is correlated with poor outcomes in patients with luminal-type breast cancer. *Ann. Surg. Oncol.*, **2011**, *18*(7), 1830-1836.

[60] Yao, S.; Zirpoli, G.; Bovbjerg, D.H.; Jandorf, L.; Hong, C.C.; Zhao, H.; Sucheston, L.E.; Tang, L.; Roberts, M.; Ciupak, G.; Davis, W.; Hwang, H.; Johnson, C.S.; Trump, D.L.; McCann, S.E.; Ademuyiwa, F.; Pawlish, K.S.; Bandera, E.V.; Ambrosone, C.B. Variants in the vitamin D pathway, serum levels of vitamin D, and estrogen receptor negative breast cancer among African-American women: a case-control study. *Breast Cancer Res.*, **2012**, *14*(2), R58.

[61] 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 Horn, L.; Wactawski-Wende, J.; Whitlock, E.; Anderson, G.L.; Assaf, A.R.; Barad, D.; Women's Health Initiative Investigators. Calcium plus vitamin D supplementation and the risk of fractures. *N. Engl. J. Med.*, **2006**, 354(7), 669-683.

[62] 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 Horn. L.; Wallace, R.B.; Whitlock, E.; Bassford, T.l; 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.; Women's Health Initiative Investigators. Calcium plus vitamin D supplementation and the risk of colorectal cancer. *N. Engl. J. Med.*, **2006**, *354*(7), 684-96.

[63] 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 dataset. *Am. J. Clin. Nutr.*, **2011**, *94*(4), 1144-1149.

[64] Chlebowski, R.T.; Pettinger, M.; Kooperberg, C. Caution in reinterpreting the Women's Health Initiative (WHI) Calcium and Vitamin D Trial breast cancer results. *Am. J. Clin. Nutr.*, **2012**, *95*(1), 258-259.

[65] Institutes of Medicine, Food and Nutrition Board. Dietary reference intakes: calcium, phosphorus, magnesium, vitamin D and fluoride. Washington DC: National Academy Press, **1997**.

[66] Gallagher, J.C.; Sai, A.; Templin, T.; Smith, L. Dose response to vitamin D supplementation in postmenopausal women. *Ann. Intern. Med.*, **2012**, *156*(6), 425-437.

[67] 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.

[68] Crew, K.D.; Shane, E.; Cremers, S.; McMahon, D.J.; Irani, D.; Hershman, D.L. High prevalence of vitamin D deficiency despite supplementation in premenopausal women with breast cancer undergoing adjuvant chemotherapy. *J. Clin. Oncol.*, **2009**, *27*(13), 2151-2156.

[69] Neuhouser, M.L.; Sorensen, B.; Hollis, B.W.; Ambs, A.; Ulrich, C.M.; McTiernan, A.; Bernstein, L.; Wayne, S.; Gilliland, F.; Baumgartner, K.; Baumgartner, R.; Ballard-Barbash, R, Vitamin D insufficiency in a multiethnic cohort of breast cancer survivors. *Am. J. Clin. Nutr.*, **2008**, *88*(1), 133-9.

[70] Cescon, D.W.; Ganz, P.A.; Beddows, S.; Ennis, M.; Mills, B.K.; Goodwin, P.J. Feasibility of a randomized controlled trial of vitamin D vs. placebo in women with recently diagnosed breast cancer. *Breast Cancer Res. Treat*, **2012**, *134*(2), 759-767.

[71] Heaney, R.P.; Holick, M.F. Why the IOM recommendations for vitamin D are deficient. *J. Bone Miner. Res.*, **2011**, *26*(3), 455-457.

[72] Holick, M.F.; Binkley, N.C.; Bischoff-Ferrari, H.A.; Gordon, C.M.; Hanley, D.A.; Heaney, R.P.; Murad, M.H.; Weaver, C.M. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab*, http://www.ncbi.nlm.nih.gov/pubmed?term=Holick%20MF%2C%20Binkley%20NC%2C%20Bischoff-Ferrari%20HA%2C%20 **2011**, *96*(7): 1911-1930.

[73] Hathcock J.J.; Shao, A.; Vieth, R.;, Heaney, R. Risk assessment for vitamin D.*Am. J. Clin. Nutr.*, **2007** Jan, *85*(1), 6-18

[74] Curhan, G.C.; Taylor, E.N. 25-h uric acid excretion and the risk of kidney stones. *Kidney Int.*, **2008**, *73*(4), 489-496.

