SOMATO PUBLICATIONS

Journal of Clinical Nutrition and Food Science

Review Article

A Basic Science and Clinical Review of Vitamin D

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Received: 01 October 2018; **Accepted:** 24 October 2018; **Published:** 27 October 2018

Citation of this article: Mills, SA., Dueno, S., Wallace, GH. (2018) A Basic Science and Clinical Review of Vitamin D. J Clin Nutr Food Sci, 1(2): 040-049.

Abbreviations

DBP: Vitamin D Binding Protein; VDR: Vitamin D Receptor; 7-DHC: 7-dehydrocholesterol; 25-(OH)D_3: 25-hydroxyvitamin D_3; 1,25α-$(OH)_2D_3$: 1,25α-dihydroxyvitamin D_3; IOM: Institute of Medicine; PTH: Parathyroid Hormone; DRI: Dietary Recommended Intakes; CaSR: Calcium Sensing Receptors; HPLC: High Pressure Liquid Chromatography; GC: Gas Chromatography; MS: Mass Spectrometry; UV/Vis: Ultraviolet and Visible Spectroscopy; Camp: Cyclic Adenosine Monophosphate; IDDM: Insulin-dependent Diabetes Mellitus

Introduction

Vitamin D was discovered in the early 20th century [1]. Since that time, it has been the subject of extensive research by both the scientific and clinical communities. Vitamin D is commonly known for its regulation of serum calcium and phosphorous levels and its ability to prevent rickets [1,2]. The development of molecular and genetic technology has allowed researchers to look at the effects of vitamin D on other organ systems. Vitamin D receptors have been found on various organs in the human body and it has been estimated that vitamin D regulates around 250 genes [3,4]. Vitamin D research continues to be an open field where clinicians look to achieve a complete understanding of vitamin D function and its benefits in human health.

A more thorough understanding of the metabolism and physiology of vitamin D can reveal its role in disease and overall health. Although much progress has been made in our understanding of vitamin D, many challenges remain. Techniques such as high- pressure liquid chromatography (HPLC), with either mass spectrometry (MS) or UV/Vis spectroscopy as detectors, have been refined to quantify the amount of vitamin D, and its metabolites, in specific tissues, such a serum [5]. However, it would be more important to know a patient's total store of Vitamin D in all tissues, which is much more difficult to determine. Vitamin D can bind to albumin, vitamin D binding protein (DBP), the vitamin D receptor (VDR), accumulate in adipose tissue, and exist in a free form within the serum [6]. Extraction and detection methods still struggle to account for all those forms. In addition to measurement challenges, questions remain about what levels of vitamin D are sufficient [7]. Even with that in mind, there is a growing consensus that vitamin D deficiency is widespread throughout the world's population [7]. However, there is not consensus as to the most effective dosage and means of administering vitamin D to increase and maintain healthy vitamin D levels [7]. When the relationship between dose administration and physiological response can be clearly defined, vitamin D dosing can be standardized so that a vitamin D deficiency can be alleviated.

Vitamin D was initially discovered as a cure for the symptoms of rickets, leading to the conclusion that the disease was caused by a vitamin D deficiency [2]. Since its initial discovery, vitamin D has become primarily associated with regulating bone and mineral health [2]. Research on vitamin D expanded during the late 20th century when it was proposed that vitamin D acted more like a hormone than a vitamin. The discovery of the vitamin D receptor (VDR) in 1976 suggested a broader role for vitamin D in health [2]. The VDR is found in the nucleus of a variety of organs not associated with the skeletal system, such as the parathyroid gland, pancreas, colon, brain, kidney, lymphocytes, and skin [8]. Further studies of VDR showed that it could bind to DNA, pointing to its role as a transcriptional regulator [8].

Vitamin D is a prohormone that can be made from 7-dehydrocholesterol (the immediate precursor to cholesterol in

the cholesterol biosynthetic pathway) in the skin by irradiation with UVB photons (290-315 nm) and can be obtained from the diet or as a dietary supplement [9]. Few foods naturally contain vitamin D, exceptions being fatty fish, fish liver oil and egg yolk [1]. Other foods are fortified with vitamin D, such as milk in the United States [1]. "Vitamin D" is often used to refer to both forms of the vitamin: vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol). These two forms only differ in the structure of one of their side chains [1], where ergocalciferol has an extra methyl group and a double bond which cholecalciferol does not have (Figure 1). In most cases, vitamin D_2 and vitamin D_3 are equivalent in their biological effects, however some differences in potency have been reported that may be related to dosage amount and frequency [1,10,11]. Animal studies have suggested that vitamin D_2 may be less toxic than vitamin D_3, but no human studies have shown this [1]. At low doses, D_3 and D_3 are equally effective, however at high dosages, D_3 may be more effective than D_2 [1].

