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The Vitamin D and Cancer Conundrum: Aiming at a Moving Target

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ABSTRACT

The case for the influence of vitamin D on health, including cancer prevention, is increasingly compelling. While some are calling for increases in the Tolerable Upper Intake Level, fortification, and dietary supplementation, questions regarding dose and individual response variability continue to merit attention. Colorectal cancer risk reduction with adequate vitamin D status is well documented. Protection has also been observed for cancer at all sites, skin, prostate, and breast. At the same time, some individuals may be adversely affected by elevated 25(OH)D concentrations with respect to risk of cancers of the prostate, breast, pancreas, and esophagus, and in some cases a U- or J-shaped association has been suggested. Future research should seek to clarify if and for whom there may be an increased risk for cancer at particular sites with high 25(OH)D concentrations, and the concentrations at which risk increases. Fundamentally, prospective longitudinal studies of these relationships are warranted. The health status, life stage, adiposity, estrogen exposure, and nutritional status of study participants should be taken into account. Continued investigation is necessary to ensure that vitamin D recommendations are appropriately targeted to individuals who stand to benefit most, while protecting vulnerable subgroups from risk of overexposure.
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Emerging evidence makes an increasingly compelling case that the importance of vitamin D for health extends far beyond bone. As the nuances of vitamin D metabolism are elucidated, the excitement around its potential to protect against cancer (1-15), metabolic syndrome (16-18), diabetes (19-24), hypertension (25-27), multiple sclerosis (28,29), and other health conditions is rising. It is no surprise, therefore, that exceptionally high attention is being given to vitamin D research, including the decision by the Institute of Medicine to revisit the Dietary Reference Intakes for vitamin D and calcium (30), increased availability of fortified foods and dietary supplements, proposed inclusion of vitamin D in the calcium/osteoporosis health claim on food labels (31), and mass media coverage. Some are calling for increasing the Tolerable Upper Intake Level, fortification, and dietary supplementation (2,32-34). Yet caution is noted, as questions regarding "how much" and "for whom" continue to merit attention (35-42).

The nutrition paradigm shifted in the 1980s and 1990s from preventing deficiency to optimizing health, and is adjusting once again in the early 21st century. The quest for optimal health at first fueled the mantra, more is better. Evidence has emerged, however, to suggest that the quest for optimal health is not without risks. More is not always better. One need look no further than antioxidants, which were touted as insurance against, if not a cure for, cancer, but are now recognized as playing a much more complex, and sometimes detrimental, role in cancer. Examples include findings in the mid-1990s of increased lung cancer incidence among smokers consuming exaggerated amounts of beta carotene as a dietary supplement (43,44).

Vitamin D is, indeed, an integral player in bone health, and may be determined to be as important in certain

cancers. Numerous workshops and review articles focusing on the associations between low vitamin D status and increased cancer risk are available in the literature (45-50). There has been little attention to the data that have suggested a potential for increased risk among certain individuals with high vitamin D status, although the International Agency for Research on Cancer did note in its review the need to further assess the potential for a J- or U-shaped association between vitamin D status and risk of cancers of the prostate, pancreas, and esophagus (50). This article briefly reviews studies germane to the consideration of a potential J- or U-shaped association between vitamin D and cancer, as factors that may indicate the need for caution against high-dose supplementation with vitamin D for vulnerable subgroups of the general population are particularly relevant to the work of dietetics practitioners.

DOES VITAMIN D HAVE A U-SHAPED DOSE–RESPONSE ASSOCIATION?

For many, if not all, nutrients, there are risks associated with both deficient and excess levels of intake. At the high end, disease and/or toxicity risk increases. At the low end, deficiency disease risk increases. Such a dose–response relationship generates a J- or U-shaped curve, characterized by increased risk in regions of both deficiency and excess and a mid-range of doses associated with decreased risk.

In considering the possibility of a J- or U-shaped association between vitamin D and cancer risk, three classes of biomarkers will be considered in this review. Biomarkers are essential for characterizing the exposures required to bring about a desired response, the biological target(s) or effects attributing to a change, and susceptibility in terms of genetics and nutrient–nutrient interactions. The association between vitamin D and health has been largely understood with respect to 25-hydroxyvitamin D (25[OH]D) as the biomarker of exposure, although there is emerging evidence addressed in this paper regarding potential confounders of 25(OH)D with respect to exposure. Biomarkers of vitamin D's effects have included parameters of toxicity (eg, hypercalcemia and hypercalciuria) or impaired bone function (eg, fracture incidence and bone mineral density). Finally, an examination of the biomarkers of susceptibility with respect to vitamin D and cancer are just beginning to emerge and must be carefully examined as public health recommendations are revised.

