

## The Role of the Parent Compound Vitamin D with Respect to Metabolism and Function: Why Clinical Dose Intervals Can Affect Clinical Outcomes

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**Context:** There is no doubt that vitamin D must be activated to the hormonal form 1,25-dihydroxyvitamin D to achieve full biological activity or that many tissues participate in this activation process—be it endocrine or autocrine. We believe that not only is 25-hydroxyvitamin D important to tissue delivery for this activation process, but also that intact vitamin D has a pivotal role in this process.

**Objective:** In this review, evidence on the vitamin D endocrine/autocrine system is presented and discussed in relation to vitamin D-binding protein affinity, circulating half-lives, and enzymatic transformations of vitamin D metabolites, and how these affect biological action in any given tissue.

**Conclusions:** Circulating vitamin D, the parent compound, likely plays an important physiological role with respect to the vitamin D endocrine/autocrine system, as a substrate in many tissues, not originally thought to be important. Based on emerging data from the laboratory, clinical trials, and data on circulating 25-hydroxyvitamin D amassed during many decades, it is likely that for the optimal functioning of these systems, significant vitamin D should be available on a daily basis to ensure stable circulating concentrations, implying that variation in vitamin D dosing schedules could have profound effects on the outcomes of clinical trials because of the short circulating half-life of intact vitamin D. (*J Clin Endocrinol Metab* 98: 4619–4628, 2013)

Extraordinary advances in vitamin D research have occurred since Adolf Windaus was awarded the Nobel Prize for its discovery in 1928 (1). Over the next several decades vitamin D was studied mostly in dietary terms and later in metabolic studies, culminating in the discovery of 25-hydroxyvitamin D [25(OH)D] by the Deluca lab in 1968 (2). Intense study of 25(OH)D over the next 2 years resulted in the discovery of the hormonal form of vitamin D—1,25-dihydroxyvitamin D [1,25(OH)<sub>2</sub>D]—by various research groups (3–6). At that point, research into any potential biological function/contribution of circulating intact vitamin D basically ceased because 1,25(OH)<sub>2</sub>D became the major focus, followed by a later change in focus in the mid to late 1980s when circulating 25(OH)D

was deemed the best nutritional status indicator for vitamin D and was shown to be associated with secondary hyperparathyroidism and cancer incidence in a way that circulating 1,25(OH)<sub>2</sub>D was not (7, 8). Thus, outcome relationships with circulating 25(OH)D have been an intense focus of research while the potential role of the parent compound intact vitamin D in the circulation has not been studied in the same way, especially because measuring this compound in the circulation has been very difficult until recently.

There is no debate that to become fully active, vitamin D must be metabolized to 1,25(OH)<sub>2</sub>D; however, what has not been fully appreciated in this metabolic scheme is the importance of cellular accessibility of the parent com-

ISSN Print 0021-972X ISSN Online 1945-7197  
Printed in U.S.A.

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Received June 26, 2013. Accepted October 2, 2013.

First Published Online October 8, 2013

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; 1,25(OH)<sub>2</sub>D, 1,25-dihydroxyvitamin D; RCT, randomized controlled trial; VDBP, vitamin D binding protein.

pound vitamin D, as well as that of 25(OH)D. The current literature shows clearly that vitamin D is vastly more accessible than 25(OH)D for internalization into any given cell type with the exception of cells expressing the megalin-cubilin system (9), the kidney and parathyroid gland, that provide the vitamin D endocrine system. In contrast, most other cell types depend on their paracrine/autocrine environment, but the availability of circulating 25(OH)D to these cells is reduced because it is tightly bound to the vitamin D binding protein (VDBP). Intact vitamin D is bound to VDBP much less strongly (10–12), with ample evidence in the literature to support this concept, showing that only the “free” metabolite can enter the cell easily for activation (13, 14). This fact becomes very important when designing clinical studies for vitamin D in the selection of dosing regime, ie, whether to give supplements at daily, weekly, monthly, or longer intervals, because such different regimes could well explain much of the discordance in outcomes that has been observed in interventional studies on infection and cancer (15–27). These data might prove more consistent with consideration of the provision of intact vitamin D as a major substrate for local activation of vitamin D in target tissues, in addition to the availability of circulating 25(OH)D. A further problem is that it is not yet known how much vitamin D is required and what is the dosing frequency to ensure a stable circulating concentration of intact vitamin D that is adequate for optimal tissue function.

