ELSEVIER

REVIEW ARTICLE

Vitamin D for Cancer Prevention: Global Perspective

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PURPOSE: Higher serum levels of the main circulating form of vitamin D, 25-hydroxyvitamin D (25(OH)D), are associated with substantially lower incidence rates of colon, breast, ovarian, renal, pancreatic, aggressive prostate and other cancers.
METHODS: Epidemiological findings combined with newly discovered mechanisms suggest a new model of cancer etiology that accounts for these actions of 25(OH)D and calcium. Its seven phases are disjunction, initiation, natural selection, overgrowth, metastasis, involution, and transition (abbreviated DINOMIT). Vitamin D metabolites prevent disjunction of cells and are beneficial in other phases.
RESULTS/CONCLUSIONS: It is projected that raising the minimum year-around serum 25(OH)D level to 40 to 60 ng/mL (100–150 nmol/L) would prevent approximately 58,000 new cases of breast cancer and 49,000 new cases of colorectal cancer each year, and three fourths of deaths from these diseases in the United States and Canada, based on observational studies combined with a randomized trial. Such intakes also are expected to reduce case-fatality rates of patients who have breast, colorectal, or prostate cancer by half. There are no unreasonable risks from intake of 2000 IU per day of vitamin D_3, or from a population serum 25(OH)D level of 40 to 60 ng/mL. The time has arrived for nationally coordinated action to substantially increase intake of vitamin D and calcium.
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KEY WORDS: Vitamin D, Breast Neoplasms, Colorectal Neoplasms, Ovarian Neoplasms, Dose-Response, Calcium, Etiology, Prevention, Treatment, Survival.

INTRODUCTION

Approximately 3,000 research studies have been published in biomedical journals investigating the inverse association between vitamin D, its metabolites, and cancer, including 275 epidemiological studies, according to a PubMed search.* Most epidemiological studies have reported that higher serum 25-hydroxyvitamin D (25(OH)D) levels are associated with lower incidence rates of various cancers (1–8) and higher 25(OH)D and 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) with lower incidence rates of aggressive prostate cancer (9, 10), with occasional exceptions (11–17) or borderline results (18). There are similarly supportive results for oral intake of vitamin D (19–27), with some exceptions, and for solar ultraviolet B (UVB) exposure.

Women with higher solar UVB exposure in the Third National Health and Nutrition Examination Survey (NHANES III) had only half the incidence of breast cancer as those with lower solar exposure (relative risk 0.50, 95% confidence interval [CI] 0.29–0.86) (24), whereas men in another national survey who had higher residential solar UVB exposure had only half the incidence rate of fatal prostate cancer (odds ratio 0.51, 95% CI 0.33–0.80) (28). High recreational solar exposure also was associated with 50% lower mortality from prostate cancer (relative risk, 0.47; 95% CI 0.23–0.99) (29). Higher solar exposure in childhood and adolescence also are associated with a similar reduction in lifetime incidence of prostate cancer (relative risk 0.49, 95% CI 0.27–0.90) (29).

Almost all laboratory studies using tissue culture systems have reported inhibition of growth of malignant cells and many have identified redifferentiation in response to vitamin D metabolites, particularly $1,25(OH)_2D$ and, to some degree, other vitamin D metabolites, such as 25(OH)D (30–38). The level of 25(OH)D in the serum is important, mainly because $1,25(OH)_2D$ is readily synthesized from it by CYP27B1, a 25(OH)D-1-alpha-hydroxylase enzyme that is ubiquitous in epithelial tissues of most organ systems (39, 40).

*National Library of Medicine PubMed Search conducted February 12, 2009. The search used the intersection of the terms "vitamin D" and "neoplasms." Epidemiological studies were identified using the terms"cohort," "case-control," "clinical trial," "ecological," "epidemiological" and "geographic." The search strategy excluded items identified by the National Library of Medicine as review, editorial, letter, comment or case report. A sample of 100 reports was reviewed, and it was determined that 10% were not research studies as such, so 10% was deducted from the counts of articles.

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Selected Abbreviations and Acronyms

25(OH)D = 25-hydroxyvitamin D
UVB = ultraviolet B
NHANES III = Third National Health and Nutrition Examination Survey
CI = confidence interval
VDR = vitamin D receptor
PLCO = Prostate, Lung, Colon and Ovarian [cancer screening project]
RCT = randomized controlled trial
DINOMIT = disjunction–initiation–natural selection–overgrowth–metastasis–involution–transition
NAS-IOM = National Academy of Science–Institute of Medicine
AI = adequate intake
UL = upper limit

Serum 25(OH)D Levels and Survival of Cancer Patients

Breast cancer patients with serum 25(OH)D levels higher than 29 ng/mL (72 nmol/L) at diagnosis had a 42% lower 15-year death rate than those with less than 20 ng/mL (50 nmol/L) (hazard ratio 0.58, 95% CI 0.35–0.95, $p < 0.02$) (41). Incidence of metastases was only half as high in women with 25(OH)D greater than 29 ng/mL than in those with less than 20 ng/mL (hazard ratio 0.51, 95% CI 0.31–0.86, $p < 0.02$) (41).

Colorectal cancer patients from the Dana Farber Cancer Center with serum 25(OH)D greater than 32 ng/mL (80 nmol/L) at diagnosis had only half the overall age-adjusted 6.5-year death rate as those with less than 20 ng/mL (50 nmol/L) (odds ratio 0.52, 95% CI 0.29–0.94, $p < 0.02$) (42). These studies confirmed earlier research that found lower case-fatality rates in patients with breast (43), colon (44), prostate (44), and lung cancer (45) who were diagnosed in summer or early fall, when serum 25(OH)D levels are highest (46).

The case-fatality rate of prostate cancer patients with high serum 25(OH)D (>32 ng/mL) is only one sixth as high as in those with low serum 25(OH)D (odds ratio 0.16, 95% CI 0.05–0.43, $p < 0.001$) (47).

Epidemiological studies that identified beneficial associations of serum 25(OH)D with incidence and case-fatality rates of breast and colon cancer are supported by confirmatory laboratory results from studies that have investigated the biological mechanisms accounting for the action of vitamin D and its metabolites in prevention of malignancy (34, 48–50). For example, oral administration of vitamin D_3 substantially reduced incidence of colon cancer in rats fed high-fat diets (51). Another study found that administration of either UVB irradiance or the raising of vitamin D metabolites with oral supplementation blocked growth of mammary cancer in mice inoculated with cancer xenografts that express vitamin D receptor (VDR) (52). There have been a few exceptions to the vitamin D–cancer inverse association in epidemiological studies in recent years (11–17, 53–55), but most of these may be accounted for by methodological limitations such as inadequate duration of follow-up, or limits upon generalizability due to use of study participants from regions with exposures that are of local and regional interest, but are not necessarily representative of the general world population. Generalizabilty also has been limited in some studies by use of populations such as heavy smokers (53), whose risk of cancer may be dominated by heavy use of tobacco and alcohol.

