

## What is the Dose-Response Relationship between Vitamin D and Cancer Risk?

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An inverse association between serum 25-hydroxyvitamin D [25(OH)D], and risk of cancers of the colon, breast, and ovary has been reported in well-conducted observational studies.<sup>1</sup> These studies have been supported by numerous natural experiments, specifically, studies that examine differences in incidence rates according to naturally occurring variations among populations in their ambient exposure to solar ultraviolet B irradiance, the main source of vitamin D.<sup>2–6</sup> The presence of a dose-response gradient is one of the key criteria for determining whether an association is causal. This review describes the dose-response gradient between serum 25(OH)D and risk of these cancers. It also projects dose-response gradients for cancers of several other sites and suggests a possible mechanism for the dose-response gradient of vitamin D in cancer.

Combining data from observational studies revealed an inverse association of serum 25(OH)D with risk of colon<sup>7</sup> and breast cancer<sup>8</sup> (Figures 1 and 2). A dose-response gradient for ovarian cancer and 25(OH)D concentrations was obtained from a recent cohort study.<sup>9</sup> The gradients were confirmed by an analysis of modeled and reported winter serum 25(OH)D levels and estimated age-standardized incidence rate estimates for 177 countries for 2002 from the International Agency for Research on Cancer (IARC) GLOBOCAN database. Serum 25(OH)D levels in each country were obtained from previous studies or modeled based on winter solar ultra-

violet B irradiance by country, adjusted for winter cloud cover data obtained from the NASA international satellite climatology cloud climatology project (ISCCP).

An inverse, monotonic dose-response gradient between serum 25(OH)D and risk of cancers of the colon and breast beginning at levels from 24 to 32 ng/mL was observed. The asymptotic (flat) portion on the left side of the dose-response curve was shortest for colon cancer (from 0 through 12 ng/mL) and longest for breast and ovarian cancer (from 0 through 25 ng/mL) and most other vitamin D-sensitive cancers. Based on observational studies, the first visible increment in prevention of colorectal cancer occurs with serum 25(OH)D levels  $\geq$  22 ng/mL, while the first visible increment in prevention of breast cancer occurs with serum 25(OH)D levels  $\geq$  32 ng/mL. Serum 25(OH)D variation below these levels generally would have little or no influence on cancer risk.

The lower limit for any benefit of vitamin D would correspond to 1000 IU/d of vitamin D<sub>3</sub> for the first meaningful increment of colorectal cancer prevention and 2000 IU/d for the first meaningful increment of breast cancer prevention. We estimated that 50% of colon cancer incidence in North America could be prevented by maintenance of a serum 25(OH)D level of  $\geq$  34 ng/mL (Figure 1). This did not require extrapolation beyond known data points. Prevention of 30% of breast cancer incidence in North America would be expected with lifelong maintenance of a substantially higher serum 25(OH)D level of  $\geq$  42 ng/mL (Figure 2). Based on a prediction involving linear extrapolation, a projected 50% reduction of breast cancer incidence could potentially be achieved by lifelong maintenance of serum 25(OH)D level  $\geq$  52 ng/mL.