### Control of Circulating Concentrations of Vitamin D, 25(OH)D, and 1,25(OH)<sub>2</sub>D and Tissue Delivery

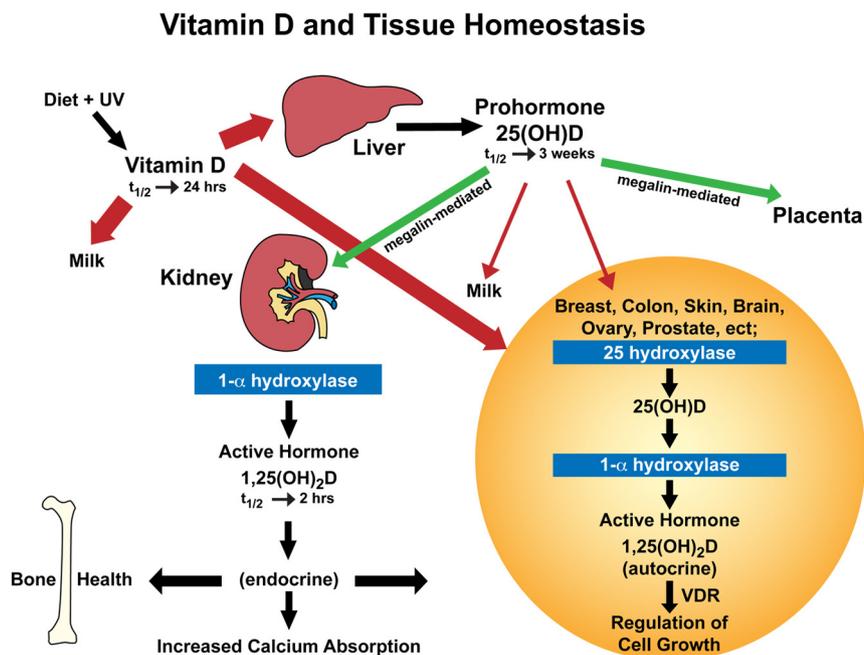
The control of circulating concentrations of vitamin D, 25(OH)D, and 1,25(OH)<sub>2</sub>D is a complex matter, affected by many disease states such as malabsorption syndromes, aberrant vitamin D metabolism as in sarcoidosis and/or disruptions in the calcium homeostatic system, etc (28–30). Although these are all important, they are beyond the scope of this short review and are not considered further; consideration is given only to what happens to these compounds under normal conditions when vitamin D is obtained through the diet or UV light induction and how they enter normal cells.

In humans, vitamin D<sub>3</sub> is naturally obtained when sunlight in the UVB range strikes the skin and causes 7-dehydrocholesterol to be converted into vitamin D<sub>3</sub>, which then diffuses into the circulation through the capillary bed (31). Vitamin D also is obtained orally through the diet as either vitamin D<sub>2</sub> or D<sub>3</sub>. As far as can be determined from the literature, this absorption process is primarily diffu-

sion-based, is dependent on bile acid solubilization, and is not saturable (32, 33). When vitamin D<sub>3</sub> enters the circulation after UV exposure, it is primarily associated with VDBP. In contrast, after intestinal absorption, it is coupled with both VDBP and lipoproteins (34). Vitamin D from either route is delivered primarily to the liver, where 25(OH)D is produced, becomes associated with VDBP, and is discharged into the circulation (35). However, vitamin D is circulated not only to the liver but also to all tissues in the body; many of these tissues are now known to contain not only the activating hydroxylase but also the vitamin D 25-hydroxylase that converts vitamin D into 25(OH)D, thus achieving autocrine production of 25(OH)D in those tissues (36–40) (Figure 1). We believe this to be an underappreciated and very important event that has not yet been adequately considered or investigated.

On reaching the circulation, the primary determinant of how long a vitamin D metabolite will stay in circulation is its affinity for VDBP (10–12). Vitamin D, 25(OH)D, and 1,25(OH)<sub>2</sub>D have vastly different dissociation constants with regard to VDBP: for 25(OH)D, it is approximately 10<sup>-9</sup> M, and for vitamin D and 1,25(OH)<sub>2</sub>D, it is approximately 10<sup>-7</sup> M (10–12); in addition, for vitamin D, it is probably reduced to approximately 10<sup>-8</sup> M by its relative insolubility *in vitro* (41). These dissociation constants also contribute to the circulating half-lives of these compounds, where for 25(OH)D, it is weeks; for vitamin D, 1 day; and for 1,25(OH)<sub>2</sub>D, a few hours (42). These dissociation constants also dictate the “free” concentration of compound that is available to diffuse across a cell membrane into cells to be metabolized or to modulate cell activity (see Figure 1). In the case of these three compounds, the “free” circulating concentrations are greater for 1,25(OH)<sub>2</sub>D than for intact vitamin D, which in turn are larger than that of 25(OH)D, matching their relative circulating half-lives.