Both forms of vitamin D use the same metabolic pathways to produce the active form of vitamin D, 1,25-dihydroxyvitamin D, or 1,25-$(OH)_2$D. An overview of vitamin D biosynthesis can be seen in Figure 2. Biosynthesis of vitamin D_3 begins with irradiation of 7-dehydrocholesterol (7-DHC) by UVB light in the epidermis [9]. The light causes opening of the B ring of the steroid ring structure to form previtamin D_3. Thermal isomerization converts previtamin D_3 to vitamin D_3. The newly formed vitamin D is released from the plasma membrane into the extracellular space, where it binds to the vitamin D binding protein (DBP) and enters the circulating blood [12]. After entering the circulating blood, the DBP transports the vitamin either to the liver for further activation or to storage tissues [1].

In this form, vitamin D is an inactive pro-hormone. Vitamin D undergoes two hydroxylations to become the active 1,25α-dihydroxyvitamin D (calcitriol). The first hydroxylation takes place in the liver while the second occurs primarily in the kidney [13]. The first hydroxylation can be carried out by several different cytochrome P450 enzymes in the liver, including the product of the gene CYP4502R1 [1,13] to make 25- hydroxyvitamin D_3 (25-(OH) D_3, calcidiol). 25-hydroxyvitamin D has no known biological activity but is the major circulating form of vitamin D and the form of vitamin D most often used to assess biological levels of vitamin D [6,7]. The second hydroxylase enzyme that is produced by the gene CYP27B1 on 25-hydroxyvitamin D to make 1,25α-dihydroxyvitamin D (1,25$(OH)_2D_3$, calcitriol) [1,13]. 1,25α-dihydroxyvitamin D is the active vitamin D form that interacts with the VDR to initiate

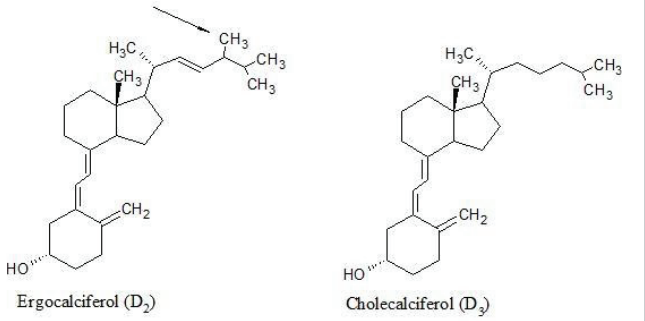

Figure 1: Structures of vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol) [6].

biological reactions, such as calcium and phosphorous absorption [1]. Once activated, 1,25α-dihydroxyvitamin D binds to the VDR on the nucleus of a various cells to induce transcriptional alterations [6]. The breakdown of 1,25α-dihydroxyvitamin D is initiated when 1,25α-dihydroxyvitamin D activates a 24- hydroxylase enzyme. The resulting metabolites are then excreted through the bile into the feces [1].

Vitamin D

Vitamin D is primarily known for its role in regulating serum calcium and phosphorous levels [1]. After 25-hydroxyvitamin D is formed in the liver, it is bound to DBP and travels in the blood until low calcium or phosphorous levels trigger its release into the kidney [1]. Low calcium levels activate the parathyroid gland to produce parathyroid hormone (PTH). PTH acts in the kidney to activate the 1-α-hydroxylase which forms the active hormone 1,25α-dihydroxyvitamin D (1,25-$(OH)_2D_3$). At the same time, PTH inactivates the 24-hydroxylase which breaks down the active hormone. These two effects lead to an increase in the concentration of the active form of vitamin D. The increased levels of 1,25-dihydroxyvitamin D increase serum calcium levels in three ways. First, 1,25$(OH)_2D_3$ initiates active calcium transport in the small intestine. Second, in conjunction with PTH, 1,25$(OH)_2D_3$ activates osteoblasts to mobilize calcium from bone to the plasma. Third, 1,25$(OH)_2D_3$, with PTH, activates reabsorption of filtered calcium in the distal renal tubule [14].

