How to Optimize Vitamin D Supplementation to Prevent Cancer, Based on Cellular Adaptation and Hydroxylase Enzymology

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Abstract. The question of what makes an ‘optimal’ vitamin D intake is usually equivalent to, ‘what serum 25-hydroxyvitamin D [25(OH)D] do we need to stay above to minimize risk of disease?’. This is a simplistic question that ignores the evidence that fluctuating concentrations of 25(OH)D may in themselves be a problem, even if concentrations do exceed a minimum desirable level. Vitamin D metabolism poses unique problems for the regulation of 1,25-dihydroxyvitamin D [1,25(OH)2D] concentrations in the tissues outside the kidney that possess 25(OH)D-1-hydroxylase [CYP27B1] and the catabolic enzyme, 1,25(OH)2D-24-hydroxylase [CYP24]. These enzymes behave according to first-order reaction kinetics. When 25(OH)D declines, the ratio of 1-hydroxylase/24-hydroxylase must increase to maintain tissue 1,25(OH)2D at its set-point level. The mechanisms that regulate this paracrine metabolism are poorly understood. I propose that delay in cellular adaptation, or lag time, in response to fluctuating 25(OH)D concentrations can explain why higher 25(OH)D in regions at high latitude or with low environmental ultraviolet light can be associated with the greater risks reported for prostate and pancreatic cancers. At temperate latitudes, higher summertime 25(OH)D levels are followed by sharper declines in 25(OH)D, causing inappropriately low 1-hydroxylase and high 24-hydroxylase, resulting in tissue 1,25(OH)2D below its ideal set-point. This hypothesis can answer concerns raised by the World Health Organization’s International Agency for Research on Cancer about vitamin D and cancer risk. It also explains why higher 25(OH)D concentrations are not good if they fluctuate, and that desirable 25(OH)D concentrations are ones that are both high and stable.

In December, 2008, the World Health Organization, through its International Agency for Research in Cancer (IARC) published a major review of cancer and vitamin D (1). The authors of the IARC report found no compelling reason to change existing public advice about vitamin D. However, the IARC has joined the National Institutes of Health in calling for randomized clinical trials to address vitamin D treatment and cancer prevention (1, 2).

Ecologic studies suggest that more environmental ultraviolet light exposure or lower latitude are related to lower risk of prostate cancer (3-5), and pancreatic cancer (6). Findings such as this have generally been attributed to the vitamin D hypothesis, which proposes that vitamin D plays a protective, anticancer role in cellular biology. However, this theory has not been confirmed by any of the case-control studies that relate concentrations of serum 25-hydroxyvitamin D [25(OH)D, the objective measure of vitamin D nutritional status] to risk of prostate or pancreatic cancer. Higher serum 25(OH)D levels either have no effect, or they may be associated with greater risk of prostate cancer (7, 8). A similar concern was raised in relation to pancreatic cancer in a cohort of smokers living in the north, in whom Stolzenberg-Solomon et al. found that men with the lowest serum 25(OH)D levels appeared to be protected against pancreatic cancer (9). More recently, Stolzenberg-Solomon et al. obtained a similar observation for pancreatic cancer when they analyzed data from various regions of the United States (10). The phenomenon that higher 25(OH)D was related to greater risk of pancreatic cancer was evident only in the more northern US cities with low environmental UVB exposure. In contrast, in regions with high environmental UVB there was no association between serum 25(OH)D and pancreatic cancer (Figure 1).
A further argument against the ‘vitamin D hypothesis’ as an explanation for higher cancer rates northward in the world is that average 25(OH)D concentrations are similar and sometimes higher in northern Europeans than they are in southern Europeans (1, 11). In short, an inadequate vitamin D supply per se is not suitable as an explanation for the positive latitudinal correlation with prostate cancer incidence.

Table I lists a summary of problems that cast doubt on the suitability of the traditional form of the vitamin D hypothesis for cancer prevention that implies more is inherently better. The rest of this paper describes how an understanding of the enzymology of the vitamin D system may help to resolve the apparently contradictory issues surrounding the roles of vitamin D, latitude, and ultraviolet light in the context of certain cancers.