A RELIABLE ASSESSMENT OF VITAMIN D EXPOSURE

Vitamin D is unique in nutrition history, as its essentiality was not discovered based on the usual observation of deficiency resulting from a lack of dietary consumption. Rather, an understanding of human need for vitamin D was derived from the observation of rickets in children who were exposed to minimal sunlight (51). Whether obtained through ultraviolet (UV) radiation-facilitated cutaneous production of cholecalciferol (vitamin D-3) or through dietary intake of cholecalciferol and ergocalciferol (vitamin D-2) (Figure), it is clear that vitamin D is essential for the adequate production of the physiologically

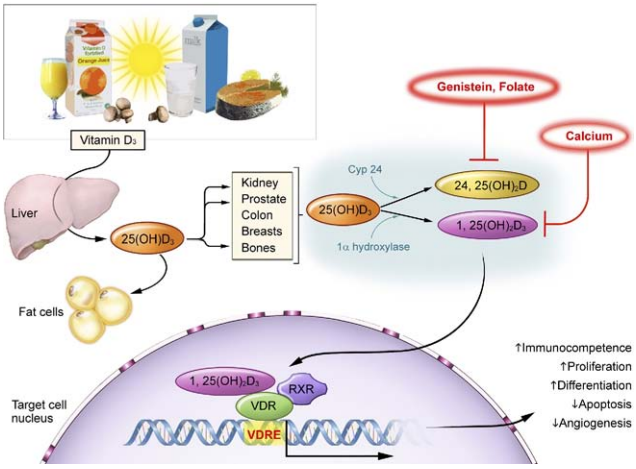

Figure. Vitamin D sources, metabolites, and mechanisms of action with respect to carcinogenesis. VDR=vitamin D receptor. VDRE=vitamin D response element. RXR=retinoid X receptor.

active hormone, 1,25-dihydroxyvitamin D (1,25[OH]$_2$D). A highly active steroid hormone with potent cell signaling activity, 1,25(OH)$_2$D is tightly regulated at the tissue level.

The intermediate metabolite of vitamin D from both UV radiation and diet, 25(OH)D, is commonly utilized as a biomarker of exposure. It is the primary circulating form of vitamin D, has a half life that is considerably longer than that of 1,25(OH)$_2$D (15 days vs 15 hours) (52), and has been correlated with total vitamin D exposure from both endogenous production and the diet (53-56).

Nonetheless, a reliable assessment of exposure to vitamin D is complicated by the existence of dual sources. Numerous factors, such as highly variable sun avoidance practices, clothing worn, latitude, air pollution, season of year, skin pigmentation, and ambiguity regarding the implications of acute vs chronic UV exposures, contribute to sunlight measurement complexity (57). Assessment of usual dietary intake of vitamin D is also challenged by several factors. For example, the availability of vitamin D in fortified foods and dietary supplements is highly variable (58).

There is also debate regarding the bioavailability of vitamin D-2 vs vitamin D-3. Vitamin D-3 supplementation has elicited greater increases in 25(OH)D than vitamin D-2 in some (59,60) but not all studies (61). In human subjects, the effects of single doses of 1,250 μg (50,000 IU) vitamin D-3 and D-2 on 25(OH)D were compared over 14 days (60). Although initially similar, 25(OH)D concentrations returned to baseline levels in the D-2 group, whereas concentrations continued to rise throughout the study period in the D-3 group (60). In a separate study, daily doses of 100 μg (4,000 IU) vitamin D-3 and vitamin D-2 were compared over 14 days (59). Vitamin D-3 increased 25(OH)D concentrations 1.7 times more than vitamin D-2 (P=0.03) (59). Holick and colleagues (61), however, found that 25 μg (1,000 IU) vitamin D-2 or D-3 to be comparably effective in raising 25(OH)D concentrations, suggesting that the efficacy of D-2 vs D-3 may depend on the dose provided.