With the discovery of VDR throughout the body in the early 1980's, researchers began to examine roles of vitamin D beyond its traditional roles in serum calcium and phosphorous levels [15]. Since that time, vitamin D has been shown to have important roles in many other organ systems, especially the immune system and in controlling gene expression in various tissues. VDR is a ligand-dependent transcription factor that, when bound to vitamin D, controls expression of approximately 250 genes. VDR is known to upregulate genes for extracellular bone matrix formation, such as osteocalcin and osteopontin. VDR also upregulates genes for cell adhesion (β_3 integrin), tumor suppression (p21) and cell differentiation (Involucrin), among others. At the same time, VDR down- regulates genes for inflammation, including IL-2, TNF-α, and interferon-γ, and genes for cell proliferation, including EGF-R and c-myc. The collection of genes controlled by VDR/vitamin D explain how vitamin D can have anti-inflammatory and immunomodulatory effects and have effects on cell proliferation [4,15]. In this context, vitamin D is associated with autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, type I diabetes, inflammatory bowel disease, and others. Vitamin D is also associated with psoriasis, cardiovascular disease, and cancers such as prostate, breast and colon cancer.

Vitamin D Detection

Vitamin D levels in the body are typically evaluated by measuring the levels of 25- hydroxyvitamin D_3 in serum, either by chromatographic methods (HPLC or GC) or by antibody binding assays [1]. Each of these methods for detecting vitamin D has biases, advantages and disadvantages [1,16,17]. Since the 2000's, the method of choice for measuring vitamin D is HPLC with detection by mass spectrometry (MS). However, this method is expensive and may not always be available. Recent work has shown that HPLC with detection