Accounting for the Paradoxes of Table I

One needs to think about vitamin D differently from the rest of endocrinology. Metabolism in the vitamin D system behaves according to enzyme-kinetic principles that are very different from those underlying other hormone control systems. The hydroxylase enzymes that metabolize 25(OH)D in vivo behave according to first-order reaction kinetics. In essence, a doubling in availability of substrate to the enzyme results in a transient doubling in the rate of product (i.e., 1,25(OH)2D) synthesis (Figure 2). After a time, an increase in 25(OH)D produces an increase in the rate of catabolism, by inducing 1,25(OH)2D-24-hydroxylase (CYP24, or 24-OHase). Tissue levels of both 1-hydroxylase [CYP27B1, or 1-OHase] and 24-hydroxylase need to be balanced according to the prevailing supply of 25(OH)D. The inverse relationship between these enzymes has been shown in vivo in rats (23) (Figure 3).

The metabolism of vitamin D behaves in a manner consistent with the model illustrated in Figure 4, in which a series of virtual compartments are represented for each metabolite and through which the flow needs to be regulated. Passage of vitamin D metabolites through the first compartment, at the level of 25-hydroxylase in the liver, is relatively unregulated. Passage of 25(OH)D at the kidney into the hormone, 1,25(OH)2D, is regulated tightly, mainly...
according to the need for calcium. At peripheral tissues, the regulation of 1,25(OH)_2D production is poorly understood, largely because the 1,25(OH)_2D generated in peripheral tissues is not normally released into the circulation. In Figure 4, the valves represent the regulated hydroxylases of the vitamin D system. In both the circulation and within peripheral tissues, the concentration of 1,25(OH)_2D needs to be regulated according to serum 25(OH)D concentration. Adjustments are made at the levels of both 25(OH)D-1-hydroxylase and 1,25-(OH)_2D-24-hydroxylase (Figure 2 and 3). This situation is not unlike the need for someone who is taking a shower to have to regulate the hot and cold water taps in response to fluctuating temperatures of the water coming in through the pipes. The situation is the same as the classic engineering problem of feedback control. Basic to feedback control is the lag time – the time it takes for a system to sense a change in input, to initiate the appropriate response, and for the response mechanism to fully complete the necessary correction (24).

In biochemistry, the time required for enzymes to respond to changes in environment (e.g. a change in vitamin D supply) has implicitly been assumed to be so rapid that any duration of disequilibrium is too insignificant to matter. To my knowledge, only a few publications have addressed the rate of adaptation of the vitamin D hydroxylases to changes in vitamin D supply (23, 25-27). That work shows that endocrine adjustments to 1,25(OH)_2D in response to calcium or to altered input of 25(OH)D can be achieved within about three days (23, 25, 28). However, the endocrine secretion of 1,25(OH)_2D (i.e. what we measure in serum or plasma) has the advantage of being tightly regulated at the kidney by at least three mechanisms: by plasma calcium, parathyroid hormone [PTH], and through direct feedback by the product, 1,25(OH)_2D. In contrast, regulation of paracrine, non-renal 1,25(OH)_2D production is not well understood at all. Unlike at the kidney, there is no regulation by calcium or PTH (29). Because they lack the multiple regulatory systems that control renal 25(OH)D and 1,25(OH)_2D metabolism, tissues such as the prostate and pancreas probably do exhibit longer adaptive lag times than does the kidney.

Non-renal tissues produce 1,25(OH)_2D for paracrine purposes. The intracellular/intra-tissue concentration of 1,25(OH)_2D is mediated by the balance between its synthesis and catabolism i.e. the ratio between 25(OH)D-1-hydroxylase and 1,25(OH)_2D -24-hydroxylase (25, 30, 31). In fact, 24-OHase [CYP24] is commonly described as the product of an ‘oncogene’ (32-34), because it breaks down the 1,25(OH)_2D that promotes cellular differentiation and
reduces replication (35, 36). In contrast, 1-OHase has been described as ‘a tumor suppressor’ (37). Prostate cancer cells, both primary cultured cells and cell lines, possess lower 1-OHase activity than normal cells from the prostate; as a result, they are somewhat resistant to the tumor suppressor activity of circulating 25(OH)D (38-40). If 1-OHase and 24-OHase need to be maintained in a ratio that compensates for changes in circulating 25(OH)D levels, then the reportedly lower cellular 1-OHase within prostate cancer cell lines suggests that those cells have lost some of their ability to adapt to low 25(OH)D concentrations.