RECENT OBSERVATIONAL STUDIES

Breast Cancer

Freedman and associates (56) recently reported that women in the NHANES III cohort with serum 25(OH)D levels higher than 25 ng/mL (62 nmol/L) had only about one fourth the age-standardized mortality rate from breast cancer as those with levels less than 25 ng/mL (relative risk 0.28, 95% CI 0.08–0.93, $p < 0.05$) (Fig. 1).

A pooled analysis of two studies of breast cancer that reported odds ratios by quintiles (4, 5) found that a median serum 25(OH)D level greater than 38 ng/mL (95 nmol/L) (top quintile) was associated with 58% lower risk of breast cancer in women with serum 25(OH)D greater than 38 ng/mL than those with 25(OH)D less than 15 ng/mL (bottom quintile) (odds ratio 0.42, 95% CI 0.31–0.55, p trend $<$ 0.02) (Fig. 2) (57). Findings from a subsequent case-control study found similar statistically significant trends for premenopausal (58) and postmenopausal (7) breast cancer.

A nested case-control study in the Prostate, Lung, Colorectal and Ovarian (PLCO) cancer screening trial failed to detect an association of serum 25(OH)D with short-term risk of breast cancer (15), possibly because of methodological issues, including lack of matching controls to cases on the date that the serum was collected, and the short median length of follow-up of 3.9 years.

Colorectal Cancer

A profound inverse association of serum 25(OH)D with age-standardized colorectal cancer mortality also was found in the National Health and Nutrition Examination Survey III (NHANES III) cohort (56). Individuals with serum 25(OH)D greater than 32 ng/mL (80 nmol/L), the current definition of vitamin D adequacy (59, 60), had approximately one fourth the risk of dying of colon cancer as those with poor vitamin D status (<20 ng/mL or 50 nmol/L) (relative risk 0.28, 95% CI 0.11–0.68) (56). This corresponds to a reduction of nearly three fourths in mortality compared to individuals with poor serum 25(OH)D status (Fig. 3).

A pooled analysis of the five studies of serum 25(OH)D and risk of colorectal cancer that reported incidence rates

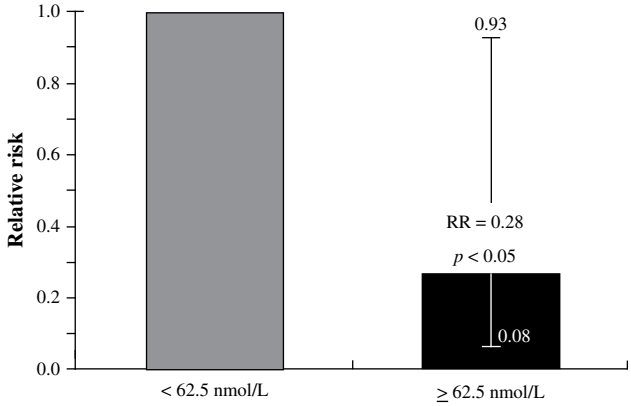

FIGURE 1. Relative risk of breast cancer mortality, by baseline serum 25(OH)D concentration, divided at the median, NHANES III cohort, 1988–2000. (Source: Drawn from data in Freedman et al. [56].)

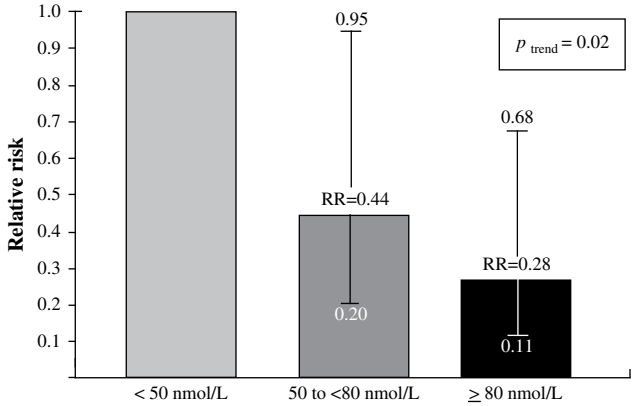

FIGURE 3. Relative risk of colon cancer mortality, by baseline serum 25(OH)D concentration, in tertiles, NHANES cohort, 1988-2000. (Source: Drawn from data in Freedman et al. [56].)

by quantiles of serum 25(OH)D provided a clear linear dose-response gradient (61) (Fig. 4). A serum 25(OH)D level greater than 38 ng/mL (95 nmol/L) (top quintile) was associated with an odds ratio of 0.45 (95% CI 0.28–0.69), corresponding to 55% lower risk of colorectal cancer compared to individuals with 25(OH)D of less than 16 ng/mL (40 nmol/L) (bottom quintile) (61).

Prostate Cancer

Observational studies of the inverse association of prediagnostic serum 25(OH)D with prostate cancer were recently reviewed by Giovannucci (62). The geographic epidemiology of prostate cancer is not as clearly linked with solar irradiance levels as it is for cancer of the breast, colon, ovary, endometrium and kidney.

Recent observational studies of the incidence of prostate cancer have had promising, although mixed, results. A study by Li and colleagues of the Physicians' Health Study cohort (10) found that physicians whose 25(OH)D and $1,25(OH)_2D$ levels were both below the median, 25(OH)D of 28 ng/mL (70 nmol/L) and $1,25(OH)_2D$ of 32 pg/mL (77 pmol/L) had twice the incidence of aggressive prostate cancer (odds ratio 2.1, 95% CI 1.2–3.4, $p < 0.05$) as men whose levels were above the median.

