

Vitamin D: Marker or Mechanism of Action?

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Abstract

The field of vitamin D and cancer research has been moving forward quickly. However, some challenges remain regarding the interpretation and integration of data collected from epidemiological investigations and laboratory experiments. These include consideration of vitamin D biology, a better understanding of characteristics that affect concentrations of the biomarker of vitamin D status, 25(OH)D, and elucidation of variation in response to vitamin D supplementation. To further the field of vitamin D and cancer prevention, future studies will need to bridge the gap between the epidemiology and molecular biology of vitamin D activity in carcinogenesis. *Cancer Epidemiol Biomarkers Prev*; 20(4); 585–90. ©2011 AACR.

Introduction

Vitamin D has been associated with a variety of disease endpoints, including cancer. In spite of the widespread enthusiasm and public health interest related to vitamin D, questions remain regarding the role of this vitamin in chronic disease. The Institute of Medicine (IOM) recently released its report on recommendations for vitamin D and calcium intake (1). This report found no evidence of a causal link between vitamin D and cancer, and therefore, recommendations for intake could not be based on the hypothesized protective effects of vitamin D on cancer (1). However, more targeted research in this area was urged (1). This finding reflects a number of issues related to the vitamin D and cancer literature, including the challenges of reconciling the results from epidemiological and laboratory studies. One specific issue is related to the integration of our knowledge of the biochemistry and molecular biology of vitamin D with information obtained from epidemiological studies. Evidence for the mechanism of action of vitamin D in carcinogenesis from laboratory studies is mainly derived from experiments using the hormonal form of vitamin D, 1,25(OH)₂D. In contrast, the vitamin D metabolite 25(OH)D is the most commonly employed biomarker in epidemiological investigations of cancer. Another aspect that is perhaps not fully appreciated is that 25(OH)D concentrations correlate with

several risk factors, discussed further below, which themselves are related to cancer risk. Thus, it is unclear whether the associations between 25(OH)D concentrations and cancer risk are due to the direct action of this molecule, to one or more of its related risk factors, or to a combination of these constituents. Given the potential impact of this nutrient/hormone on health, it is crucial to fully understand the unique separate and combined contributions of epidemiological and laboratory studies in order to make accurate public health recommendations. In this commentary, we provide a review of the following: (1) vitamin D biology and the evaluation of vitamin D insufficiency; (2) the paradox in epidemiological and laboratory studies related to vitamin D and cancer; and (3) the possible challenges to improving vitamin D status through supplementation.

Vitamin D Biology

Vitamin D can be obtained via endogenous synthesis upon UV exposure to the skin, or it can be consumed through the diet or supplements (2). After vitamin D enters circulation, it is hydroxylated in the liver to form 25(OH)D (3), the most frequently used biomarker of vitamin D status in clinical settings and epidemiological studies. Concentrations of 25(OH)D account for both endogenous synthesis and vitamin D intake (4), and this biomarker has been shown to vary widely in human populations, as discussed further below. However, this form of vitamin D has far less biological activity than 1,25(OH)₂D, which is formed in the kidney after the enzymatically catalyzed addition of another hydroxyl group to 25(OH)D (2). Due to its potent hormonal effects, 1,25(OH)₂D is under tight homeostatic control, and therefore, does not vary as widely as 25(OH)D in circulation; nor is it generally thought to be as strongly affected by the same factors which influence 25(OH)D, such as sun exposure and vitamin D intake (2). For these reasons, the metabolite 1,25(OH)₂D has rarely been investigated in epidemiological studies of vitamin D and cancer. In contrast, due to its potent transcriptional effects, 1,25(OH)₂D is the vitamin D

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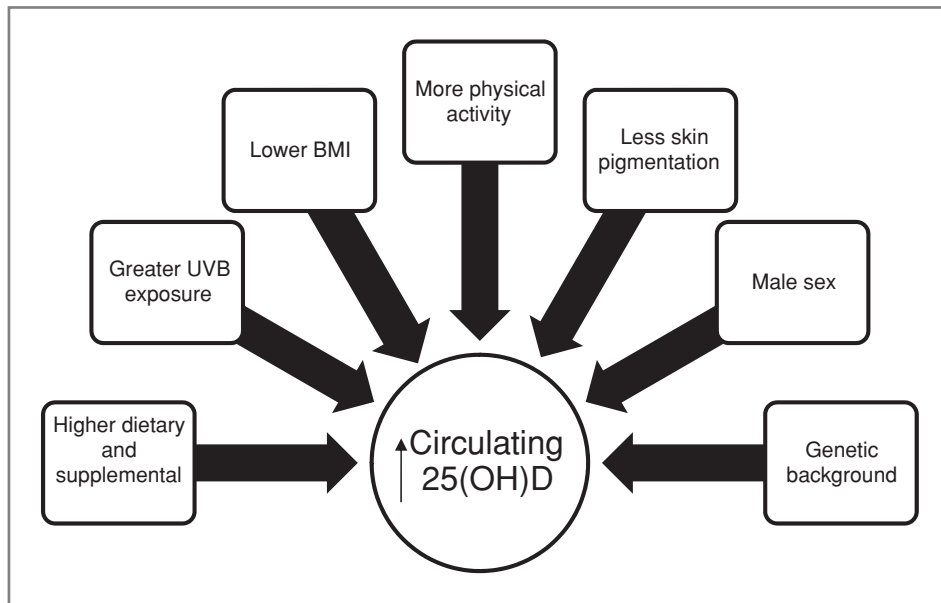