If prostate and pancreas do not adapt rapidly to declining 25(OH)D concentrations, then the vitamin D hypothesis can explain why rates of these types of cancer increase with latitude despite average 25(OH)D concentrations that may not necessarily trend downwards with latitude. In essence, at latitudes distant from the equator, persons who exhibit the highest serum 25(OH)D concentrations during the summer will as a consequence suffer the largest absolute and relative declines in 25(OH)D through the ‘vitamin D winter’, when at high latitudes UV is not sufficiently intense to generate vitamin D in skin (41, 42). Those who avoid exposing skin to summer sunlight will exhibit the smallest amplitude fluctuations in serum 25(OH)D. As shown in an elegant review by Kimlin, there is minimal seasonal variability in environmental ultraviolet light radiation capable of generating vitamin D near the equator, but the relative variability increases dramatically with latitude (43). Serum
25(OH)D concentrations cycle in a pattern and amplitude that is closely linked to fluctuations in UVB light intensity throughout the seasons. Humans are hairless primates, suited to a natural environment in which their dermal vitamin D factory is fully exposed (surely, our evolution was over before we started to wear clothes). We are a species optimally designed through evolution to suit tropical latitudes where serum 25(OH)D concentrations should remain high and stable. Consequently, it is reasonable to infer that perpetually fluctuating inputs of vitamin D are not something to which evolution could have adapted all aspects of our biology.

What needs to be established is whether a slow rate of adaptation of the vitamin D hydroxylases can be enough of a problem to affect cancer risk – recent evidence suggests that it is. In their 2009 paper relating cancer of the pancreas to 25(OH)D in US populations, Stolzenberg-Solomon et al. suggested the effect shown here in Figure 1 may be due to an unknown molecular agent (10). The hypothesis proposed here is a more viable explanation, because it is consistent with what is known about vitamin D and ultraviolet light. I had outlined the present hypothesis in 2004 in the context of prostate cancer before becoming aware that a similar relationship exists with pancreatic cancer (9). The 2004 publication predicted that a cancer-risk relationship for those with higher 25(OH)D should be specific to higher latitude (lower environmental UV) and that at lower latitude, the risk relationship would not be evident (44). The results presented in Figure 1 are consistent with that prediction. The present hypothesis is also logically consistent with the evidence that some antineoplastic drugs suppress expression of 24-OHase (45). If suppression of 24-OHase is a good thing, then surely, then inducing 24-OHase with seasonal cycles of 25(OH)D or pulse doses of vitamin D must be a bad thing. Not all vitamin D-responsive tissues are likely to behave in the manner proposed here for the prostate. Certainly,
cancers of the breast and colon have been well validated epidemiologically as being protected against by higher 25(OH)D concentrations (1, 5, 46). There is little doubt remaining that the vitamin D system is the mechanism by which latitudinal gradients affect incidence of these carcinomas. However, the epidemiology of prostate (8,12) and pancreatic cancers (9,10) suggests that these tissues are inefficient at adapting to seasonal dynamics of UV light and the consequent fluctuations in serum 25(OH)D.

I have shown that renal 1-OHase and 24-OHase do adjust according to 25(OH)D supply (23, 25) (Figure 3). However, adaptation of non-renal tissues to moderate change in serum

Figure 5. Effects of lag time on peripheral tissue level of 1,25(OH)_{2}D (dark lines) in response to the dynamic circulating level of 25(OH)D (grey lines). The shaded regions highlight tissue 1,25(OH)_{2}D levels that are below the set-point; these indicate the times when there is a relative excess of the catabolic, oncogenic enzyme, 24-OHase, a situation synonymous with a relative deficiency in the ‘tumor suppressor’ enzyme, 1-OHase. Note that deflection of 1,25(OH)D below the setpoint is less when the average 25(OH)D is maintained at a high level (Panel A vs. C), or if the amplitude is minimal (Panel A vs. B). The rate constants used to calculate the enzyme curves (variable K, Equation 2) are the same for all panels; however, K was a conditional number that was assigned a value of 1 during the rising phase in 25(OH)D, and a value of 0.02 during the declining phase as explained in the text. The panels illustrate the effects of alterations to the mean and the amplitude of 25(OH)D (A-C), and the effects of four large intermittent doses of vitamin D that would raise the level of 25(OH)D abruptly at 180-day intervals (D). In each panel, the model also shows the effects of holding 25(OH)D constant from Day 900 onward. The tissue ratio of the 25(OH)D-1-hydroxylase and 1,25(OH)_{2}D-24-hydroxylase is abbreviated as (1-OHase/24-OHase). The tissue 1,25(OH)_{2}D levels are calculated as (1-OHase/24-OHase) times 25(OH)D level. The targeted tissue 1,25(OH)_{2}D level is maintained at a set-point as indicated by the block arrows (which represent the same thing in Figure 4). The ratio of the enzymes at any given time is calculated according to Equation 2. The levels of 25(OH)D in Panels A-C were calculated using a Microsoft Excel spreadsheet, using a sine function assigned a wavelength period of 365 days and allowing for entry of the variables to define the mean and amplitude of the sine wave. The concentrations of 25(OH)D in Panel D were calculated using a Microsoft Excel spreadsheet, based on step increases in 25(OH)D above a baseline (to mimic pulse doses), followed by an exponential decline based upon the 25(OH)D half-life of 60 days (54). The spreadsheets used to calculate and produce these figures are available by email request from the author.
25(OH)D has never been characterized. The only thing known is that the vitamin D hydroxylases outside the kidney do not respond to PTH or to calcium (47). Until recently there was no strong reason to study the dynamics of vitamin D metabolism because there was no reason to imagine that rates of change in vitamin D would have played any role in maintaining health or in the risk of disease. Today, the concerns that were laid out by the WHO/IARC report concerning vitamin D and the risks of cancer of the pancreas and prostate, along with calls from many fronts to undertake vitamin D intervention trials, make it all the more important to understand the unique, dynamic aspects of vitamin D metabolism.