In a nested case-control study of 90 Kaiser Foundation cases and 91 controls matched on age, race, and day of serum storage, the estimated relative risk of prostate cancer was 0.41 (not significant) in men in the top quartile of serum vitamin D metabolites, specifically, 25(OH)D greater than 28 ng/mL (70 nmol/L) and $1,25(OH)_2D$ greater than 39 pg/mL (94 pmol/L). The risk of aggressive prostate cancer (palpable mass or Gleason score 7–10) in men in the top quartiles of serum 25(OH)D and $1,25(OH)_2D$ was extremely low (relative risk 0.03, not significant) in men older than 57 years of age (the median age of the cohort) (9). These effects were not present in younger men or for

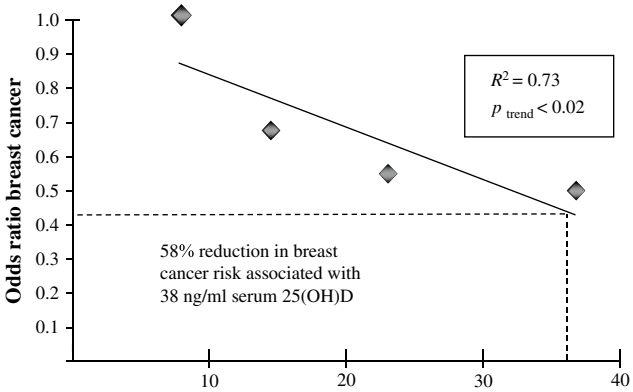

FIGURE 2. Pooled odds ratio for breast cancer, according to serum 25(OH)D concentration, meta-analysis, 2008. (Sources: Bertone-Johnson et al. [5], Lowe et al. [4], Garland et al. [57].) (Graphic: E. D. Gorman.)

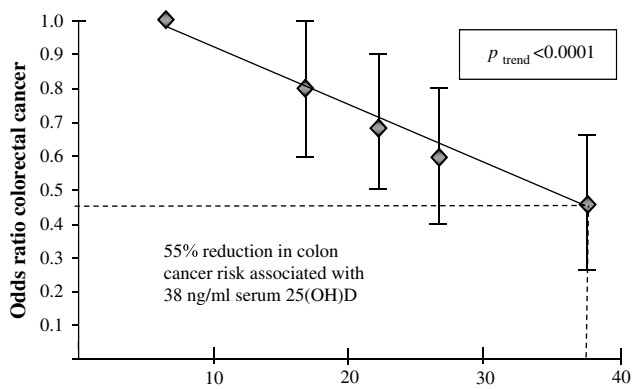

FIGURE 4. Pooled odds ratio for colorectal cancer, according to serum 25(OH)D concentration, meta-analysis, 2007. (Source, meta-analysis of six studies: Gorham et al. [61].) (Graphic: E. D. Gorham, S. B. Mohr.)

serum 25(OH)D alone. Earlier studies of vitamin D status and prostate cancer risk had mixed results (62).

A nested case-control study of the PLCO cohort by Ahn et al. (16) found no association between serum 25(OH)D and odds ratios for prostate cancer. The results from the PLCO study of prostate cancer conflict with studies that used longer median periods of follow-up, such as 11 to 12 years (9, 10), suggesting that the follow-up interval in the PLCO cohort studies may have been too short for reliable detection of the association.

Cancer of the Ovary

Ecological studies first identified higher ovarian cancer mortality rates in areas of higher latitude and lower levels of solar irradiance (63–66). These have been supported by observational studies of dietary intake of vitamin D (25) and of prediagnostic serum 25(OH)D (6). Lower prediagnostic serum 25(OH)D was associated with high risk of ovarian cancer in overweight women, although not in thinner women (6).

Cancer of Other Sites

Ecological studies also have reported inverse associations of total solar or UVB irradiance with risk of renal (67) and endometrial (68) cancers. These associations have generally persisted after adjustment for potential confounders, such as dietary factors and/or per capita health care expenditures. A complete listing of cancers inversely associated with UVB irradiance in the United States is available that includes multivariate adjustment using multiple regression to control for several pertinent demographic and behavioral covariates (69)

Clinical Trials

Geographic studies of the inverse association of sunlight with colon cancer (70) and breast cancer mortality (71–74) and of dietary vitamin D and calcium with colon cancer incidence (19–22) stimulated initiation of randomized controlled prevention trials that provided measurements of the effects of vitamin D and calcium on human cancer incidence (55, 75, 76). The most recent randomized controlled trial (RCT), by Lappe et al. (76), found that supplementation of postmenopausal women with 1,100 IU/day of vitamin D_3, in conjunction with 1,450 mg/day of calcium, yielded a 60% reduction in incidence of all invasive cancers combined (relative risk 0.40, 95% CI 0.20–0.82, $p < 0.03$) (Fig. 5). There was a 77% reduction in incidence when cases diagnosed during the first year of follow-up were excluded (relative risk 0.23, 95% CI 0.09–0.60, $p < 0.01$) (Fig. 6) (76). These profound reductions in incidence of all invasive cancers occurred within the 4-year duration of the study.

There were parallel trends in the Lappe et al. RCT (76) for cancers of the breast, colon, lung, and hematopoietic

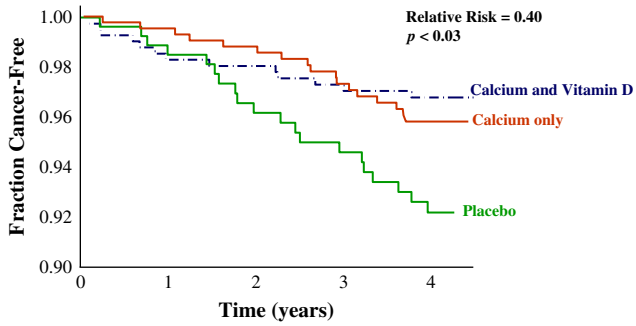

FIGURE 5. Cancer-free survival in 1,179 women (age 66.7 ± 7.3 years at baseline) after 4 years of follow-up, according to random allocation to placebo, calcium (1,450 mg calcium per day), or calcium and vitamin D_3 (1,100 IU vitamin D_3 and 1,450 mg calcium per day). Survival was 60% higher in the calcium and vitamin D group than the placebo group. (Source: Redrawn from Lappe et al. [76].)

malignancies, although the trends are based on fewer events than all cancers (76). About half the reduction in incidence of all cancers combined appeared to be due to vitamin D_3, and about half to calcium supplementation. Two earlier negative trials examined data derived from the same study (55, 75) that used a minimal dose of vitamin D (400 IU) that was too little to increase serum 25(OH)D by more than approximately 1 to 3 ng/mL (2–7 nmol/L) by mid-study in the intervention group compared to the control group. This trial also experienced substantial noncompliance and lost considerable power because of a factorial design that did not take into account an unexpected interaction for colorectal cancer between the vitamin D–calcium intervention and a hormone replacement intervention (77).