Besides simple cellular diffusion of free compound, there exists another important tissue transport mechanism for these steroids—the megalin-cubilin endocytotic system (9). This system is key in the delivery of 25(OH)D to the 25-hydroxyvitamin D-1- $\alpha$ -hydroxylase in the kidney (9), and it also exists in the parathyroid glands, so that its important role in the endocrine function of vitamin D is self-evident (43). The megalin-cubilin system also functions in the placenta (43), which we will revisit later. However, where tissues lack this endocytotic system, diffusion of vitamin D compounds in relation to free circulating concentrations becomes inherently important. Interestingly, VDBP-knockout animal models show normal survival when given dietary vitamin D on a daily basis (44, 45); because vitamin D metabolite cellular access could



**Figure 1.** Diagram of the metabolic processes providing vitamin D and its metabolites to various tissues of the body. Tissue distribution of vitamin D and 25(OH)D based on simple diffusion (red arrows) or endocytosis (green arrows). Endocytosis requires the tissue-specific megalin-cubilin system, whereas simple diffusion is primarily controlled by the dissociation constant of the vitamin D compound for the VDBP. Bolder red lines indicate greater diffusion rates due to a higher dissociation constant.  $t_{1/2}$ , half life.

only be by diffusion in these animals, this shows that the parent compound vitamin D is normally transferred in wild-type animals through simple membrane diffusion.

## Dosing with Vitamin D and Relative Sustainability of the Compounds in the Circulation

Vitamin D is introduced into the human body in two ways, orally or by UV exposure. UV exposure is the natural way by which humans receive their vitamin D, but now, due to a variety of lifestyle changes, it is mostly obtained from oral sources. Adams et al (46) first described the magnitude and time course by which vitamin D<sub>3</sub> is produced and released into the circulation. This work clearly demonstrated that the human body could produce and release thousands of IU of vitamin D<sub>3</sub> into the circulation within 24 hours of modest UV exposure (46), whereas orally ingested vitamin D<sub>3</sub> appears in the circulation at a more rapid pace, with a 12-hour peak rather than a 24-hour peak (34, 46, 47). Furthermore, there is a different plasma distribution of vitamin D in oral vs UV-stimulated release (34); yet, the circulating half-life of the compound by either route is of the order of 12–24 hours (34). Because of this short half-life, even large bolus vitamin D doses of 50 000 to 100 000 IU are cleared from the circulation within a week, making vitamin D basically undetectable in

the circulation (46–49). It was largely assumed that after a large bolus dose or UV exposure the rapidly disappearing vitamin D was stored in adipose tissue to be released at a later time. This has recently been shown not to occur, however, because it does not reappear in the systemic circulation at detectable concentrations (49). Early work has demonstrated vitamin D to be excreted in bile, feces, and urine (50). Thus, the only known way to sustain constant circulating vitamin D concentrations is by daily supplementation and/or chronic UV exposure (51, 52).

From a supplementation standpoint, one would have to supply an adult with a dose that would achieve increases in circulating vitamin D. For instance, a 400 IU/d dose for several months will achieve little or no detectable effect on the circulating concentration of vitamin D (51, 53).

However, if one supplements adults for extended periods with 2000–6000 IU/d vitamin D<sub>3</sub>, stable circulating concentrations of vitamin D are maintained in the range of 10–40 ng/mL in a linear fashion (51, 53), although what constitutes a minimal dosage of vitamin D for achieving an optimal circulating vitamin D concentration remains to be determined.

What is the time course for the systemic appearance of 25(OH)D after acute or chronic dosing of vitamin D<sub>3</sub>? 25(OH)D does not appreciably increase in the systemic circulation until about 24 hours after oral supplementation with 100 000 IU vitamin D<sub>2</sub>, and then by only 1–4 ng/mL (54). Chronic daily dosing of vitamin D will result in a slow, sustained rise in circulating 25(OH)D that will reach a steady state at 3–4 months (55, 56), whereas acute, interval, or large bolus dosing with vitamin D results in a variety of appearance and disappearance rates (15, 49, 57–60).