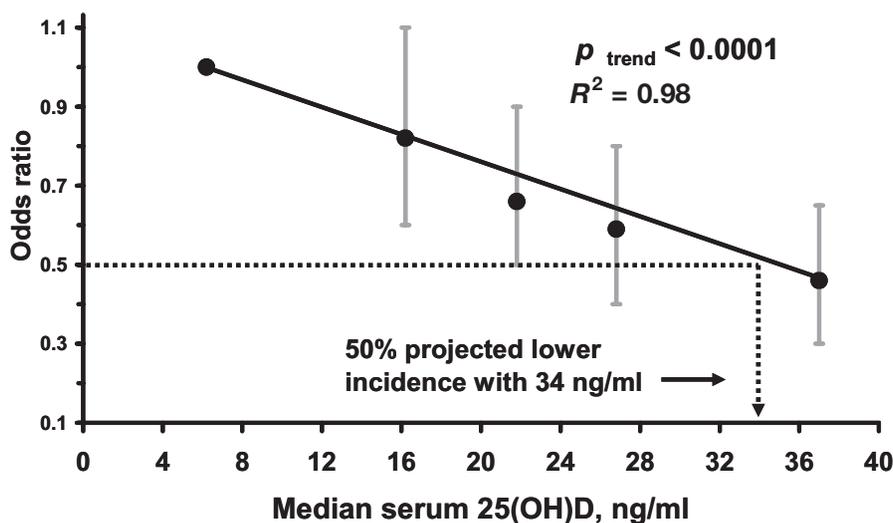
The dose-response gradients described here provide a quantitative basis for formulating recommendations to the medical community and general public for primary prevention of colorectal and breast cancer, and should be used for that purpose. In North America, a projected 50% reduction in colon cancer incidence would require universal intake of 2000 IU/d of vitamin D<sub>3</sub>, while a similar reduction in breast cancer incidence would require 3500

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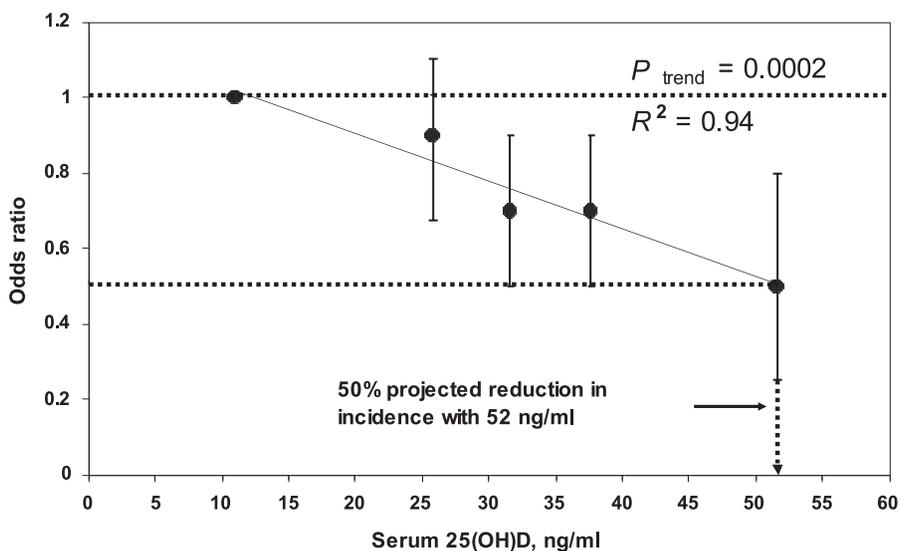


**Figure 1.** Pooled analysis of studies of serum 25 (OH)D level and risk of colorectal cancer. Bars denote 95% confidence intervals. (Used with permission from Gorham ED, Garland CF, Garland FC, et al. Optimal vitamin D status for colorectal cancer prevention: A quantitative meta analysis. *Am J Prev Med.* 2007;32:210–216.)

IU/d. The intake expected to prevent half of breast cancer incidence would be above the 2000 IU/d upper limit established by the National Academy of Sciences.<sup>10</sup> These gradients for cancer risk suggest that the upper limit should be revised upward, since there is considerable benefit, and no established adverse effect of vitamin D<sub>3</sub> intake below 10,000 IU/d.<sup>11,12</sup> In the meantime, 2000 IU/d of vitamin D<sub>3</sub> for all individuals aged 12 years and older

would be far safer than the present median adult intake in the US of approximately 230 IU/d. Safe and appropriate intake at age 6 months to 11 years would be 1000 IU/d.<sup>10</sup> Use of ergocalciferol (vitamin D<sub>2</sub>), which is popular in Europe and is used in some major US brands of multi-vitamins, should be discontinued immediately in favor of vitamin D<sub>3</sub>.<sup>13-15</sup>