Dose-Response

In parallel with classical nutritional deficiency diseases, the dose-response relationship for breast cancer appears to be

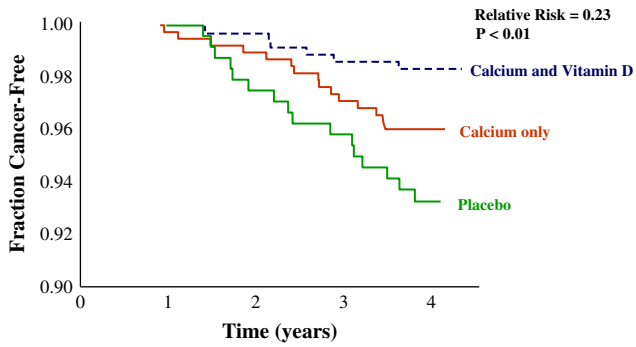

FIGURE 6. Cancer-free survival in 1,179 women, excluding first-year cases, after 4 years of follow-up, according to random allocation to placebo, calcium (1,450 mg calcium per day), or calcium and vitamin D_3 (1,100 IU vitamin D_3 and 1,450 mg calcium per day). Cancer-free survival was 77% higher in the calcium and vitamin D group than the placebo group. (Source: Redrawn from Lappe et al. [76].)

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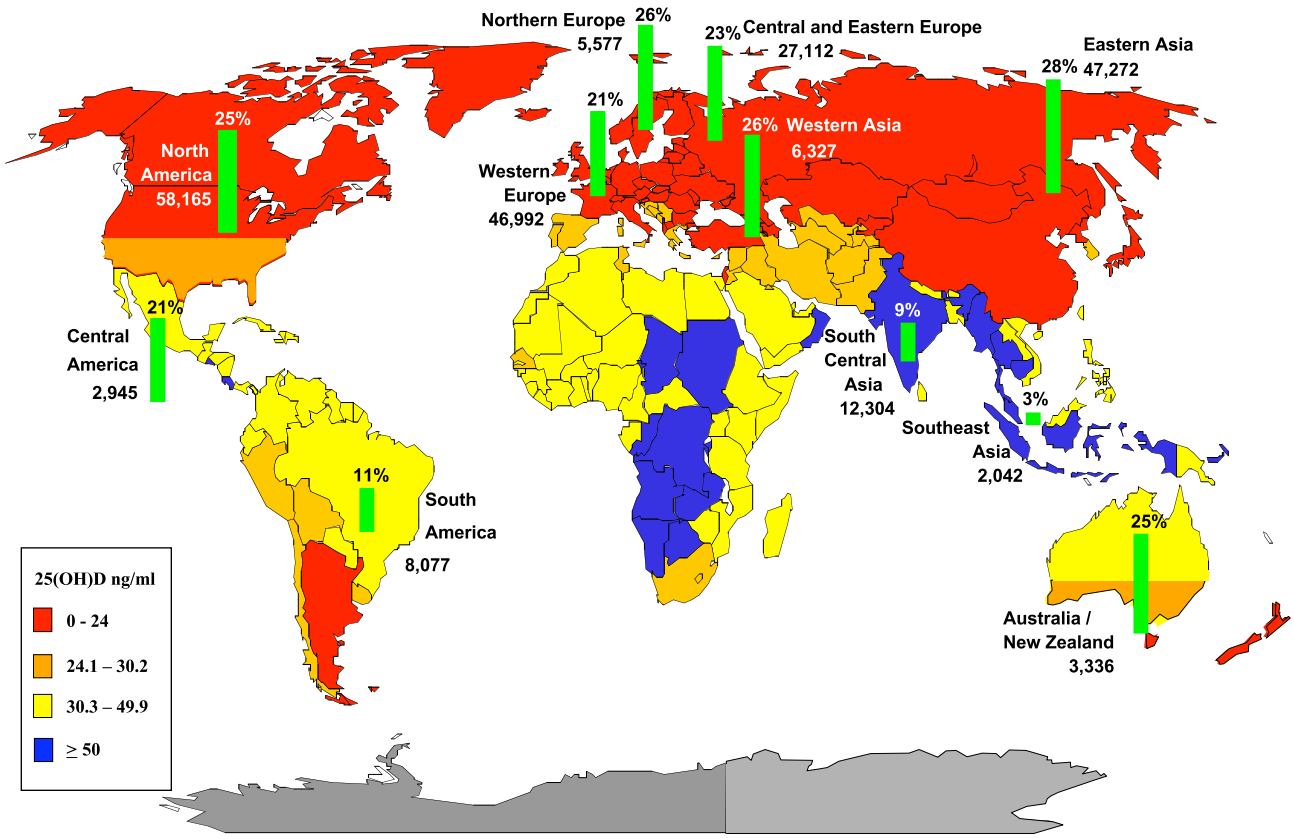

FIGURE 7. Estimated 25(OH)D serum levels and projected annual reduction in new breast cancer cases with 2,000 IU per day of vitamin D_3, and, when feasible, modest sun exposure (< 10 minutes/day) not exceeding threshold for erythema. (Graphic: S. B. Mohr, MPH.)

linear and downward through at least 60 ng/mL (150 nmol/L) (4). Cohort studies of colon cancer have not included serum 25(OH)D levels this high (60), although it is likely that the downward slope is linear, similar to that of breast cancer, based on ecological studies that have examined incidence of colon cancer according to latitude and solar UVB irradiance (79, 80). New nested-case control studies would be desirable to determine the shape of the dose-response gradient in geographic areas where serum levels of 25(OH)D greater than 40 ng/mL are commonly encountered.

VITAMIN D AND GLOBAL CANCER PREVENTION

Intake of 2,000 IU/day of vitamin D_3 would lead to 25% reduction in incidence of breast cancer (Fig. 7) and 27% reduction in incidence of colorectal cancer (Fig. 8) in North America. A dosage of 2,000 IU/day of vitamin D is the National Academy of Sciences upper limit (UL) for intake on a daily basis (81). Approximately 220,149 new cases of breast cancer (see Fig. 7) and 254,105 new cases of colorectal cancer (see Fig. 8) would be prevented annually in the world by raising serum 25(OH)D concentrations to approximately 40 to 60 ng/mL, which is, in general, associated with oral intake of 2,000 IU of vitamin D_3 per day. It is projected based on a meta-analysis (57), that approximately 58,000 cases of breast cancer would be prevented in the United States and Canada each year with this serum level (see Fig. 7). It is also projected that approximately based on data from the NHANES III cohort 49,000 cases of colorectal cancer would be prevented in the United States and Canada each year (61). This action would also prevent three fourths of deaths from breast and colorectal cancer in the United States and Canada.