As a biomarker of vitamin D status, 25(OH)D is not without shortcomings. The concentration of 25(OH)D may be influenced by a complex interplay between adiposity (sequestration), skin pigmentation (less substrate), and physical activity (less substrate and/or mobility) (53,62,63). Of particular significance is the reality that 25(OH)D concentrations are influenced by certain metabolic and disease processes, including carcinogenesis and several dietary components (eg, calcium, genistein and folate).

There are also measurement issues that should be taken into account when interpreting serum 25(OH)D values. Specifically, 25(OH)D is usually measured at just one point in time; therefore, it is not an indicator of long-term exposure status (64). While some analytic techniques separate $25(OH)D_2$ from $25(OH)D_3$, the distinction may or may not be relevant from efficacy or safety perspectives. The inconsistency in measuring and reporting these two forms of vitamin D can lead to confusion and misinterpretation (65). Accreditation of laboratories to improve the methodology and interpretation of vitamin D measurements is being undertaken through the vitamin D External Quality Assessment Scheme (www.deqas.org). In addition, the National Institute of Standards and Technology has collaborated with the National Institutes of Health's Office of Dietary Supplements to develop standard reference material for the validation of serum $25(OH)D_2$ and $25(OH)D_3$ measurement methods (66).

CONNECTING VITAMIN D EXPOSURE TO HEALTH OUTCOMES

The association between vitamin D (25[OH]D) and health risk is best understood with respect to bone health. However, even in this extensively studied area, there is considerable debate about the specific break points where concentrations are linked with either negative or positive health outcomes. According to Vieth and colleagues (67), 100 μg (4,000 IU) per day for 5 months was well tolerated, and no adverse events such as hypercalcemia were observed. A review of human clinical trials by Hathcock and colleagues (68) concluded that doses even higher than 250 μg (10,000 IU) per day were found to produce no toxic effects. On the other hand, the Institute of Medicine estimated the Tolerable Upper Intake Level to be 50 μg (2,000 IU) for individuals aged 14 years or older (69). It should also be noted that in the Women's Health Initiative those taking 10 μg (400 IU) vitamin D reported a 17% increase in renal calculi (70). Whether this complication occurred secondary to other health-related issues or high calcium intake, or the participants represented a particularly vulnerable subpopulation remains to be determined. It is certainly conceivable that individuals vary considerably in the dosage of vitamin D that brings about benefits or induces ill consequences.

A dose–response for the multiple nonskeletal health outcomes that may be influenced by vitamin D intake, such as some cancers (eg, colon, prostate, and breast), diabetes, hypertension, and multiple sclerosis, are even less well defined. What are the appropriate biomarkers of effect? Questions also arise as to whether exposure levels should be considered for each disease or health endpoint of interest. Indeed, it will be critical to take these other potential health outcomes into account in some manner as vitamin D needs are continually reassessed.

VITAMIN D AND CANCER

The vitamin D and cancer interrelationship surfaced with observations that increased sun exposure was associated with a reduction in colon cancer mortality (71), and later with a reduction in risk of prostate cancer (72). Further evidence has emerged to suggest a protective effect of vitamin D with respect to risk of cancer incidence, progression, and/or mortality. This protection has been observed for cancer at all sites (4), skin (5,6), colon and/or rectum (7-11), prostate (12), and breast (13,14,15). At the same time, some individuals may be adversely affected by elevated 25(OH)D concentrations, with respect to risk of cancers of the prostate (39,40), breast (15), pancreas (36,37), and esophagus (41,42) (Table). Examination of the evidence for a negative effect of vitamin D on carcinogenesis in various tissues and among certain subgroups suggests a complex situation in which the potential for both harm and benefit may depend on dose, timing and duration of exposure, tissue specificity, lifestyle factors, and genetic polymorphisms.

Skin

Since vitamin D exposure is primarily derived from UV exposure, discussion of cancer risk must include melanoma. Although UV exposure is the greatest risk factor for melanoma, it is possible that vitamin D may be protective against melanoma progression (5,6). Notably, Nürnberg and colleagues (5) found that lower 25(OH)D concentrations were associated with progression of malignant melanoma. Moan and colleagues (6) noted that although melanoma incidence is higher in southern vs northern latitudes, melanoma prognosis is actually better for populations in regions closer to the equator. These studies point to the importance of assessing the effects of vitamin D not only in primary prevention, but also in the early and late stages of carcinogenesis.