[75] Launay-Vacher, V.; Gligorov, J.; Le Tourneau, C.; Janus, N.; Spano, J.P.; Ray-Coquard, I.; Oudard, S.; Pourrat, X.; Morere, J.F.;

Deray, G.; Beuzeboc, P. The Renal Insufficiency and Anticancer Medications (IRMA) Study Group: Prevalence of renal insufficiency in breast cancer patients and related pharmacological issues. *Breast Cancer Res. Treat*, **2009**, *70*(2), 124-133.

[76] Trukova, K.P.; Grutsch, J.; Lammersfeld, C.; Liepa, G. Prevalence of vitamin D insufficiency among breast cancer survivors. *Nutr. Clin. Pract.*, **2012**, *27*(1), 122-128.

[77] Vashi, P.G.; Trukova, K.; Lammersfeld, C.A.; Braun, D.P.; Gupta, D. Impact of oral vitamin D supplementation on serum 25-hydroxyvitamin D levels in oncology. *Nutr. J.*, **2010**, *9*, 60.

[78] Peppone, L.J.; Huston, A.J.; Reid, M.E.; Rosier, R.N.; Zakharia, Y.; Trump, D.L.; Mustian, K.M.; Janelsins, M.C.; Purnell, J.Q.; Morrow, G.R. The effect of various vitamin D supplementation regimens in breast cancer patients. *Breast Cancer Res. Treat*, **2011**, *127*(1), 171-177.

[79] Chlebowski, R.T.; Johnson, K.C.; Lane, D.; Pettinger, M.; Kooperberg, C.L.; Wactawski-Wende, J.; Rohan, T.; O'Sullivan, M.J.; Yasmeen, S.; Hiatt, R.A.; Shikany, J.M.; Vitolins, M.; Khandekar, J.; Hubbell, F.A. 25-hydroxyvitamin D concentration, vitamin D intake and joint symptoms in postmenopausal women. *Maturitas*, **2010**, *68*(1), 73-78.

[80] Chlebowski, R.T.; Johnson, K.C.; Kooperberg, C.; Hubbell, F.A.; Lane, D.; O'Sullivan, M.; Cummings, S.; Hiatt, R.; Shikany, J.M.; Rohan, T.; Yasmeen, S.; Khandekar, J.; for the Women's Health Initiative (WHI) Investigators. The Women's Health Initiative randomized trial of calcium plus vitamin D: breast cancer and arthralgia effects. *Proc. Amer. Soc. Clin. Oncol.*, **2006**, *25*, 6.

[81] Chlebowski, R.T. Aromatase inhibitor-associated arthralgias. *J. Clin. Oncol.*, **2009**, *27*(30), 4932-4934.

[82] Waltman, N.L.; Ott, C.D.; Twiss, J.J.; Gross, G.J.; Lindsey, A.M. Vitamin D insufficiency and musculoskeletal symptoms in breast cancer survivors on aromatase inhibitor therapy. *Cancer Nurs.*, **2009**, *32*(2), 43-150.

[83] Singh, S.; Cuzick, J.; Mesher, D.; Richmond, B.; Howell, A. Effect of baseline serum vitamin D levels on aromatase inhibitors induced musculoskeletal symptoms: results from the IBIS-II, chemo-prevention study using anastrozole. *Breast Cancer Res. Treat*, **2012**, *132*(2), 625-629.

[84] Amir, E.; Simmons, C.E.; Freedman, O.C.; Dranitsaris, G.; Cole, D.E.; Vieth, R.; Ooi, W.S.; Clemons, M. A phase 2 trial exploring the effects of high dose (10, 000 IU/day) vitamin D(3) in breast patients with bone metastases. *Cancer*, **2010**, *116*(2), 184-191.

[85] Khan, Q.J.; Reddy, P.S.; Kimler, B.F.; Sharma, P.; Baxa, S.E.; O'Dea, A.P.; Klemp, J.R.; Fabian, C.J. Effect of vitamin D supplementation on serum 25-hydroxy vitamin D levels, joint pain, and fatigue in women starting adjuvant letrozole treatment for breast cancer. *Breast Cancer Res. Treat*, **2010**, *119*(1), 111-118.

[86] Rastelli, A.L.; Taylor, M.E.; Gao, F.; Armamento-Villareal, R.; Jamalabadi-Majidi, S.; Napoli, N.; Ellis, M.J. Vitamin D and aromatase inhibitor-induced muscoloskelatal symptoms (AIMSS): a phase II, double-blind, placebo-controlled, randomized trial. *Breast Cancer Res Treat*, **2011**, *129*(1), 107-116.