Mechanisms of Vitamin D in Cancer Prevention

Ten mechanisms have been reported that account for the role of vitamin D and calcium in reducing cancer incidence and mortality. Most studies that have discovered mechanisms have cited epidemiological findings as an important source of ideas. The mechanisms are (a) up-regulation of adherence and signaling between epithelial cells (82–84), (b) contact inhibition of proliferation (82–84), (c) differentiation (30, 31, 83, 85), (d) cell cycle stabilization (86), (e) promotion of apoptosis (87–89), (f) anti-neoangiogenesis (90–92), (g) down-regulation of glycogen synthase kinase 3 (GSK-3) which reduces proliferation of colorectal,

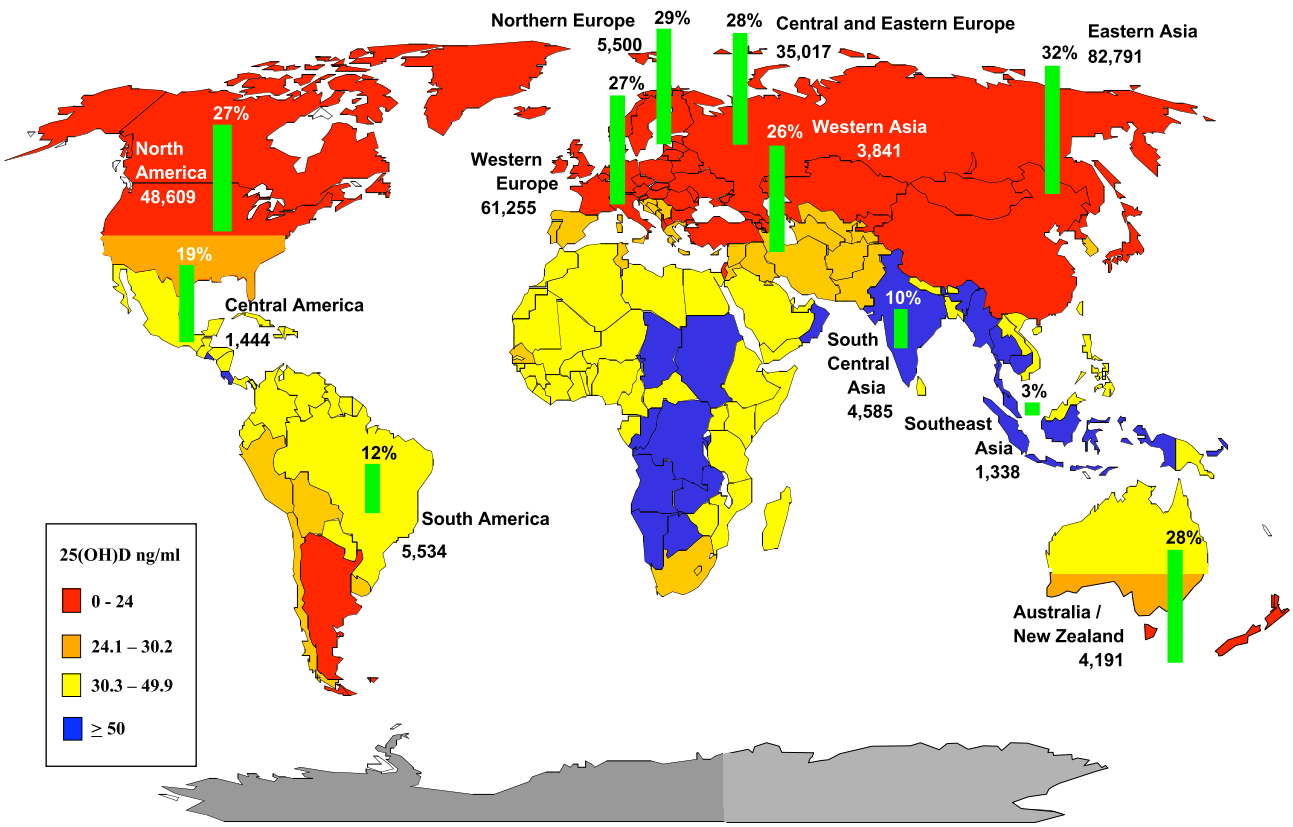

FIGURE 8. Estimated 25(OH)D serum levels and projected annual reduction in new colorectal cancer cases with 2,000 IU per day of vitamin D_3, and, when feasible, modest sun exposure (<10 minutes/day) not exceeding threshold for erythema. (Graphic: S. B. Mohr, MPH.)

prostate, and pancreatic cancers in vitro (93), (h) down-regulation of the canonical Wnt signaling pathway that is active in colorectal and other cancers (83), (i) increased expression of DKK-1 protein, a tumor suppressor in colon cancer cells having mutations in the Wnt/beta-catenin pathway (94), (j) down-regulation of DKK-4 transcription; DKK-4 is a target of the Wnt/beta-catenin pathway and is up-regulated in colorectal cancer, increasing cellular autonomy, mobility and invasiveness (94). The VDR-1,25(OH)2D complex binds to the promoter of DKK-4, largely preventing its transcription (94).

Vitamin D metabolites such as $1,25(OH)_2D$ up-regulate transcription of E-cadherins, the principal epithelial intercellular adherence proteins (82, 95) and induce translocation to the plasma membrane of beta-catenins, proteins whose activity results in anchoring of intercellular junctional proteins to the cytoskeleton, helping maintain the typically cuboidal, polarized shape of most epithelial cells (95).

Vitamin D is not an antioxidant, so it does not prevent reactive oxygen species from attacking DNA. Its activity in preventing and reducing cancer incidence and death rates could not have been deduced from classical two-hit (96) or multi-hit carcinogenesis models (97, 98). These mainly deterministic initiation-promotion models primarily address accumulation of defects in (or, more recently, hypermethylation of) DNA, advancing toward malignancy. They do not readily accommodate reversal of the time sequence. Calcium also is not an antioxidant, although it may bind oxidants.

An integrative model has been proposed for cancers of epithelial origin that accommodates the actions of vitamin D and calcium (60). This model encompasses results of tissue culture research on cell lines and epidemiological findings. A group led by Munoz, Palmer, and their colleagues identified a role of E-cadherin and beta-catenin in the action of vitamin D metabolites against colon cancer cells in tissue culture and identified several relevant genes and signaling pathways (83).