Colon and Rectum

The most compelling role of vitamin D in cancer prevention comes from studies involving the colon and rectum (1,7-11). Most epidemiologic evidence points to a lower colorectal cancer risk in those with greater vitamin D exposure. Dietary vitamin D intake greater than 16 μg/day (≥645 IU/day) vs intake less than 4.5 μg/day (≤180 IU/day) has been observed to be inversely associated with advanced colonic neoplasia (odds ratio [OR] 0.61; 95% confidence interval [CI] 0.39 to 0.97) (7). In a case-control study, a pooled analysis of individuals in the Health Professionals Follow-up Study and the Nurses' Health Study with 25(OH)D concentrations in the highest (median 39.4 ng/mL [98 nmol/L]) vs lowest (median 18.4 ng/mL [46 nmol/L]) quintile had a lower risk of colorectal cancer (OR 0.66; 95% CI 0.42 to 1.05; P_{trend}=0.01) and colon cancer risk (OR 0.54; 95% CI 0.34 to 0.86; P_{trend}=0.002) (9). Analysis of the Health Professionals Follow-up Study cohort alone revealed an association with colon cancer (OR 0.46; 95% CI 0.24 to 0.89; P_{trend}=0.005) (9). Colorectal cancer mortality was 72% lower in those with serum 25(OH)D concentrations of 32 ng/mL (80 nmol/L) or higher, compared with those with concentrations of 20 ng/mL (50 nmol/L) or less (95% CI 32% to 89%, P_{trend}=0.02) (10). A meta-analysis of

Table. Summarized findings from seven studies that observed an association between high 25(OH)D serum concentration and an increased risk for cancer

Author(s) and year	Study design	Participants (n)	Duration of study	Outcome or biomarker	Summary of findings (odds ratio[OR] [95% confidence interval], unless otherwise noted)
Breast					
Goodwin and colleagues 2009 (15)	Prospective Cohort	Women with early breast cancer (512)	Mean 11.6 y	Distant recurrence and death	<20 ng/mL vs 29-32 ng/mL, multivariate HR[a] for distant recurrence=1.71 (1.02-2.86) and for death=1.6 (0.96 to 2.64); >44 ng/mL vs 32-44 ng/mL, nonsignificant increase in risk for death
Esophageal					
Abnet and colleagues 2007 (42)	Cohort	Chinese men and women (720)	Cross-sectional analysis	Esophageal squamous dysplasia	Highest vs lowest quintile, RR[b] 1.86 (1.35-2.62, P_{trend}=0.002); In males, RR 1.74 (1.08-2.93, P_{trend}=0.0373); In women, RR 1.96 (1.28-3.18, P_{trend}=0.0021)
Chen and colleagues 2007 (41)	Prospective, stratified (age and sex), case-control	Chinese men and women, 40-69 y (545 cases; 1,071 controls)	6 y	Esophageal squamous cell carcinoma	In men, highest vs lowest quartile, HR 1.77 (1.16-2.70, P_{trend}=0.0033); In men and women combined, highest vs lowest quartile, HR 1.3 (0.97-1.73, P_{trend}=0.013)
Pancreatic					
Stolzenberg-Solomon and colleagues 2006 (36)	Prospective, nested, case-control	Finnish male smokers, 50-69 y (200 cases; 400 controls)	Median 11.8 y	Pancreatic cancer incidence	Cases more likely to have higher 25(OH)D (P=0.03); Highest quartile (≥65 nmol/L) vs lowest (≤32 nmol/L), multivariate adjusted OR 2.92 (1.56-5.48; P_{trend}=0.001)
Stolzenberg-Solomon and colleagues 2009 (37)	Prospective, nested, case-control	US men and women (184 cases; 368 controls)	Mean 11.7 y	Pancreatic cancer incidence	Not associated with pancreatic cancer, OR 1.45 (0.66-3.15; P_{trend}=0.49); For those with low vs moderate/high residential sun exposure, 25(OH)D associated with increased pancreatic cancer risk, multivariate adjusted OR 4.03 (1.38-11.79; $P_{interaction}$=0.015) Cases vs controls more often reported being current smoker (P<0.0002)
Prostate					
Ahn and colleagues 2008 (40)	Stratified (stage of prostate cancer), nested, case-control	US men (749 cases; 781 controls)	2-10 y	Prostate cancer risk	Increasing quintile of season-standardized 25(OH)D and prostate cancer risk, OR 1.32 (0.94-1.84; P_{trend}=0.04); high-stage aggressive disease, OR 1.83 (0.95-3.5; P_{trend}=0.02); aggressive disease with stringent definition, OR 1.78 (1.01-3.14; P_{trend}=0.03)
Tuohimaa and colleagues 2004 (39)	Longitudinal, nested, case-control	Nordic men (622 cases; 1,451 controls)	14-15 y	Prostate cancer incidence	Both low (≤19 nmol/L) and high (≥80 nmol/L 25(OH)D associated with increased risk (OR 1.5 [0.8-2.7] and 1.7 [1.1-2.4], respectively; 40-59 nmol/L 25(OH)D associated with lowest risk.