Given the pharmacokinetics of vitamin D and 25(OH)D, investigators considering performing a clinical trial with vitamin D should consider the above information and what the circulating profiles of vitamin D and 25(OH)D might be on dosing of 400, 1000, 2000, or 4000 IU/d; 28 000 IU/wk; 120 000 IU/mo; 360 000 IU every 3–4 months; or some higher annual dose. All of these schedules have been utilized in various clinical trials (15–27, 51, 53, 57–77). All of these dosing schedules appear to

be well tolerated in normal adults from a toxicity standpoint because none results in hypercalcemia or hypercalciuria (15–27, 51, 53, 57–77). However, these doses will differ greatly in their impact on circulating concentrations of vitamin D and 25(OH)D; daily doses of vitamin D result in stable circulating concentrations of both compounds (51), whereas weekly or longer interval dosing will result in large fluctuations in circulating vitamin D but stable concentrations of 25(OH)D (77–79). Indeed, any high-dose, long-interval dosing schedule can be considered pharmacological rather than physiological.

## Biological Roles in Human Physiology Unique to the Parent Compound, Vitamin D

### Lactation and pregnancy

If circulating vitamin D truly has unique roles independent of circulating 25(OH)D, what might they be? The clearest example of this occurs during human lactation. Our laboratory has been involved in this area of investigation during the last three decades. We published the first comprehensive report that quantified vitamin D and its various metabolites in human milk (80) and remain active in this area of work. Our interest in this area was sparked during undergraduate training (B.W.H.) when human milk was described as the perfect food for the newborn infant, except that it was deficient in vitamin D and breastfeeding could result in rickets to the nursing infant. Sadly, this fact is still true today in that the solely breastfed infant can exhibit severe vitamin D deficiency (81). For dietitians, nutritionists, and primary medical practitioners, the solution was simple—provide supplemental vitamin D drops for the nursing infant and the problem will be solved (82). But why should this problem exist, and how was it avoided in early humans?

Our initial work on the vitamin D content of human milk showed it to be woefully inadequate in vitamin D, confirming earlier work using bioassay methodology (83). In hindsight, this error was easy to make because only 25(OH)D was assayed and detected in human milk, and the parent vitamin D could not be measured due to inadequate methodology (80, 84). The basic problem, though, was that there was little circulating vitamin D in the mothers because they had poor oral intake and little UV exposure. Thus, there was little vitamin D for transfer into the mother's milk, and incorrect conclusions about human milk emerged as a result (80), which we corrected only when we performed experiments demonstrating that both UV exposure and increased maternal vitamin D supplementation could produce profound increases in both cir-

culating and milk concentrations of vitamin D but minimal changes in concentrations of 25(OH)D (85, 86).

Our most intriguing observation on this topic was on a hypoparathyroid patient taking 100 000 IU/d vitamin D<sub>2</sub> who went through two normal pregnancies and deliveries and breastfed her infants. Her milk contained nearly 8000 IU/L of antirachitic activity, mostly as vitamin D<sub>2</sub> with relatively little 25(OH)D<sub>2</sub> content (86); her milk contained vitamin D<sub>2</sub> at 28% of the circulating concentration as compared to 25(OH)D<sub>2</sub> at only 1.3% of the circulating concentration (86), an observation confirmed in lactating women with “normal” vitamin D status (87). Thus, the parent compound is clearly transferred from the circulation into the breast milk much more efficiently than 25(OH)D; a simple calculation suggests that for every 1000 IU/d vitamin D<sub>3</sub> provided to a lactating woman, about 80 IU/L will appear in her breast milk. Thus, to provide 400–500 IU/d for their infants, nursing mothers will appear to need to acquire 6000 IU/d of vitamin D<sub>3</sub>, something not yet investigated because, until recently, recommended intakes for nursing mothers were 400 IU/d, and intakes above 2000 IU/d were considered harmful (88).