[87] Prieto-Alhambra, D.; Javaid, M.K.; Servitja, S.; Arden, N.K.; Martinez-Garcia, M.; Diez-Perez, A.; Albanell, J.; Tusquets, I.; Nogues, X. Vitamin D threshold to prevent aromatase inhibitor-induced arthralgia: a prospective cohort study. *Breast Cancer Res. Treat*, **2011**, *125*(3), 869-878.

[88] Prieto-Alhambra, D.; Servitja, S.; Javaid, M.K.; Garrigós, L.; Arden, N.K.; Cooper, C.; Albanell, J.; Tusquets, I.; Diez-Perez, A.; Nogues, X. Vitamin D threshold to prevent aromatase inhibitor-related bone loss: the B-ABLE prospective cohort study. *Breast Cancer Res. Treat*, **2012**, Mar 21 [Epub ahead of print].

[89] McCullough, M.L.; Weinstein, S.J.; Freedman, D.M.; Helzlsouer, K.; Flanders, W.D.; Koenig, K.; Kolonel, L.; Laden, F.; Le Marchand, L.; Purdue, M.; Snyder, K.; Stevens, V.L.; Stolzenberg-Solomon, R.; Virtamo, J.; Yang, G.; Yu, K.; Zheng, W.; Albanes, D.; Ashby, J.; Bertrand, K.;, Cai, H.; Chen, Y.; Gallicchio, L.; Giovannucci, E.; Jacobs, E.J.; Hankinson, S.E.; Hartge, P.; Hartmuller, V.; Harvey, C.; Hayes, R.B.; Horst, R.L.; Shu, X.O. Correlates of circulating 25-hydroxyvitamin D: Cohort consortium vitamin D pooling project for rare cancers. *Am. J. Epidemiol.*, **2010**, *172*(1), 21-35.

[90] Millen, A.E.; Wactawski-Wende, J.; Pettinger, M.; Melamed, M.L.; Tylavsky, F.A.; Liu, S.; Robbins, J.; LaCroix, A.Z.; LeBoff, M.S.; Jackson, R.D.; Predictors of serum 25-hydroxyvitamin D concentrations among postmenopausal women: the Women's Health Initiative calcium plus vitamin D clinical trial. *Am. J. Clin. Nutr.*, **2010**, *91*(5), 1324-1335.

[91] Lucas, J.A.; Bolland, M.J.; Grey, A.B.; Ames, R.W.; Mason, B.H.; Horne, A.M.; Gamble, G.D.; Reid, I. Determinants of vitamin D status in older women living in a subtropical climate. *Osteoporosis Int.*, **2005**, *16*(12), 1641-1648.

[92] Lappe, J.M.; Davies, K.M.;, Travers-Gustafson, D.; Heaney, R.P.; Vitamin D status in a rural postmenopausal female population. *J. Am. Coll. Nutr.*, **2006**, *25*(5), 395-402.

[93] Bjelakovic, G.; Gludd, 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**, *6*(7), CD007470.

[94] LaCroix, A.Z.; Kotchen, J.; Anderson, G.; Brzyski, R.; Cauley, J.A.; Cummings, S.R.; Gass, M.; Johnson, K.C.; Ko, M.; Larson, J.; Manson, J.E.; Stefanick, M.L.; Wactawski-Wende, J. Calcium plus vitamin D supplementation and mortality in postmenopausal women: the Women's Health Initiative calcium-vitamin D randomization controlled trial. *J. Gerontol. A. Biol. Sci. Med. Sci.*, **2009**, *64*(5), 559-567.

[95] Autier, P.; Gandini, S. Vitamin D supplementation and total mortality: a meta-analysis of randomized controlled trials. *Arch. Intern. Med.*, **2007**, *296*(16), 2257-2264.

[96] 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.

[97] Brigham and Women's Health Press Release. Largest study of vitamin D and omega-3s set to begin soon at Brigham and Women's Hospital. [http://www.brighamandwomens.org/Pressreleases/PressRelease.aspx?PageID=508 (assessed October 20, **2010**)].

[98] Internet Cmmunication: National Insitute of Health Research (NIHR); http://www.hta.ac.uk/funding/clinicaltrials/Outcomestableforweb.pdf. Accessed on 16-2-**2011**.

[99] Melamed, M.L.; Michos, E.D.; Post, W.; Aston, B. 25-hydroxyvitamin D levels and the risk of mortality in the general population. *Arch. Intern. Med.*, **2008**, *168*(15), 1629-1637.

[100] Rosen, C.J. Vitamin D insufficiency. *New Engl. J. Med.*, **2011**, *364*, 284-354.

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