[a]HR=hazard ratio.
[b]RR=relative risk.

epidemiologic studies suggested that individuals with 25(OH)D ≥33 ng/mL (82 nmol/L) may have a 50% lower risk of colon cancer than individuals with values ≤12 ng/mL (30 nmol/L) (P_{trend}<0.0001) (8).

Experimental evidence regarding the relationship between vitamin D supplementation and colon cancer is beginning to emerge and suggests the need for additional research. There is promising evidence from a preclinical

model suggesting that supplementation with a combination of calcium and vitamin D reduced colon tumor incidence (89% fewer intestinal tumors, $P=0.01$; 100% fewer colonic tumors, $P=0.05$) and multiplicity (91% reduction in the intestine, $P=0.01$; 82% reduction in the small intestine, $P=0.02$; 82% reduction in the colon, $P=0.02$) that are otherwise observed over the course of 24 months in mice fed a diet with characteristics that have been associated with increased risk of colon cancer (high in dietary fat and low in vitamin D, calcium, folic acid, choline, and methionine) (11). Clearly, the effects of vitamin D vs the combination with calcium will need to be sorted out, and the usual caveats to the extrapolation of animal data to the human situation apply. Human experimental data have been inconclusive. In the Women's Health Initiative study, vitamin D-3 supplementation (10 μg [400 IU]) did not affect colon cancer risk (35). It is possible that in this population a daily dietary supplement of 10 μg (with adherence at approximately 65%) was insufficient to maintain protective concentrations of 25(OH)D or that higher than average baseline vitamin D intakes may have reduced the differences between controls and cases. Calcium intakes may also have been a confounding factor. Further, as colorectal cancer latency is 10 to 20 years, the 7-year study period may have been too short to detect a benefit. It is noteworthy that a nested case-control study within the Women's Health Initiative found that lower baseline serum 25(OH)D concentrations were associated with an increased risk of colorectal cancer (35). The Women's Health Initiative and the mouse study together with observational data indicate that additional, properly controlled study of the effect of vitamin D supplementation on colon and rectal cancer risk is warranted.

Prostate

In US populations, epidemiologic studies have generally found no association between 25(OH)D concentrations and prostate cancer risk. In a middle-aged Scandinavian population, where vitamin D deficiency is frequent, Faupel-Badger and colleagues (73) found no relationship between prostate cancer risk and 25(OH)D concentrations. In a similar population, serum 25(OH)D concentrations were inversely associated with risk of earlier exposure to and more aggressive prostate cancer (OR 1.7; 95% CI 1.0 to 3.0; $P_{\text{trend}}=0.01$) (12).

Of concern is the suggestion of a U-shaped relationship between serum 25(OH)D and prostate cancer risk in a prospective case-control study by Tuohimaa and colleagues (39). Increased risk was observed at 25(OH)D concentrations both ≤8 ng/mL (20 nmol/L) (OR 1.5; 95% CI 0.8 to 2.7) and ≥32 ng/mL (80 nmol/L) (OR 1.7; 95% CI 1.1 to 2.4), compared to a reference range of 16 to 24 ng/mL (15 to 60 nmol/L). In a case-control study in which participants were stratified according to diagnosis of aggressive vs nonaggressive prostate cancer, Ahn and colleagues (40) detected a significant linear increase in risk of aggressive prostate cancer for those with 25(OH)D concentrations higher than the lowest quintile (<17 ng/mL [42 nmol/L]) ($P_{trend}=0.05$). It is notable that this study (40) included a higher ratio of cases to controls, compared to the study conducted by Tuohimaa and colleagues (39).