Figure 1. Contributors to circulating concentrations of 25 (OH)D.

metabolite most commonly studied in laboratory experiments. The relationship of 25(OH)D concentrations to 1,25(OH)₂D levels is complex and is subject to cross-regulation (5). As a result, it is difficult to reconcile the findings from epidemiological and laboratory studies given that the two vitamin D metabolites are not directly translatable. Therefore, it is important to understand what is meant by vitamin D status as well as the factors that affect circulating concentrations of vitamin D metabolites in an epidemiological context.

Vitamin D deficiency and correlates of 25(OH)D concentrations

In the past, concentrations of 25(OH)D of <10 ng/mL were considered deficient, as this is the level at which rickets and other frank deficiency syndromes become apparent (6). However, this definition was recently updated to reflect the accumulation of knowledge regarding the regulation of parathyroid hormone and other biomarkers, which resulted in a new proposed cutoff of <20 ng/mL (6, 7). Based on this new definition, a number of publications have reported that vitamin D deficiency in North America is prevalent, even in areas of high sun exposure (8–11). Furthermore, it has now been recommended that the optimal level for prevention of chronic disease is approximately 30 ng/mL of 25(OH)D (12); attaining these concentrations has been estimated to require in excess of 2000 IU/day of vitamin D (12). Because estimates of optimal 25(OH)D levels are largely based on observational studies of associations between 25(OH)D concentrations and risk for disease, it is important to understand the contributors to 25(OH)D status that may confound these relationships.

Blood concentration of 25(OH)D is employed as a biomarker for overall vitamin D status for good reason. It accounts for both dietary and supplemental vitamin D

intake as well as endogenous synthesis of vitamin D in the skin upon UV irradiation (13). The challenge with the use of this biomarker is that it is also correlated with several factors which themselves are related to numerous health outcomes. These include age, race/ethnicity, sex, body size, physical activity, and genetic background (8, 14–18; Fig. 1). To illustrate this point, we used physical activity and body mass index (BMI) data from participants with a history of adenomatous polyps (19, 20) to compare 25(OH)D concentrations of a group of individuals with the healthiest profiles (i.e., low BMI and high physical activity) versus those with a higher risk profile (i.e., higher BMI and lower physical activity). As shown in Figure 2, individuals who have the lowest BMI and the highest level of physical activity have the highest concentrations of 25(OH)D, while those who are obese and participate in relatively little physical activity have the lowest levels. Arguably, individuals in the latter group comprise a high-risk group for several health outcomes that have been

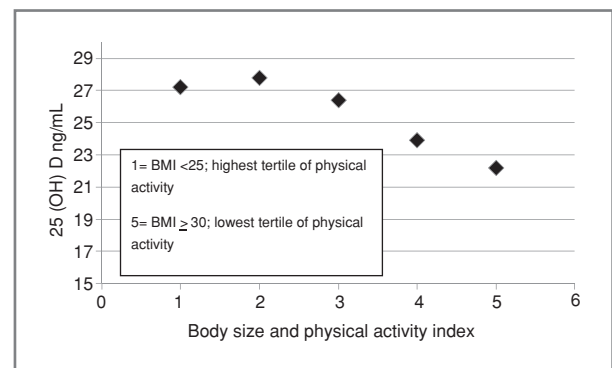


Figure 2. Body size and physical activity as they relate to 25(OH)D concentrations.