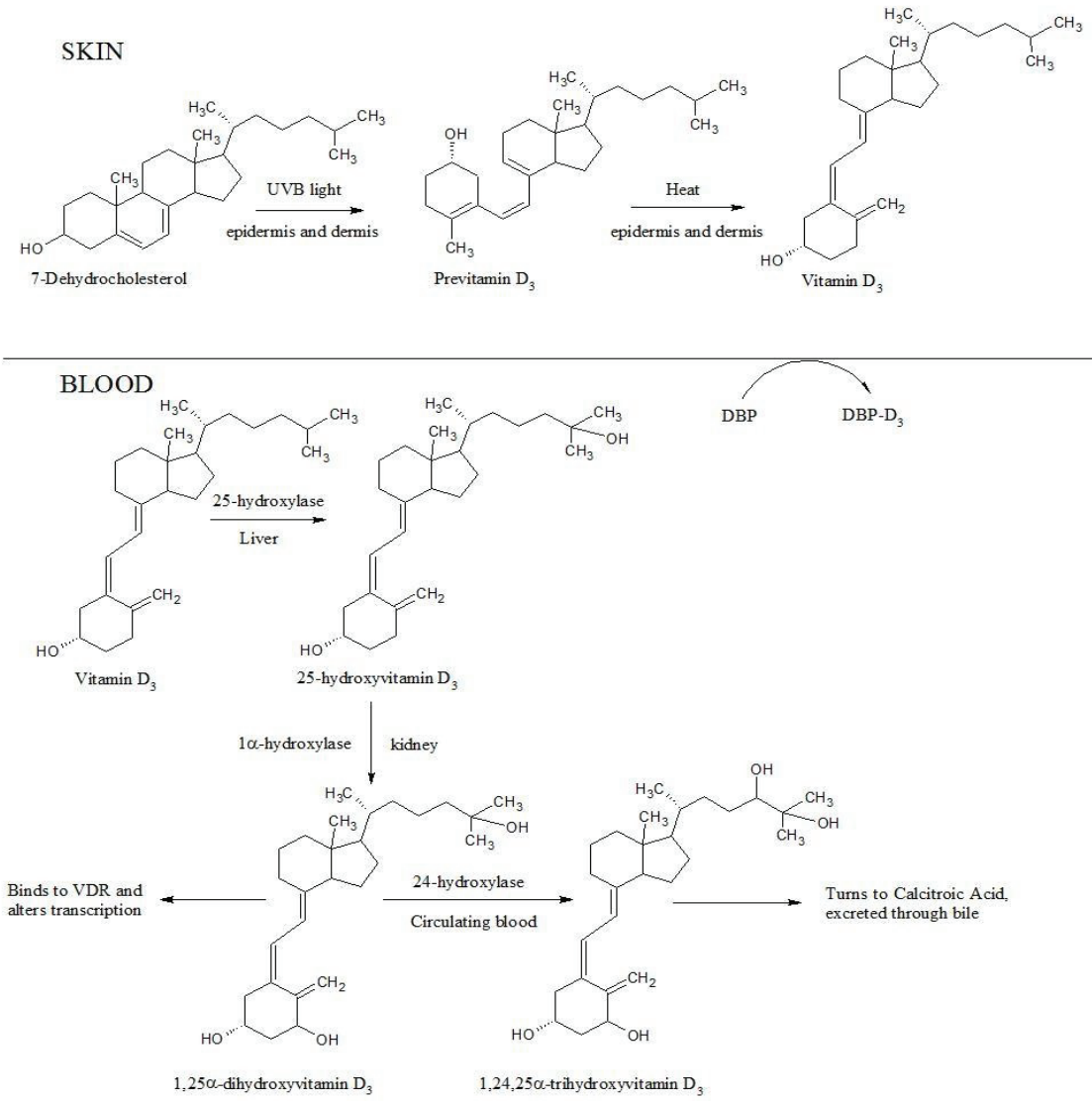

Figure 2: Biosynthesis of vitamin D3 [6].

by UV absorbance can also be used to determine vitamin D levels and that this method is superior to antibody binding assays (ELISA) [18]. When reading reports in the literature, it is important to identify which method was used to determine vitamin D levels in a particular study and to be aware of the possible errors associated with that method of analysis [1].

Vitamin D Deficiency

Although there is some discrepancy as to what an optimal level of vitamin D is, most studies indicate a serum level of 25-hydroxyvitamin D greater than 30 ng/mL indicates sufficient vitamin D levels [7,19,20]. A serum level of 25-hydroxyvitamin D below 30 ng/mL is considered insufficient, while levels of 25-hydroxyvitamin D below 20 ng/mL is considered vitamin D deficiency [21]. Severe deficiency is a level of 25- hydroxyvitamin D below 10 ng/mL. Vitamin D deficiency is prevalent throughout the world [22]. In the United States, vitamin D deficiency has become a common diagnosis. From 2001-2004, only 23% of US adults and adolescents had what is considered optimal levels of 25-hydroxyvitamin D (>30 ng/mL) [23].

Misdiagnosis of Vitamin D deficiency

In a physiological context, vitamin D deficiency results in decreased serum calcium and phosphate levels, which subsequently leads to secondary hyperparathyroidism, leading to bone mineral degradation [9,24]. Symptoms of vitamin D deficiency may be generic and overlap with symptoms of other medical conditions. When a patient presents with nonspecific symptoms like muscle weakness and pain, many times it is initially considered to be fibromyalgia [25,26]. It is important that clinicians also look for the indicators of vitamin D deficiency. As previously stated, the best indicator of vitamin D levels is to measure serum levels of 25-hydroxyvitamin D [1]. When vitamin D deficiency causes symptoms of hypocalcemia, such as

muscle pain and weakness, clinicians often miss the underlying vitamin D deficiency and incorrectly replenish calcium stores by administering calcium supplements. Misdiagnosis of vitamin D deficiency has become more prevalent in the hospital setting. In a 1998 study of a Massachusetts hospital, 57% of the 290 patients had vitamin D deficiency [27]. When patients have unrecognized vitamin D deficiencies that are not corrected by supplementation, they are prone to extended hospital stays because of decreased bone mineralization. These conditions can be seriously disadvantageous for elderly patients that have frail skeletal systems.

One proposal to reduce the large vitamin D deficit in the population is to identify those who are most prone to deficiency. Risk factors for vitamin D deficiency are outlined below, but include people that are: not taking vitamin supplements, older, darker skinned, obese, not well exposed to the sun, taking drugs with anti-hormonal effects and osteoporotic [28]. Many cases of vitamin D deficiency will come from the elderly population since increased age allows for less 7-DHC to be synthesized in the skin [29], as well as issues with osteoporosis and increased non-exposure to the sun. Elderly females going through menopause should be considered as well because of their mineral dysregulation. Holick MF [30] reported that more than 50% of postmenopausal women taking medicine for osteoporosis have vitamin D levels below 30 ng/mL.