Vitamin D status can be enhanced with very brief



**Figure 2.** Pooled analysis of studies of serum 25(OH)D level and risk of breast cancer. Bars denote 95% confidence intervals. (Used with permission from Garland CF, Gorham ED, Mohr SB, et al. Vitamin D and prevention of breast cancer: pooled analysis. *J Steroid Biochem Mol Biol.* 2007;103:708–711.) This meta-analysis was based on data from Bertone-Johnson ER, Chen WY, Holick MF, et al. Plasma 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D and risk of breast cancer. *Cancer Epidemiol Biomarkers Prev.* 2005;14:1991–1997; Lowe LC, Guy M, Mansi JL, et al. Plasma 25-hydroxy vitamin D concentrations, vitamin D receptor genotype and breast cancer risk in a UK Caucasian population. *Eur J Cancer.* 2005;41: 1164–1169.

solar exposures.<sup>16</sup> This benefit can be achieved, where feasible, with time outdoors on sunny days in the range of 3 to 15 min/d within an hour of noon with  $\geq 40\%$  of skin area exposed; the duration should be based on skin type and age.<sup>17</sup> Shorts, a brief top for women, and a cap with a broad brim should be worn during time spent outdoors for vitamin D synthesis. Sunscreens should not be used during this brief interval to allow vitamin D synthesis.<sup>17,18</sup>

We believe it would also be prudent to measure serum 25(OH)D in late winter every 2 to 3 years in all individuals in the United States, Canada, Europe, and similar latitudes in both hemispheres, and to maintain a serum 25(OH)D level between 55 and 90 ng/mL in everyone 5 years of age and older (between 55 and 80 ng/mL in children 1 through 4 years of age). Likewise, we believe this should be combined with intake of 1000 mg/d of calcium in males and 1200 mg/d in females, ideally from food but if necessary from a formula containing citrate. Intake of 6 to 8 glasses of fluids per day should be ensured for adequate hydration.

Some consideration of mechanisms is appropriate. Vitamin D is essential for the expression of proteins involved in expression of intercellular junctions such as E-cadherin.<sup>19,20</sup> Tissue culture systems of normal epithelial cells have confirmed that tight junctions, desmosomes, and gap junctions are the most common junctions between epithelial cells.<sup>20</sup> The proteins that constitute junctional systems decline when the concentration of vitamin D metabolites is low.<sup>19,20</sup> In the absence of intact intercellular junctions, epithelial cells may separate, lose their normal cuboidal architecture, and acquire an increasingly amorphous architecture, with loss of function and apical-basal polarity.<sup>21</sup> This phenomenon has been termed decoupling.<sup>22</sup> Decoupling can also be produced by reducing the calcium concentration in the culture medium.<sup>22</sup>

The tight junction consists of proteins including E-cadherin, an intercellular glue that is up-regulated in response to activation of a vitamin response element in a gene that regulates its synthesis.<sup>19,20</sup> This forms a binding matrix that includes calcium. The relevant response element is activated by a heterodimer consisting of the combined vitamin D receptor, the retinoid X receptor proteins, and ligands.<sup>23</sup> The vitamin D receptor up-regulates a large complement of other genes.<sup>24</sup> It is closely involved in regulatory pathways related to the p53 gene, among other tumor suppressor genes.<sup>25</sup> It also down-regulates a large complement of other genes, including many proto-oncogenes and promoters of tumor angiogenesis, including vascular endothelial growth factor (VEGF).<sup>26</sup>

The mechanism of vitamin D in cancer is most easily explained by the recognition that malignancies are

characterized by a continuum of progressive evolutionary changes from the normal to the malignant cell. The role of vitamin D in genesis of cancer is most easily understood in terms of its pivotal role in an evolutionary process that begins at the level of decoupled epithelial cells. Cells that are coupled to their neighbors cannot compete with one another for resources because they are limited in migration by neighboring cells. When the cells decouple, population dynamics become operative. Through natural selection, cells that have acquired somatic mutations that confer a reproductive advantage will eventually become predominant in their tissue compartment.

The intestinal epithelial cells in high-risk individuals reproduce much (about 4 times) faster than those in other people.<sup>27</sup> Unfortunately, rapid reproduction comes at a cost, generally loss of fidelity of reproduction of the DNA. This occurs when there is not enough time between cell cycles for repair of the nearly inevitable loss of structural integrity of DNA that occurs during replication.