associated with 25(OH)D including cancer, cardiovascular disease, and diabetes (21–23). Race/ethnicity is another factor that can be considered. For example, Black/African-American race has been consistently linked to significantly lower circulating concentrations of 25(OH)D and hence a higher prevalence of vitamin D deficiency compared to other racial/ethnic groups (8, 9, 24, 25), perhaps due to greater skin pigmentation (26). Overall, the contribution of characteristics such as physical activity, race/ethnicity, and body size to concentrations of 25(OH)D appears to be equal to, if not greater than, that of dietary and supplemental intake of vitamin D (8, 15–17). An individual's genetic background also influences circulating 25(OH)D concentrations; variation in the gene for the vitamin D binding protein (*DBP* or *GC*) in particular seems to be related to 25(OH)D (14, 27). Fu et al. (28) showed that the presence of a single nucleotide polymorphism (SNP) in *GC* (rs4588) was significantly related to the degree to which 25(OH)D concentrations increased in response to vitamin D supplementation. Results from a genome-wide linkage study also indicate a significant impact of genes on serum levels of 25(OH)D (29). Thus, it is clear that while high, or "optimal" concentrations of 25(OH)D may be achieved via dietary or supplemental sources of vitamin D, a strong argument can be made supporting the concept that circulating 25(OH)D concentrations may be as strongly influenced by other characteristics which themselves are associated with the risk for many illnesses.

Does vitamin D have a mechanism of action or is it a biomarker of risk?

Another consideration with regard to the epidemiological data for vitamin D and health outcomes is that it is not 25(OH)D itself, but rather 1,25(OH)₂D which is thought to be the most biologically active vitamin D metabolite (30). The latter molecule is synthesized by the addition of a hydroxyl group to form 1,25(OH)₂D by CYP27B1 (30), and upon binding to the vitamin D receptor (VDR) is estimated to have effects on up to 1000 genes (31). Since 1,25(OH)₂D is the form of vitamin D that is most often used in laboratory experiments, its anticarcinogenic effects have been well-documented (32–35) and form the basis for a potential mechanism of action. However, the relationship between blood levels of 25(OH)D and 1,25(OH)₂D is complex (5). Treatment with 1,25(OH)₂D has been shown to significantly reduce the half-life of circulating 25(OH)D concentrations (5). Supplementation with 4,000 IU/d of vitamin D had no effect on concentrations of 1,25(OH)₂D after 6 months in one study (36), though in another investigation in an older population there was a significant increase in 1,25(OH)₂D concentrations after eight weeks on three different cholecalciferol-dosing regimens of approximately 45,000 IU/month (37). One interpretation of these results is that concentrations of 1,25(OH)₂D plateau after 25(OH)D reaches concentrations of approximately 30 ng/mL (37); therefore, supplementation to higher 25(OH)D concentrations is unlikely to elicit further benefit. Another

possibility is that circulating concentrations of 1,25(OH)₂D do exhibit greater variation than has previously been noted, and that measurement of this hormone in epidemiological studies of vitamin D and cancer will help elucidate the potential mechanism of action.

Perhaps more important biologically than circulating concentrations of these metabolites, it has been hypothesized that local tissue-level conversion of 25(OH)D to 1,25(OH)₂D may have a substantial impact on the action of vitamin D in carcinogenesis. The presence of CYP27B1 in sites other than the kidney allows for conversion of 25(OH)D to 1,25(OH)₂D at the tissue level; hence, higher circulating levels of 25(OH)D could also lead to greater local concentrations of 1,25(OH)₂D that cannot be detected in circulation using the standard epidemiological methodology for measuring blood levels of vitamin D metabolites (3). An example of the paradox related to the form of vitamin D used in epidemiological studies versus laboratory experiments can be illustrated in the case of African-Americans, who tend to have the lowest circulating 25(OH)D concentrations compared to those of other races or ethnicities, but who have been shown to have similar, or higher, circulating 1,25(OH)₂D levels (38–42). In our study population of subjects with a previous history of adenomatous polyps (8), individuals who reported African-American race ($n = 18$) had significantly lower 25(OH)D concentrations (18.5 ± 7.5 ng/mL) as compared to non-Hispanic whites ($n = 539$; 26.7 ± 9.1 ng/mL); however, 1,25(OH)₂D concentrations were higher for African-Americans (38.4 ± 7.0 ng/mL) than for non-Hispanic whites (35.3 ± 10.0). Nonetheless, as mentioned above, measures of circulating vitamin D metabolites may not effectively capture information regarding 25(OH)D to 1,25(OH)₂D conversion at the cellular level. Lower 25(OH)D concentrations may not provide enough substrate to produce adequate localized 1,25(OH)₂D, as has been shown by Liu et al. in macrophage activation by TLR2/1 in serum from African-Americans (43).