In 2001, Vieth et al (55) demonstrated that the 2000 IU/d upper safe limit for vitamin D assigned by the Institute of Medicine (IOM) in 1997 was incorrect and that daily intakes of vitamin D up to 4000 IU/d were indeed safe (55, 56). We utilized the findings of the 1999 Vieth study (89) to gain Institutional Review Board approval for a series of studies that clearly demonstrated our hypothesis (53, 90, 91). In addition, in our initial pilot studies, we found that maternal adherence (compliance) with vitamin D supplementation exceeded 90%, whereas adherence rates for giving infant vitamin D drops was only 70% (53, 90)—data that allowed us to proceed to a National Institute of Child Health and Human Development 6-year, two-site, double-blind, randomized controlled trial (RCT) that fully confirmed our initial observation (91).

Thus, our proposed approach safely addressed the needs of both the lactating woman and her recipient breastfeeding infant while reconfirming that the use of infant vitamin D supplementation is far below the universal supplementation recommended by the American Academy of Pediatrics that should begin within the first few days after birth (82): ie, of the 319 exclusively breastfeeding mother-infant dyads, 31 (9.7%) were receiving a vitamin D supplement at 1 month of age (our unpublished data). Sadly, if one follows the 2010 IOM recommendations for lactating women of 400 IU/d (92), we will see the same problems as in 1975 when we posed the initial question, “How can ingesting human milk cause vitamin D

deficiency?” Breastfeeding is, therefore, a clear example of a situation where circulating vitamin D is critical in assuring vitamin D provision for the infant and where circulating 25(OH)D does not substitute for the intact vitamin.

Why should the transfer of vitamin D rather than 25(OH)D be favored in the mammary gland? We postulate that it is simply controlled by the relative freedom of vitamin D, as compared to 25(OH)D, from VDBP binding, which allows more vitamin D than 25(OH)D to diffuse across cell membranes into the milk. This situation is in contrast to the relative transfer of 25(OH)D and vitamin D from mother to fetus across the placenta. Here, 25(OH)D is much better able to navigate the crossing because fetal concentrations of 25(OH)D are about 70% that of the mother (93), whereas vitamin D concentrations are only about 10% of maternal concentrations (51), similar to the mammary gland (93, 94). What are the mechanisms here? Again, no one really knows, but a good hypothesis is that vitamin D is going across again by simple diffusion, whereas 25(OH)D is being specifically transported attached to the VDBP through the megalin-cubilin endocytotic system, which is very active in the placenta (43) and is key to the conversion of 25(OH)D to 1,25(OH)<sub>2</sub>D, as it is in the kidney (9).

### Other tissues

It is possible that circulating vitamin D has a physiological function other than simple diffusion advantages in tissues other than the mammary gland, as suggested by some recent evidence. Such a mechanism would take advantage of the ability of vitamin D to diffuse into cells and then become activated by already known enzymatic steps because the cytochrome P<sub>450</sub> enzymes that convert vitamin D to 25(OH)D are dispersed throughout the body, eg, in prostate, skin, intestine, brain, and of course, in liver (36–39). It is important to reemphasize that 25-hydroxyvitamin D-1- $\alpha$ -hydroxylase is even more widely dispersed in tissues (95) so that intact vitamin D can be metabolized to the active hormone in these tissues; being widely dispersed in various tissues, it is likely that they serve some useful biological function.

Of all cell types in the body already examined for vitamin D-axis enzymes, dermal keratinocytes are the best characterized (39). Indeed, the isolation and molecular cloning of the 25-hydroxyvitamin-D-1- $\alpha$ -hydroxylase was first achieved using keratinocytes (96), which are known to metabolize the intact compound into hormonal 1,25(OH)<sub>2</sub>D (39, 96). This function is unsurprising because huge amounts of vitamin D<sub>3</sub> are made in the skin in response to UV light exposure. This process enhances anti-inflammatory and antimicrobial protection. When this

system is short of vitamin D<sub>3</sub> due to avoidance of sunlight or wearing sunscreen, serum intact vitamin D content falls (97).

Orally administered vitamin D<sub>3</sub> can substitute for UVB replete keratinocytes; Hata et al (98) gave 4000 IU/d vitamin D<sub>3</sub> orally to subjects for a period of 21 days and found marked increases in cathelicidin expression in keratinocytes from normal patients and those with atopic dermatitis, with only modest rises in circulating 25(OH)D concentrations from 22.5 to 35.5 ng/mL. Circulating vitamin D<sub>3</sub> concentrations were not measured. This cathelicidin response might result from the small rise in circulating 25(OH)D, but might well have been due to the much larger rise in circulating vitamin D<sub>3</sub> that would have occurred on such doses (from < 2 ng/mL to concentrations > 20 ng/mL) (51), able to diffuse into the keratinocyte ready for activation (39, 96).