An example of a first step toward cancer due to vitamin D deficiency may be loss of a protein coded by a growth suppressor gene, such as the p53 tumor suppressor protein. The function of this gene can be reduced or lost due to harm to the gene, or to weak activation of the gene. Since the p53 gene is up-regulated by vitamin D metabolites, the production of p53 protein is reduced when these metabolites are insufficient. This can occur in response to dietary or environmental factors, such as vitamin D deficiency. Since p53 inhibits replication, its loss or reduction cuts the doubling time of the cell, causing the cell's progeny to advance through generations faster. This confers a selective reproductive advantage on the progeny.

If a decoupled epithelial cell acquires a 1% selective advantage over neighboring cells in reproduction, its progeny will eventually consist of a clone occupying 99% of the tissue compartment in which the malignancy arose. This requires 9000 generations, equivalent to approximately 25 years, if the reproductive rate is one generation per day, as it is for colonocytes in cancer-prone individuals. Consistent with this evolutionary sequence, the median induction period for colonic (and most solid tumors) is 20 to 25 years.<sup>28</sup> If the cellular reproductive rate is normal, the same 9000 generations would grow a malignant clone of similar mass, but it would take 99 years. At that late point in the life cycle of the individual, it is likely that another disease would have claimed the person's life. Concern about such a slow-growing malignancy would be irrelevant.

The mechanism of the dose-response relationship between vitamin D and cancer risk is that vitamin D and

its metabolites exert substantial control on the rate of evolution of cancer in epithelial tissues. When an individual's vitamin D status is very high, the reproductive rate of epithelial cells will be the minimum needed to maintain health. The epithelial cells are reliably self-adherent and undergo a normal life cycle. When vitamin D status is low, the reproductive rate of epithelial cells increases abnormally, leading to loss of fidelity in DNA replication and acquisition of somatic mutations. If the early genetic victims of replication defects include tumor suppressor genes such as p53, the evolutionary process is further accelerated. Vitamin D is pleiotropic and also prevents cancer by several other mechanisms, including maintenance of normal differentiation, enhancement of apoptosis, and prevention of tumor angiogenesis.

The microevolutionary progression of cancer is best avoided. It is wiser to prevent cancer from its earliest stage by maintaining vitamin D adequacy (serum 25(OH)D  $\geq$  55 ng/mL). Before a massive degree of microevolution of the cancer has occurred, and when tumor-suppressor genes that respond to the vitamin D receptor-ligand complex are still present, arrest of proliferation and metastasis of the malignancy may be possible and should be attempted.

Maintaining and restoring vitamin D adequacy has the potential to play a unique role in primary prevention and as an adjunct to existing treatments for cancer. An approach to using vitamin D to prevent cancer may be to maintain a serum 25(OH)D level of  $\geq$  55 ng/mL throughout life. It would also be wise to promptly restore the usually deficient serum 25(OH)D level of all individuals with newly diagnosed invasive cancers of the colon, breast, and ovary to  $\geq$  55 ng/mL unless hypercalcemia is present.

It has been shown that the necessary 55 ng/mL concentration of 25(OH)D can be easily maintained, as it is in healthy lifeguards.<sup>29</sup> Any physiological state that predisposes to cancer is unsafe, including a low serum level of 25(OH)D. Monitoring tools are now available that support maintaining the 25(OH)D level in a range that is high enough for safety but low enough to avoid major risks.

Based on the dose-response curves identified in this report and modeled national baseline median population winter 25(OH)D levels, the projected number of cases that could be prevented in North America with universal attainment of a serum 25(OH)D level of  $\geq$  55 ng/mL would be at least 60,000 cases per year of colorectal cancer and 85,000 cases per year of breast cancer. The projected number of cases that could be prevented annually in the world with this serum level of 25(OH)D would be approximately 250,000 cases of colorectal cancer and 350,000 cases of breast cancer.

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