Breast

The totality of evidence regarding the association between breast cancer risk and vitamin D status is unclear (74). Garland and colleagues (13) have asserted that breast cancer incidence may be reduced by 50% ($P<0.001$) with a 25(OH)D concentration of 52 ng/mL (130 nmol/L) compared to 10 ng/mL (25 nmol/L) or less. The findings of two recent prospective cohorts were consistent with this assertion. Participants in the Iowa Women's Health Study who reported consumption of >20 μg (800 IU) vitamin D per day had lower breast cancer risk than those who reported consuming less than 10 μg per day (400 IU) (adjusted relative risk 0.66, 95% CI 0.46 to 0.94, $P_{\text{trend}}=0.02$) (14). Goodwin and colleagues (15) reported that women with early breast cancer and 25(OH)D concentrations <20 ng/mL (50 nmol/L) were found to be at increased risk of distant recurrence (multivariate hazard ratio [HR] 1.71, 95% CI, 1.02 to 2.86) and death (multivariate HR 1.6, 95% CI 0.96 to 2.64) compared to those whose concentrations were greater than 29 ng/mL (72 nmol/L). However, the maximum benefit occurred within the range of 32 to 44 ng/mL (80 to 110 nmol/L), suggesting that a U-shaped relationship might exist between 25(OH)D and cancer survival (15). The finding that survival may decrease with 25(OH)D concentrations >44 ng/mL (110 nmol/L) was not statistically significant; however, it does raise awareness of the need for additional research. It is also noteworthy that some recent studies have found no association between breast cancer risk and vitamin D exposure (75) or 25(OH)D concentrations (76).

Pancreas

In a prospective study, higher prediagnostic 25(OH)D concentrations have been reported to be associated with a three-fold higher risk for pancreatic cancer in Finland among smokers (OR 2.92; 95% CI 1.56 to 5.48; $P_{\text{trend}}=0.001$) (36). Several potentially confounding variables in this study, including a lower long-term vitamin D status than the global average and concerns about organochlorines in vitamin D–rich fish consumed, may have influenced the observed interrelationships. In another nested case-control study in the United States, 25(OH)D concentrations were not associated with risk of overall pancreatic cancer among older adults (37). However, among the study participants living in low, vs moderate or high, residential UVB exposure areas, there was an increased risk of pancreatic cancer with higher 25(OH)D concentrations (OR 4.03; 95% CI 1.38 to 11.79; $P_{\text{interaction}}=0.015$) (37).

Esophagus

Prospective epidemiologic evidence has also revealed potential concerns regarding vitamin D and esophageal cancer. Namely, a direct association was found between highest vs lowest quartiles of serum 25(OH)D concentrations and esophageal squamous cell carcinoma in Chinese men (HR 1.77; 95% CI 1.16 to 2.70, $P_{\text{trend}}=0.0033$) and in men and women combined (highest vs lowest quartile, HR 1.3; 0.97 to 1.73, $P_{\text{trend}}=0.013$), but not in women (41). Further, 25(OH)D concentrations were associated with increased risk of esophageal squamous dysplasia, an ab-

normality that precedes esophageal squamous cell carcinoma, in men (relative risk [RR] 1.74; 95% CI 1.08 to 2.93, P_{trend}=0.0373), in women (RR 1.96; 95% CI 1.28 to 3.18, P_{trend}=0.0021), and in men and women combined (RR 1.86; 95% CI 1.35 to 2.62, P_{trend}=0.002) (42). The authors speculate that vitamin D increases phase I enzyme activity and, thereby, promotes the bioactivation of environmental polycyclic aromatic hydrocarbons.

MECHANISMS OF ACTION

The vitamin D receptor binds the biologically active form of vitamin D, 1,25(OH)$_2$D, and interacts with specific nucleotide sequences (response elements) of target genes to produce a variety of biological effects (Figure). These include the induction and or inhibition of multiple genes that influence proliferation, invasiveness, angiogenesis, metastatic potential, differentiation, and apoptosis (77,78). More than 470 single nucleotide polymorphisms have been identified in the vitamin D receptor (79), some of which may eventually serve as biomarkers of susceptibility for cancer risk.