Although a recent short-term RCT using a daily dose of vitamin D<sub>3</sub> was negative (99), other studies using smaller oral doses of vitamin D<sub>3</sub> have reported clinical improvement in atopic dermatitis (75, 100, 101), the best example of which is a recent study by Samochocki et al (101) where patients were given 2000 IU/d vitamin D<sub>3</sub> for 3 months and significant clinical improvement in atopic dermatitis was seen with increases in circulating 25(OH)D to 13 ng/mL from 7 ng/mL (101), although immeasurable baseline vitamin D would have increased to approximately 10 ng/mL (51), providing significant increases in substrate vitamin D<sub>3</sub> to the diseased keratinocyte.

Recent work demonstrates that intact vitamin D has effects on other tissues; for example, bolus doses of vitamin D<sub>2</sub> (100 000 IU) decrease circulating hepcidin rapidly (M. Hewison, B. W. Hollis, unpublished data, 2013). Hepcidin is an antibacterial protein that regulates the absorption, tissue distribution, and extracellular concentration of iron by suppressing ferroportin-mediated export of cellular iron and is produced by hepatocytes and monocytes (M. Hewison, B. W. Hollis, unpublished data, 2013). This report demonstrated that circulating hepcidin began to decline 2 hours after a bolus of vitamin D<sub>2</sub> before circulating 25(OH)D begins to rise and at the time orally administered intact vitamin D appears in the circulation (M. Hewison, B. W. Hollis, unpublished data, 2013).

Further evidence about the role of vitamin D in cancer suppression/elimination comes from our interventional trial on the progression of low-grade prostate cancer (27). In this study, men with low-grade prostate cancer were given 4000 IU/d vitamin D<sub>3</sub> for 1 year. After this, repeat biopsies staged by a pathologist blinded to treatment showed improved outcome (in comparison with matched historical controls) (27), with increases in circulating 25(OH)D from 32 to 66 ng/mL. The men with the highest baseline circulating 25(OH)D concentrations showed the greatest benefit in clinical outcome

with supplementation, whereas tissue slice analysis of prostate glands from men receiving 4000 IU/d vitamin D<sub>3</sub> for 2 months before prostatectomy, using matrix-assisted laser desorption/ionization mass spectrometry, exhibited large changes in membrane lipids (B. W. Hollis, S. Gattoni-Celli, unpublished data). Again, the relative role of changes in 25(OH)D vs those of intact vitamin D on tumor progression remains to be tested.

Studies of the type reported by Bischoff-Ferrari et al (103) could answer many of the questions posed here. These investigators provided study subjects with 20 µg/d of either vitamin D<sub>3</sub> or 25(OH)D<sub>3</sub> and examined a variety of outcome variables after 4 months; circulating 25(OH)D<sub>3</sub> was raised more than vitamin D<sub>3</sub>, but doses could be adjusted to allow comparison of their relative biological efficiency, although such studies would have to be performed outside the United States because we no longer have any available source of 25(OH)D<sub>3</sub>.

## Dosing of Vitamin D and How It May Affect Study Outcomes

In 2010, the IOM committee on vitamin D suggested that the only data useful for advising changes in policy would have to originate from RCTs (92); however, RCTs are extremely expensive, and for vitamin D compounds, there is no financial incentive for their use. Furthermore, designing RCTs for nutrients is more challenging than for new medications because nutrients are normally taken in the diet, and for vitamin D, it is provided by diet as well as sunlight, confounding RCT data (104). Thus, we tend to see small trials performed on specific topics or in specific diseases (23–27, 64–76) or at certain times in the lifecycle, such as pregnancy (51, 62, 105) and lactation (53, 90, 106).