The newly proposed model of cancer pathogenesis is termed the Disjunction–Initiation–Natural selection–Overgrowth–Metastasis–Involution–Transition (DINOMIT) model (Fig. 9A and 9B) (60). The model includes the classical concepts of carcinogenesis, such as initiation and promotion, but encompasses the life cycle of malignancies and provides an explanation of the ability of vitamin D and calcium to prevent and potentially arrest the pathogenesis of cancer.

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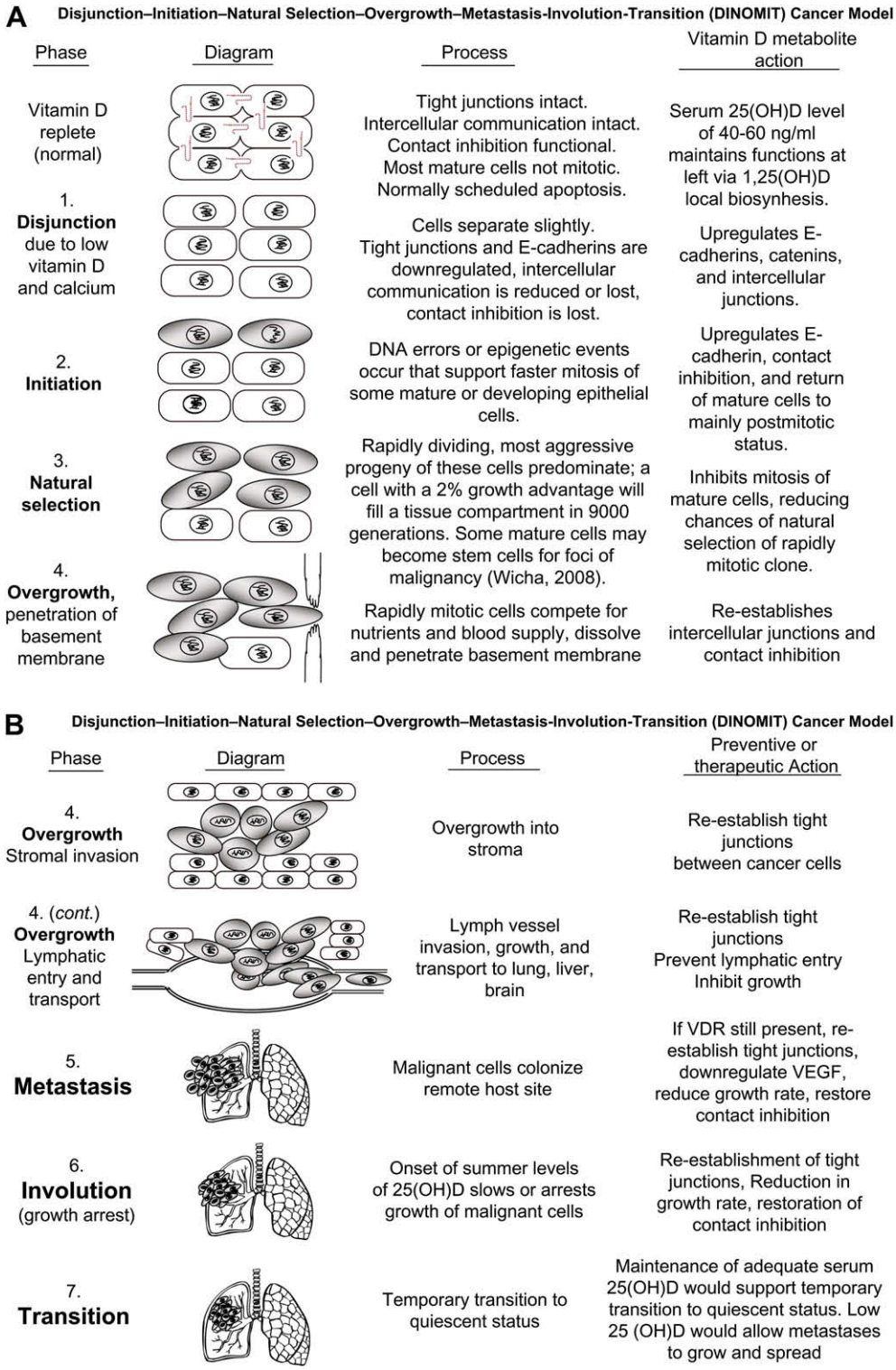

FIGURE 9. A and **B**. Disjunction–initiation–natural selection–overgrowth–metastasis–involution–transition (DINOMIT) cancer model.

Scientists' Letter on Vitamin D for Cancer Prevention

July 1, 2008

Subj: Scientists' Letter on Vitamin D for Cancer Prevention

To whom it may concern:

We are aware of substantial scientific evidence supporting the role of vitamin D in prevention of cancer. It has been reasonably established that adequate serum vitamin D metabolite levels are associated with substantially lower incidence rates of several types of cancer, including those of the breast, colon, and ovary, and other sites.

We have concluded that the vitamin D status of most individuals in North America will need to be greatly improved for substantial reduction in incidence of cancer. Epidemiological studies have shown that higher vitamin D levels are also associated with lower risk of Type I diabetes in children and of multiple sclerosis. Several studies have found that markers of higher vitamin D levels are associated with lower incidence and severity of influenza and several other infectious diseases.

Higher vitamin D status can be achieved in part by increased oral intake of vitamin D3. The appropriate intake of vitamin D3 for cancer risk reduction depends on the individual's age, race, lifestyle, and latitude of residence. New evidence indicates that the intake should be 2000 IU per day. Intake of 2000 IU/day is the current upper limit of the National Academy of Sciences, Institute of Medicine, Food and Nutrition Board. New evidence also indicates that the upper limit should be raised substantially. The levels that are needed to prevent a substantial proportion of cancer would also be effective in substantially reducing risk of fractures, Type I childhood diabetes and multiple sclerosis.

Greater oral intakes of vitamin D3 may be needed in the aged and in individuals who spend little time outdoors, because of reduced cutaneous synthesis. Choice of a larger dose may be based on the individual's wintertime serum 25(OH)D level.

For those choosing to have serum 25-hydroxyvitamin D tested, a target serum level should be chosen in consultation with a health care provider, based on the characteristics of the individual. An approximate guide-line for health care providers who choose to measure serum 25-hydroxyvitamin D in their patients would to aim for 40-60 ng/ml, unless there are specific contraindications. Contraindications are extremely rare, and are well known to physicians. No intervention is free of all risk, including this one. Patients should be advised of this, and advised in detail of risks that may be specific to the individual. The risks of vitamin D inadequacy considerably exceed any risks of taking 2000 IU/day of vitamin D3, which the NAS-IOM regards as having no adverse health effect.