Risk factors of vitamin D deficiency

Pigmentation: High levels of melanin in individuals with dark skin act to block sunlight from producing vitamin D. These individuals require longer exposure to sunlight to obtain the same amount of vitamin D as individuals with lighter skin [1,31]. In 2009, it was found in the United States that 97% of non-Hispanic blacks and 90% of Mexican Americans had vitamin D insufficiency [23]. In 2012, a US national survey concluded that 14% of the total adolescent population was vitamin D deficient, and 50% of the deficient population were non-Hispanic black adolescents [32]. In a sickle cell disease study, Buison et al. [33] recorded for their control population that the healthy black subjects had 65% lower vitamin D levels (7.2 ng/mL) than the levels of the healthy white subjects (14.8 ng/mL). Similar correlations were seen in a placebo controlled study by Rajakumar et al. [34]. Vitamin D_3 supplementation (1000 IU/d) was given to a study population composed of 84 black children and 73 white children and they found that mean levels of 25-hydroxyvitamin D were lower for the black children than the white children.

Seasonality: One of the primary causes of vitamin D deficiency is lack of sun exposure since the major source of vitamin D comes from exposure to UV light [12,23]. In a 2009 study, seasonal 25-hydroxyvitamin D levels were measured in adults living in Estonia and Northern Europe where their diet lacks vitamin D [35]. During the winter, 73% of the study population had levels below 20 ng/mL, while during the summer only 29% of the population had levels below 20 ng/mL.

Latitude: Several studies have linked latitude with Vitamin D deficiency citing that the intensity of the UVB light needed to synthesize vitamin D in the skin is much less at higher latitudes, however numerous other factors confound this connection [21]. These additional factors include environmental factors such as height of the atmosphere, duration of sunlight in the summer and winter months, cloud cover, ozone cover, and clothing [1].

Anticonvulsant medications: Patients taking anticonvulsant medications, such as phenobarbital, phenytoin, carbamazepine, primidone, valproic acid, ethosuximide, and Dilatitin® are at increased risk for vitamin D deficiency because these drugs can alter bone mineral metabolism. Anticonvulsants have been shown to increase production and catabolism of 1,25α-dihydroxyvitamin D in animal studies [31,36]. This inhibits absorption of calcium from the small intestines and leads to decreased serum calcium levels which increases the risk of osteomalacia [36]. Proposed mechanisms for the effects of antiepileptic drugs (AED) on bone metabolism include the activation of 1α-hydroxylase, which increases 1,25α-dihydroxyvitamin D levels and decreases calcium absorption [37]. There is supporting evidence that anticonvulsants can alter vitamin D metabolism by symptomatically increasing bone frailty. In an anticonvulsant study conducted by Mikati et al. [37] patients that took AEDs had low bone density compared to the control group. They found that high doses of vitamin D significantly increased bone mineral density (BMD) in certain areas of the skeletal system, but the doses of vitamin D did not consistently maintain BMD for the year the study was conducted. The decreased duration of vitamin D supplementation questions if vitamin D dosing is adequate. While the study proposed vitamin D supplementation as a possible solution, no standardized dose has been established that can consistently alleviate the symptoms of AEDs.

Corticosteroids: Like anticonvulsants, corticosteroid treatments can cause decreased serum calcium levels by suppressing calcium absorption within the intestine [38]. The mechanism of action is proposed to be similar to the mechanism of action of anticonvulsants, is due to an abnormal activation of the 1α-hydroxylase enzyme. This observation is supported by research from Bec et al. [39], where protein binding assays revealed that patients on corticosteroids had low levels of 25-hydroxyvitamin D [39]. In addition, it has been reported that corticosteroids, such as glucocorticoids, can inhibit calcium absorption in the intestine by 30% when suppressing the VDR found on the intestinal wall [40]. Although the physiological effects of corticosteroid therapy are the same as anticonvulsants, the mechanism for which it plays a role in vitamin D regulation is not clear. Some literature supports that gender and sex hormones may also play a role in the metabolism of vitamin D the same as corticosteroid.