Another challenge in interpreting data from epidemiological work is related to the potential for bias by study design. For example, numerous reports on the relationship between 25(OH)D and breast cancer have been published (44–55). By and large, the results of case-control or cross-sectional studies have shown lower concentrations of 25(OH)D among breast cancer cases as compared to controls; in contrast, prospective studies have been largely null (56). Reasons for discrepancies in the findings by study design are unclear. One explanation is the apparent larger number of premenopausal women included in the case-control or cross-sectional studies; there are biologically plausible mechanisms linked to estrogen that may result in stronger associations between 25(OH)D and cancer in premenopausal women (57). A further consideration is that women with locally advanced or metastatic breast cancer have lower 25(OH)D concentrations than women with earlier stage cancers (56, 58). It is possible that women with more advanced breast cancers may exhibit lifestyle changes that affect characteristics which themselves influence 25(OH)D

concentrations such as body size, physical activity, sun exposure, or diet (59). These changes would lead to the observation that women with breast cancer or advanced breast cancer have lower concentrations of 25(OH)D compared to controls or women with localized cancers, respectively, when a classical case-control study design is employed. Another possibility is that the disease pathology of breast cancer directly affects vitamin D concentrations (56). Thus, it is not yet clear that increasing circulating 25(OH)D concentrations via supplementation will reduce the risk of developing breast cancer. To date, only two clinical trials on this subject have been reported, with conflicting results (52, 60). The largest was the Women's Health Initiative (WHI), in which participants who were supplemented with 1000 mg of calcium plus 400 IU of vitamin D exhibited no reduction in breast cancer incidence over 7 years compared to women given a placebo (52). In a trial by Lappe et al. (60), there was a statistically significant reduction in overall cancer risk and fewer breast cancer cases among women receiving 1,400 to 1,500 mg of calcium plus 1,100 IU of vitamin D per day compared to placebo (60). While the number of incident breast cancer cases in the trial was low and these results must be interpreted with caution, they may indicate that supplementation with vitamin D at doses higher than that used in the WHI trial may be required for prevention of breast cancer (61). Nonetheless the possibility remains that the impact of both endogenous synthesis and intake of vitamin D on circulating 25(OH)D concentrations is regulated by homeostatic mechanisms that cannot, or perhaps should not, be over-ridden by high-dose supplementation.

Response to vitamin D supplementation

As mentioned above, recommendations for optimal 25(OH)D concentrations have been made largely on the basis of association studies showing that individuals who have 25(OH)D concentrations of >30 ng/mL have the lowest risk for several chronic diseases (6). However, it is not clear that all individuals will exhibit the same response to vitamin D supplementation. Response will likely depend on other factors such as SNPs in vitamin D-related genes that are only now being studied in detail. In addition, recent work suggests a potential homeostatic control mechanism for 25(OH)D concentrations. Nelson et al. reported that baseline 25(OH)D concentrations were inversely related to the response to supplementation with approximately 800 IU cholecalciferol/day (62). The authors also indicated that this dose was sufficient to bring most of the participants in their study to 25(OH)D concentrations above the suggested optimal level of >30 ng/mL. Another investigation of three cholecalciferol

supplementation protocols indicated that the greatest magnitude of response to treatment was observed in those who had the lowest 25(OH)D concentrations upon study entry (37). A similar pattern was demonstrated by Bogh et al. in a study of participants exposed to UVB irradiation (63); those who had the lowest 25(OH)D concentrations at baseline exhibited the greatest increases in circulating 25(OH)D concentrations after UV exposure (63). Hence, as with other nutrients, it is likely that those who stand to benefit the most from vitamin D supplementation are those who exhibit the greatest insufficiency, with little to no benefit among those already approaching optimal 25(OH)D concentrations. In fact, the IOM report suggests that supplementation in such populations could potentially result in long term adverse health effects (1).

Summary

Overall, epidemiological data have consistently demonstrated an inverse relationship between 25(OH)D concentrations and risk for some cancers. However, a better understanding of the biological activity of vitamin D is critical to move the study of vitamin D and disease risk forward. Currently, it is unclear whether 25(OH)D is a marker of a higher risk profile for cancer; acting through a mechanism of action involving 1,25(OH)₂D; or both. Relatively high concentrations of 25(OH)D are found in individuals who are at lowest risk for cancer due to factors such as lower body size or greater physical activity. However, laboratory studies based on the hormonal form of vitamin D, 1,25(OH)₂D, have demonstrated anticarcinogenic activity, supporting a mechanism of action for at least this form of vitamin D. Therefore, future studies will need to bridge the gap between the epidemiology and molecular biology of vitamin D action so that the full spectrum of effects of vitamin D on cancer can be fully understood. Perhaps then the evidence for the link between vitamin D and cancer can be strengthened.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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