**Modeling of Tissue 1,25(OH)2D Levels during Declines in 25(OH)D**

The best previous description of the problem of cellular lag time in accommodating to external environmental influences comes not from the realm of enzymology, but rather, from the vitamin D-related field of bone biology. In the 1980s, Frost proposed a mechanostat model for bone metabolism – a mechanical feedback system that controls the adaptation of bone mass according to the mechanical forces imposed on bone over time (48). In an elegant publication, Turner expanded on the concept, and provided mathematical simulations to represent the changes in bone density in response to changes in mechanical loading (49). The concept that I am outlining here applies the principle of cellular accommodation to the need for the enzymes metabolizing 25(OH)D to adapt to changes in the input of 25(OH)D. Like Frost and Turner, this is modeled after the classic thermostat-feedback system. The rate equation for a thermostat is:

\[
\text{dT/dt} = -K(T-T_0)
\]

(Eq 1)

but in this case, T is the ratio of 1-OHase/24-OHase at a given time; time is indicated by t; the rate constant is K; and the target enzyme ratio (setpoint) required for optimal adaptation of the enzymes to a given concentration of circulating 25(OH)D is indicated by T_0. Assuming that when we activate the system, the ratio of the enzymes is T_1, the solution of Equation 1 is

\[
T(t) = (T_1 - T_0)e^{-(Kt)} + T_0
\]

(Eq 2)

Accordingly, the adaptation of 1-OHase and 24-OHase should approach the target ratio that is suitable for a given 25(OH)D concentration by following an exponential decay function, \(e^{-Kt}\). This model assumes a direct correlation between the rate of change in the 1-OHase/24-OHase ratio and the distance that the ratio is from its set-point target that suits the immediate 25(OH)D concentration. This process of adaptation of enzyme activities is by definition not instantaneous, and to my knowledge, its rate has never been characterized for any non-renal tissue.

The rate function, K, is a conditional value that can vary, depending on whether the 25(OH)D concentration is increasing or decreasing. This is analogous to the reality that the power of a heater to increase temperature is not necessarily equal to the power of a cooling unit to lower temperature. The examples illustrated in Figure 5 represent fluctuations in serum 25(OH)D over time, and the resulting tissue levels of 1,25(OH)2D. For the Figure, the rate of induction of 24-OHase in response to rising 25(OH)D concentrations was assumed to be much greater than the rate at which the enzyme adapts to declining concentrations of 25(OH)D. Hence, K is bigger during the rising phase and smaller during the declining phase of 25(OH)D. The result is that the Figure emphasizes the effect of the downward phase of 25(OH)D fluctuations.

What makes responses to 25(OH)D different from the examples of single-step change usually presented to illustrate cellular adaptation is that latitudinally related change in vitamin D status is continuous and sinusoidal in nature. Moreover, change due to the pharmacological use of pulse administration of vitamin D produces an abrupt jump in 25(OH)D followed by an exponential decline. In the vitamin D system, the only thing that mimics a single-step change would be a loading dose, followed by properly selected, regular maintenance doses of vitamin D.

Figure 5 shows that for a given seasonal amplitude in serum 25(OH)D, providing additional vitamin D as a steady supplement can reduce the magnitude of the below set-point phase in tissue 1,25(OH)2D (Panel A vs. C). The Figure also shows how it can be that northern Europeans, despite having average 25(OH)D concentrations equal or higher than those of southern Europeans, can exhibit greater risk of cancer. Northerners exhibit proportionately larger amplitudes in 25(OH)D due to the greater variability in environmental ultraviolet light at higher latitudes (Panel A vs. B).