## Infection

In addition to vitamin D trials for skeletal outcomes, the role of vitamin D in infectious disease currently is a hot topic of investigation (15–22, 63, 107, 108). Treatment/prevention of infectious disease with vitamin D must depend on its autocrine roles. Three high-profile negative studies recently have been reported (15, 22, 63). Each used periodic bolus dosing of vitamin D (15, 22, 63), so that a lack of continuing availability of intact vitamin D may have confounded the findings suggested by other work (18, 19) where, of six positive RCTs investigating vitamin D and infection (16–21), five utilized a daily dosing schedule (16, 18–21) for a range of infections, including pulmonary tuberculosis (15–17), respiratory infections (19, 20, 22, 63), influenza (18), and gingivitis (21). Indeed, a recent meta-analysis of RCTs with respect to vitamin D

supplementation and respiratory tract infections demonstrated vitamin D to be an effective treatment (109) and suggested that vitamin D should be given in a daily dose, as is the premise of this review. The vitamin D doses in these studies that were effective were given daily, in line with the present review; furthermore, the effective doses were higher than those required for skeletal health and above recent IOM recommendations (92).

## Musculoskeletal, cancer, cardiovascular, diabetes, multiple sclerosis, neurology, and pregnancy

Although all are agreed that vitamin D is required for skeletal health, an area of controversy that still persists is how much circulating 25(OH)D is required to maintain optimal skeletal homeostasis (102). Here again, long interval vitamin D dosing has proved ineffective and has even appeared to be harmful to bone health (77).

In evaluating recent vitamin D supplementation studies, it is clear that as a group they are diverse in the types of subjects and patients supplemented and in the dosing schedules used; yet, most demonstrate benefits from using vitamin D at 1200 to 40 000 IU/d, in many different conditions (16–21, 24–27, 64–76). Again, using higher vitamin D dosing than IOM bone recommendations (110), important outcomes are reported, including decreases in cancer rates (24), regression of established prostate cancer (27), reduction of blood pressure in African Americans (68), improved insulin sensitivity (69–71), and stabilization of Parkinson's disease in some patients (65). One of the most impressive study results was the ability of vitamin D<sub>3</sub> to prevent multiple sclerosis in a highly susceptible population (74); again, it may be noted that these studies used daily doses of vitamin D of 1200 to 40 000 IU and with no adverse events reported (15–22, 24–27, 57, 58, 60–76). Conversely, there are notable high-profile RCTs that failed to produce positive results (15, 22, 23, 25, 63), including the Women's Health Initiative study that failed to show vitamin D could prevent cancer (23); however, this study provided only approximately 280 IU/d vitamin D<sub>3</sub>, which would have had no effect on any circulating vitamin D metabolites (55, 56, 89). Another RCT failure with respect to cancer was the Trivedi et al (25) study that administered 100 000 IU vitamin D<sub>3</sub> on a quarterly basis, although it did demonstrate positive effects on skeletal outcomes (25). The failure of these studies with respect to cancer outcomes was predictable from the arguments we present in this review. What such studies do, however, is to support the premise that any dosing schedule that does not allow a stable circulating concentration of the parent compound will not provide an optimal autocrine environment for vitamin D metabolism in non-bony tissues.

This review considers the autocrine function of vitamin D, which is sensitive to both circulating vitamin D and 25(OH)D and where negative outcomes could be predicted due to failure to maintain circulating vitamin D over time, as with monthly, quarterly, or yearly bolus vitamin D dosing. Although these studies do not prove our view of the direct role of intact vitamin D in the tissues, they do suggest that caution and careful planning of dosing in future RCTs is required to avoid possible confounding from interval dosing.

## Conclusions

Circulating vitamin D, the parent compound for tissue vitamin D activation, likely has an important direct physiological role beyond what was originally anticipated through the local tissue autocrine system. Based on emerging data from the laboratory and from clinical trials and on available knowledge of vitamin D axis metabolism, it appears likely that for the optimal benefits of vitamin D supplementation, enough vitamin D should be provided on a daily basis to ensure that stable circulating concentrations are maintained over time. This view implies that schedules for vitamin D dosing could have profound effects on the outcomes of clinical trials, due to the short circulating half-life of vitamin D. Although further work is needed to assess the impact of intact vitamin D concentrations on clinical outcomes, the information so far available suggests that care is required in the design of dosing regimes in future RCTs, both in terms of the doses given and the intervals at which they are given.

## Acknowledgments

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This work was funded in part by the National Institute of Child Health and Human Development (Grants R01 HD043921 and HD47511); National Institutes of Health (NIH) Grant RR01070; the South Carolina Clinical and Translational Research Institute, with an academic home at the Medical University of South Carolina; NIH/National Center for Research Resources Grant UL1 RR029882; National Center for Advancing Translational Sciences Grant UL1 TR000062; the Thrasher Research Fund; and the Kellogg Foundation.

Disclosure Summary: The authors have nothing to disclose.

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