A substantially higher level of support for research on the role of vitamin D for the prevention of cancer is urgently needed. However, delays in taking reasonable preventive action on cancer by ensuring nearly universal oral intake of vitamin D3 in the range of 2000 IU/day is costing thousands of lives unnecessarily each year that are lost due to fractures, cancer, diabetes, multiple sclerosis, and other diseases for which vitamin D deficiency plays a major role.

Signed:

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FIGURE 10. Scientists' Call for Action on Vitamin D for Cancer Prevention

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FIGURE 10. (Continued)

Disjunction

The first phase of the DINOMIT model is disjunction, consisting of substantial weakening or loss of adherence between epithelial cells within a tissue compartment, such as a breast terminal ductal lobular unit or a colonic epithelial crypt (60, 99, 100). This hallmark of malignancy has been observed in time-lapse microphotography of mammary epithelium (100). It is partly due to loss of E-cadherin synthesis and may be partly

due to lack of sufficient concentrations of beta-catenin at the plasma membrane to link the E-cadherins to the cytoskeleton tightly enough to maintain adherence and the normal cellular architecture characteristic of epithelium, typically a monolayer in the intestine and a monolayer or bi-layer in the breast (83).

Existence of the capability of human cells for disjunction and rapid autonomous proliferation is not surprising, since it is needed for growth and healing of injuries (101).

Some of this capability may be left over from an earlier era, when life existed solely as unicellar organisms. Life on earth consisted of autonomous, unicellular organisms for 85% of its 4.25 billion years (102). It has been hypothesized that some of the most conserved contemporary DNA code may have existed at the time of emergence of the first multicellular organisms, approximately 637 million years ago (102). Such DNA may facilitate the capability of the human cell for autonomous life decoupled from a basement membrane, and the mobility that is needed for reproduction and functioning of the cellular immune system.

Initiation

The second phase consists of initiation, or occurrence, of variation in the DNA or in epigenetic factors that influence its transcription to RNA and translation to proteins. The critical events are most likely uncorrected errors that occur during DNA replication or, at times, action on the DNA of alkylating agents, ionizing radiation, or epigenetic factors (103). The action is not specific to a particular gene, but its hallmark is persistent alteration in the DNA molecule or in factors that influence the expression of various regions of the DNA. Genetic or persistent epigenetic variation is a condition for the next stage in evolution of a malignancy.

Natural Selection

When disjunction of cells allows mobility of a sufficient number of cells in a tissue compartment, and some variation in the DNA or epigenetic factors occurs, a competitive population dynamic is created that leads to a third phase, natural selection. This phase consists of selection of the fastest reproducing, most aggressive cells. It is a well-described process of evolution (104), yet occurring on a microscopic scale. Since its effect on the organism is adverse or even fatal, it may be distinguished from the more general term evolution by using the term "devolution". Devolution does not ordinarily occur in mature normal epithelium, but only in populations of cells that are not growth-inhibited by direct contact with adjacent cells. This phenomenon is well known from results of treatment of advanced cancers, but it has not, to our knowledge, been regarded as the principal cause of the earliest stages of carcinogenesis.

Since the driver of evolution is the gene (105), a stem cell having a gene associated with faster reproduction or aggression against other cells in competition for limited resources, will eventually be overrepresented in the tissue compartment (60). In settings where some cells are autonomous, the progeny of a cell that has a 2% advantage in mitotic rate will occupy 99% of the tissue compartment in 9,000 generations (60).

Rapidly reproducing cells that have devolved within a tissue compartment have no way of sensing that their behavior may lead to the death of the organism. Natural selection at the level of the organism would reduce this tendency, but its influence virtually stops at the end of the reproductive period. This has been suggested as a possible reason that incidence rates of cancer are usually low below approximately 50 years, after which they rise exponentially (106).

Overgrowth

The next phase is clonal expansion, or overgrowth of the tumor outside the basement membrane of the tissue and into the stromal layer; it occurs for unknown reasons. The basement membrane is composed of collagens and amino acids (107). One possible reason for invasion could be action in the peripheral cells of a clone that has become starved for essential amino acids by localized hyperproliferation and crowding. Human cells cannot synthesize these compounds, but they are present in abundance as components of the basement membrane. Aggressive cells near the basement membrane may begin to dissolve it enzymatically or by changing extracellular pH, in order to obtain needed amino acids. If this occurs at a faster rate than the repair rate, a result may be weakening of the basement membrane, eventually allowing penetration by malignant cells. This leads to the phenomenon of overgrowth of the rapidly mitotic, aggressive clone in the stromal layers. This overgrowth is clinically evident as localized cancer.

Metastasis

The next phase is metastasis, which may be regarded as analogous to colonization of a remote range by an organism. As the expansion of the tumor mass continues, a few cells from the overgrowing clone transit the lymphatic or blood circulation and ultimately lodge in remote tissues, where overgrowth continues if differentiation and intercellular communication are not restored. Invasion of distal tissues could be facilitated by disjunction in the remote tissue, reducing its barrier function.

Involution

The next phase is involution, which occurs when vitamin D status is restored by a seasonal rise in 25(OH)D; it consists of

a temporary arrest of growth of metastases and other progeny of the primary tumor whose VDR is intact. Involution accounts for the findings that diagnosis is highest in winter months for breast (108), colon (109), and prostate (44, 110). During the involution phase, malignant cells remain in the metastases, but intercellular junctions may be re-established in cells that have functional VDR. The close contact induces contact inhibition, limiting further mitoses. As a result of such mitotic arrest, new malignant cells are no longer being produced due to their cell cycle approximating normal interphase. On the other hand, seasonal loss of vitamin D status would result in loss of contact inhibition and up-regulation of growth during the winter, when the tumor expands to a critical mass large enough to palpate or otherwise attracts clinical attention. If involution does not occur, which is usual at temperate and higher latitudes, the metastatic mass grows to a volume that interferes with an essential function of a vital organ, generally lung or liver, or results in vascular penetration, causing hemorrhage.