Figure 5 also shows that pulsatile administration of vitamin D can produce large below-set-point phases in tissue 1,25(OH)2D (Panel D). While pulse doses of vitamin D can benefit bone outcomes (50,51), those outcomes rely on the renal endocrine component of the vitamin D system that surely adapts much faster to 25(OH)D than peripheral tissues where the metabolism of vitamin D is not as tightly regulated. Furthermore, the rate constants represented by variable K in Equation 2 are not necessarily the same in all tissues. Hence, some tissues, such as prostate and pancreas, may be more likely to be affected adversely by pulsatile doses of vitamin D than others.

The approach outlined here is admittedly overly simplistic, but it does lay out the evidence for why there should be no doubt that so long as serum 25(OH)D concentrations are in a
phase of decline, there can be no full achievement of tissue 1,25(OH)₂D to match its ideal set-point concentration. No matter how small the true increment below the set-point may prove to be, it is by definition, a sub-optimal concentration. This may not in itself prove harmful as a single event in an individual; however, over the lifetimes of many men and women, more than 50 annual cycles of below set-point phases in tissue 1,25(OH)₂D will have an adverse effect on the risk of promotion or progression of certain types of cancer.

To my knowledge, the hypothesis presented here is the only way to incorporate the vitamin D hypothesis in a manner that accounts for the apparent contradictions outlined in Table I. This hypothesis is based on what is known about the unusual, first-order in vivo enzyme kinetics of the vitamin D system. There is no other hormone system in which an increase in substrate can produce the kind of dramatic rise in hormone concentration shown in Figure 2. The key prediction based on this hypothesis was published in 2004 and was in relation to prostate cancer (13). The prediction has been confirmed prospectively in 2009, in the context of cancer of the pancreas as shown in Figure 1 (10). Furthermore, no other hypothesis has been put forward to address the problems listed in Table I. The present hypothesis is testable in experimental models, such as the TRAMP mouse model of prostate cancer, and using epidemiologic data. The prediction is not tenable as a primary study outcome for human clinical trials, because it predicts an increased risk of cancer of prostate and pancreas in individuals given large doses of vitamin D at dosing intervals of more than 2 months.

**Implications of the Model**

The concept outlined here has implications for clinical-trial designs using vitamin D. A major problem with any clinical trial is that poor adherence to medication results in negative findings (52, 53). For vitamin D, one way to improve adherence is to give vitamin D less often, but at larger doses (50, 54). In the realm of pharmacology, a general guideline is to provide a drug at an interval no longer than the half-life of the drug (55). For vitamin D, the effective half-life for the decline in 25(OH)D after a dose of vitamin D₃ is approximately 2 months (56). However, during the first month after a dose of vitamin D₃, serum 25(OH)D concentrations have been shown to be quite stable (57, 58). In contrast, when vitamin D₂ is given, the total serum 25(OH)D concentration during the subsequent month ends up even lower than before the dose was given (58). Incidentally, the phenomenon of a lower than baseline total serum 25(OH)D one month after giving vitamin D₂ is clinical evidence supporting the present contention that the rate of adaptation of metabolic clearance or catabolism is undesirably slow to respond to fluctuations in vitamin D supply. The conclusion from these considerations is that clinical trials using vitamin D at intermittent doses should avoid vitamin D₃ and they should avoid dosing intervals of any form of vitamin D that exceed two months. Vitamin D₃ given on a once weekly or once monthly may be an optimal strategy that will probably improve adherence compared to daily dosing (59, 60) while minimizing fluctuations in serum 25(OH)D concentration.

The hypothesis and the model presented here deal with issues that are distinct from the question of whether higher, steady 25(OH)D concentrations are in themselves preferable to lower concentrations. There are reasons to conclude there is an inherent benefit from higher 25(OH)D concentrations (21, 22, 61), but the present hypothesis is not intended to address those aspects. This hypothesis provides an explanation for the problems listed here in Table I, and it justifies vitamin D supplementation for conditions where latitude appears to increase the risk of cancer or other disease even if the average 25(OH)D concentrations are not different for populations at different latitudes. Supplementation increases the prevailing serum 25(OH)D concentrations, while reducing the effect of the seasonal amplitude in 25(OH)D on the tissue fluctuations in 1,25(OH)₂D.

**Conflict of Interest Statement**

In the past year, Dr Vieth has served as a paid consultant and/or speaker for the following: Carlson Labs, DiaSorin, Merck/MSD, Stieffel, Yoplait, Wyeth.

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