There are six vitamin D receptor polymorphisms that have been studied most frequently with respect to cancer, including *Fok1, Cdx2, Bsm1, Apa1, Taq1,* and *Poly(A)* (80). Several of the vitamin D receptor polymorphisms commonly studied are named after the restriction enzymes originally used for genotyping. For example, the *Fok1* single nucleotide polymorphism can be detected as a restriction fragment length polymorphism using the enzyme *Fok1*. A meta-analysis of case-control and nested case-control studies of the *Fok1* and *Bsm1* polymorphisms and cancer risk found a significant 14% increase in breast cancer risk (95% CI 1.03 to 1.27; P=0.006) and a nonsignificant 30% increase in skin cancer risk (95% CI 1.04 to 1.61; P=0.68) among individuals with the *Fok1 ff* vs *FF* genotypes (81). Cell culture data indicate that the *f* allele results in a vitamin D receptor protein that is functionally less active (82,83); thus, the cellular consequences of the *ff* genotype are similar to that of lower vitamin D status (81). In fact, Orton and colleagues (84) note that serum 25(OH)D concentrations may be associated with the *Fok1* single nucleotide polymorphism, as concentrations were noted to be lower among individuals with the *ff* vs *FF* genotype (64 nmol/L vs 100 nmol/L, respectively; P=0.005). The *Fok1* polymorphism is explored here as an example because it has been shown to have a functional effect. More broadly, these types of studies serve as proof of principle that genetic polymorphisms can modify the relationship between vitamin D status and cancer risk. The state of the science has been explored and documented in existing reviews and meta-analyses (85,86). Clearly, additional work is needed in this area.

The enzyme, 25-hydroxyvitamin D-1α-hydroxylase (1-α-hydroxylase), is activated by CYP27B1 and catalyzes conversion of 25(OH)D to 1,25(OH)$_2$D in many tissues throughout the body, when the substrate is present in sufficient amounts (47) (Figure). The 1,25(OH)$_2$D produced in extra-renal tissues acts locally and is subsequently degraded by 24-hydroxylase (CYP24) (47) (Figure). Local activation and degradation of 1,25(OH)$_2$D are mechanisms through which vitamin D differentially influences cancer risk at various body sites (87). These processes are also modulated by carcinogenesis in certain tissues (eg, prostate and colon), suggesting potential avenues for cancer prevention (87,88).

MODIFIERS

Activation of 1,25(OH)$_2$D is modified by carcinogenesis in certain tissues and by dietary factors. For example, in prostate cancer, there is a decrease in the ability of 1-alpha-hydroxylase to convert 25(OH)D to 1,25(OH)$_2$D (89,90). Therefore, with advanced prostate cancer, prostate cells are unable to activate 1,25(OH)$_2$D from 25(OH)D to suppress cell division and/or promote differentiation (89,90). Calcium, often consumed along with vitamin D in fortified milk and dietary supplements, also tends to reduce renal hydroxylation of 25(OH)D to 1,25(OH)$_2$D (91). The effect of calcium on nonrenal tissues remains to be clarified. Conversely, phytoestrogens and folate stimulate colonic synthesis of 1,25(OH)$_2$D from 25(OH)D via activation of CYP27B1 (91-93).

Binding of 1,25(OH)$_2$D to the vitamin D receptor is also modified by dietary factors, such as retinol (94), and carcinogenesis. Vitamin D receptor activity is lost in human beings in poorly differentiated colon tumors, rendering them unable to extract circulating 1,25(OH)$_2$D (95). Serum 25(OH)D measurements in these late stages may be misleading, as adequacy of exposure would not necessarily confer a benefit in advanced carcinogenesis of the colon or rectum.

Reduced degradation of 1,25(OH)$_2$D may also be modulated by dietary factors. Calcium, phytoestrogens (eg, genistein in soy), and folate have been observed to inhibit CYP24A1 activity and, therefore, degradation of 1,25(OH)$_2$D (96). Similarly, genistein works synergistically with 1,25(OH)$_2$D$_3$ or 25(OH)D$_3$ in vitro to inhibit growth of prostate epithelial cells and prostate cancer cells (97), potentially through inhibition of CYP24A1 activity and increased stability of the vitamin D receptor (98).