Transition

The last phase is transition. If vitamin D and calcium deficiency persist and the metastatic lesions do not irretrievably harm a vital organ, the metastatic cancer will make a transition to carcinomatosis, or disseminated malignancy. If vitamin D and calcium are repleted to adequate levels, some evidence suggests that there may be a transition from an acute to a chronic disease. This suggestion is supported by two studies that reported a reduction by approximately half in long-term death and recurrence rates in patients with breast (41) and colorectal (42) cancer. It is also, to some degree, supported by the RCT of Lappe et al. (76). The postmenopausal women who were randomized inevitably included some who had incipient breast, colorectal, and other cancers that were below the threshold for clinical detection (111) and did not exceed it during the trial. While making a favorable transition would require lifelong vitamin D and calcium repletion, it could theoretically serve in the role of a partial cure for individuals who are willing to adopt lifelong vitamin D and calcium repletion.

Key mechanistic components of the above model were explored first by Palmer and associates of the Munoz laboratory (94) and extended by Ordonez-Moran and colleagues (83), who confirmed that the VDR receptor-ligand complex activates signaling pathways that induce E-cadherin and other proteins that adhere cells to one another, including zona occludens proteins 1 and 2. Vitamin D metabolites also enhance intercellular adherence through direct effects on the plasma membrane that are not dependent on DNA transcription (84). Approximately 10% of malignancies are of nonepithelial origin, and this model may not apply to them.

Role of Calcium and Dietary Factors

Extracellular calcium ions are required for intercellular adherence (112). Intercellular junctions endocytose in response to very low concentrations of calcium in the extracellular fluid and exocytose upon its restoration (113). Some exogenous agents cause endocytosis of intercellular junctions, including linolenic acid (C18:3 n-6) and its precursor, linoleic acid (C18:2 n-6) (114). Unfortunately, n-6 linoleic acid is the most common polyunsaturated fatty acid consumed in the Western diet (median intake 15 g/day), and some popular vegetable oils contain substantial amounts of n-6 linolenic acid (115). Chenodeoxycholic acid and some other human bile acids also cause disjunction (115, 116) and high lumenal concentrations predispose humans to colon cancer (117, 118) if they do not consume sufficient calcium, consistent with a basic principle of calcium anticarcinogenesis described by Newmark et al. (118).

Implications for cancer treatment

Recent data suggest that endocytosis of intercellular junctions, the first and arguably the primary lesions preceding devolution into malignancy within a tissue may be reversible throughout the lifespan of the cell and its progeny, probably including some metastatic cells that have an intact VDR colonizing remote tissues. The VDR is a 64-kb molecule that is robust and tends to remain functional throughout the evolution of a malignancy, even as mutations accumulate (119, 120).

Extreme exposures to powerful carcinogens, such as tobacco smoke, or high intakes of ethanol and mycotoxins, may overwhelm the influence of vitamin D and calcium, as they do for cancer of the lung and bronchus in smokers (121) and, to a degree, bladder cancer (122) in smokers. Such carcinogen-driven cancers can be prevented only by eliminating exposure to tobacco or the relevant carcinogen.

Safety

There have been 748 RCTs that assigned vitamin D supplements to study participants, according to a PubMed search in August 2008. Most were performed to study the effect of vitamin D on bone disease, and all monitored the participants for safety and toxicity. Studies have included vitamin D doses, in international units per day, of 800 (123, 124), 1,100 (76), 2,000 (127), and 4,000 (124, 128). A pediatric clinical trial used 800 IU per kilogram of body weight for premature infants (130). Reports of toxicity, mainly hypercalcemia, have been rare and minor (130–133).

Dose-response gradients for cancer risk according to serum 25(OH)D levels presented in the Symposium in Print and the medical literature indicate that the National Academy of Sciences–Institute of Medicine (NAS-IOM)

recommended adequate intake (AI) should be revised upward to at least 2,000 to 4,000 IU/day. Adoption of the new AI would substantially reduce the incidence of cancer, and there are no consistently established adverse effects of vitamin D_3 intake in the range below 4,000 IU/day that would be sufficient to justify a lower AI (76, 130–135).

Rare contraindications to the new AI should be mentioned as part of the recommendations. The upper limit (UL) should be increased to at least 5,000 IU/day, based on expected benefits compared to anticipated minor risks. Some knowledgeable vitamin D scientists and physicians have recommended a higher UL of 10,000 IU/day based on a critical examination of published studies of toxicity balanced against benefits (131).

Vitamin D_3 (cholecalciferol) should replace vitamin D_2 (ergocalciferol). Vitamin D_3 is more effective in humans, at least in larger doses (136). Virtually all evidence reported to date on the efficacy of vitamin D for cancer prevention has been based on vitamin D_3. Vitamin D_3 is the normal product of biosynthesis of vitamin D in humans and other animals and is the most common form of vitamin D in the U.S. diet.

The preventive effects of higher vitamin D_3 intake have led 16 vitamin D scientists and concerned physicians in the United States and Canada to disseminate a call to action (Fig 10) recommending universal daily intake of 2000 IU of vitamin D_3. This intake is the same as the current upper limit that the NAS-IOM previously found, upon review and extensive analysis, had no adverse health effects (81). Intake of 2000 IU/day of vitamin D_3 would add 20 ng/mL the level of 25(OH)D obtained from typical exposure to the sun. The call to action comes in part from cancer prevention research reviewed in the Symposium in Print, and in part from studies that have identified inverse associations of low vitamin D status with high risk of myocardial infarction (137, 138), type 1 diabetes (139, 140), multiple sclerosis (141, 142), and falls (123). These studies have moved the scientific debate past a threshold where the risk of inadequate vitamin D status greatly exceeds any credible risk that might be associated with intake of 2000 IU/day of vitamin D_3, even when considering the possibility of this intake by large numbers of individuals.

The data presented in recent reviews provide components of an enigma that has so far resisted unitary interpretation. However, the many lines of evidence that include epidemiological and laboratory studies converge to suggest a new public health approach to cancer by identification and elimination of vitamin D deficiency to broadly reduce cancer risk.

More research should be performed to determine the extent to which the benefits of vitamin D adequacy may apply to a wider range of cancers and to describe dose-response relationships with cancer incidence and mortality rates at higher serum 25(OH)D levels, such as 60–80 ng/mL (150–200 nmol/L) and oral intakes of vitamin D_3 above 4000 IU/day.

In the meantime, populations living at or higher than 30° latitude in either the northern or southern hemisphere, or who have a mainly indoor lifestyle, should be considered at high risk of breast, colon, ovarian, and many other types of cancer as a result of highly prevalent vitamin D deficiency (143, 144). The studies described and referenced in this Symposium in Print provide the scientific basis for a new era of research and public health action using vitamin D to reduce incidence and mortality from cancer, and substantially increase treatment success.

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