In human beings, total body fat also appears to influence vitamin D status, presumably by being a storage site or "sink" for vitamin D (99). Serum 25(OH)D has been observed to be lower in individuals with a body mass index >30 (100-102). This hypothesis is supported by observations that obese and nonobese individuals have similar skin content of vitamin D-3 precursors, but individuals with obesity have a lower increase in serum vitamin D-3 following ultraviolet exposure (102). Likewise, vitamin D esters accumulate in the fat of rats as a function of time (103). It is unclear if weight loss would create a safety concern because vitamin D may be liberated from adipose stores (104).

In addition to the potential effects of adiposity on vitamin D status, it has been suggested that vitamin D status may conversely affect adiposity. Marshall (38) hypothesized that adding vitamin D to the diet modifies response to gut microbes in a way that may be contributing to obesity. The specific mechanism through which vitamin D would exert an obesogenic effect via the microbiome is unclear. Alternatively, there is evidence that baseline 25(OH)D status is positively correlated with thermic effect of a meal (105) and with body fat loss (106) in conjunction with a reduced-energy diet. The increasing prevalence of obesity worldwide emphasizes the importance of

investigating whether the relationships between 25(OH)D and body mass index and/or adiposity are simply confounding, if there is a direct relationship, and what the nature of such a relationship may be.

FRONTIERS IN VITAMIN D RESEARCH

As the relationship between vitamin D status and cancer are elucidated, it is important to consider the totality of evidence for both benefit and risk, and to consider these effects in subgroups, as well as the general public. The evidence for a protective effect of vitamin D on colorectal cancer risk is quite promising, with 25(OH)D concentrations of at least 32 ng/mL (80 nmol/L) being associated with lower risk compared to lower concentrations (1,7-11). Conversely, evidence is rather mixed for breast cancer risk (13-15,74) and is limited but suggestive of increased risk in esophageal (41,42), prostate (39,40), and pancreatic tissues (36,37), possibly for subgroups of the general population. The concentrations at which increased risk has been observed in these studies are quite varied (15, 36,37,39,40-42). In the two studies that documented a U-shaped association, increased risk for breast cancer recurrence was observed only at concentrations >44 ng/mL (110 nmol/L) (15), whereas increased risk for prostate cancer was observed at concentrations >32 ng/mL (80 nmol/L) (36). Collectively, the weaknesses of many previous studies include use of recent exposure status information as an indicator of chronic exposures, insufficient monitoring of UV radiation and dietary exposures, and inadequate consideration of modifiers of 25(OH) concentrations.

Taken together, the seven studies that have documented increased risk for cancer with higher vitamin D exposure or status do not make a case for caution for the general population. They do, however, raise flags that should be of interest to health care practitioners who are caring for individuals. There is not yet sufficient evidence to recommend either high-dose vitamin D supplementation or vitamin D avoidance for the prevention of cancer. Considerable additional research is needed to fully understand if there is an increased risk for cancer at particular sites with high 25(OH)D concentrations, who may be at increased risk for cancer with high concentrations, and what concentrations would be defined as increasing risk.

To clarify the association between vitamin D status and cancer risk at various sites, the many modifiers of 25(OH)D concentration and its relationship with cancer must be appropriately addressed. Fundamentally, prospective longitudinal studies of vitamin D exposure and its relationship to serum 25(OH)D concentrations are warranted. Future research should take into account the health status and life stage of study participants, assessing the effects of adiposity, estrogen exposure, and nutritional status. The increasing prevalence of obesity among Americans may have profound implications for vitamin D metabolism.

Clearly, the realities of vitamin D exposure and metabolism, including the potential influences of genetic polymorphisms and tissue specificity, complicate efforts to define a single level of intake that would meet the needs of most healthy individuals. Increasing knowledge regarding vitamin D receptor polymorphisms and other genetic variations may clarify inconsistencies in the data regarding vitamin D's effect on cancer risk, serving to inform the complex decisions to be made regarding appropriate delivery of vitamin D to the individuals who stand to benefit most, while protecting vulnerable subpopulations from the risks of overexposure.

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