Genetic predispositions: Genetic factors that predispose an individual to vitamin D deficiency are genetic mutations that will alter genes that are responsible for calcium and phosphate maintenance within the body as well as mineralization of bone [41]. Mutation of 1α-hydroxylase in the kidney prevents the production of the active hormonal form of vitamin D while a mutation of the VDR does not allow for vitamin D to regulate transcriptional processes [42-45].

Vitamin D Dosage Requirements

Once individuals have been identified as Vitamin D deficient or at high risk for deficiency, supplementation with Vitamin D is indicated to correct the deficiency. Vitamin D dosing will depend upon the current stores of 25-hydroxyvitamin D within the body. Clinicians prescribing vitamin D to their patients measure the current amount in the serum to estimate a dosage that will increase their vitamin D levels to a desired level. Current questions posed by researchers and clinicians concern how to define the normal range of vitamin D within the body, as well as how to find a standard dose of vitamin D that can

maintain this level. The normal range of vitamin D has been defined in the literature to be 32-100 ng/mL of 25-hydroxyvitamin D with the minimal level of sufficiency ranging between 30-32 ng/mL [21]. This range of vitamin D concentrations has been chosen based on its effect on calcium absorption, risk of rickets and risk of fractures.

The FDA and RDA both currently recommend 400-600 IU of D_3 per day. This dosage assumes that diet, latitude, seasonality, body habitus and physiology are all equal [46]. In a study of 13,987 patients about vitamin D supplementation Veugelers et al. [46] found that 2900 IU of vitamin D was necessary to achieve sufficiency of vitamin D in 97% of healthy adults, while 7200 IU was necessary daily for obese patients.

It has been estimated that 100 IU of vitamin D is necessary to increase the 25- hydroxyvitamin D level by 1 ng/mL; thus, to maintain 30 ng/mL, 3000 IU is needed daily [11,47]. This dosage can be obtained from an accumulation of sunlight exposure, diet, and supplementation [28]. In 2011, the IOM updated the dietary references of vitamin D and stated: 400 IU for 0-1 year, 600 IU for 1-70 years, and 800 IU for 70+ years [1]. Currently in the US, vitamin D doses of 1,000-5,000 IU can be found in multivitamin and single vitamin supplements that are commercially available [48].

Vitamin D dosing in children is also controversial, again the current RDA is 400 IU/day. RajaKumar et al. [34] supplemented 157 children (8-14 year) in a double-blind placebo study with 1000 IU/day of D_3. All participants had less than 20 ng/ml 25-$(OH)D_3$ at onset of the study. After 2 months, 65% of the supplemented group had levels between 20- 30 ng/ml, compared to 14% in the control group [49]. No PTH or electrolyte problems were recorded in any patient. At 6 months follow up, 47% had levels between 20-30 ng/ml and 14% were above 30 ng/ml. Mortensen et al. [50] did a similar study with 4-8 year olds and concluded that 800 IU/day of D_3 was needed per day to maintain a level of 20 ng/ml in 100% of patients. Smith et al. [51] treated 14-18 year olds in a double-blind study and concluded 1200 IU/day of D_3 was necessary to maintain 25-$(OH)D_3$ at 20 ng/ml. In none of the studies above did anyone experience any toxicity. It is necessary to keep in mind when looking at the two studies above that the Pediatric Endocrine Society feels that 30 ng/dl is the minimum level to be considered normal.

There is not a consensus among physicians as to what doses of vitamin D will lead to sufficient levels of 25-$(OH)D_3$. What is clear is that toxicity is rare. In a 15-year review from the National Poison Data System, 25,397 persons reported overdosing on D_2 or D_3, with no significant mortality or morbidity found in the reported group [52]. In some cultures, D_2 or D_3 will be used as an anti-inflammatory drug. Doses ranging from 3.6 million to 210 million IU are reported over a 1-4 month time span. At these very-high dosages, some patients have adverse effects such as weakness, hearing loss, polyuria, nausea and vomiting. In some cases, patients may present with hypertension, renal failure, renal stones or altered consciousness. To prevent too much vitamin D intoxication, reports conclude that serum 25-hydroxyvitamin D levels should not exceed 150 ng/mL [21]. Another question that arises is the recommended testing frequency for vitamin D levels. It is suggested to wait about 3 months after dosing to read a patient's vitamin D levels [28].

Rickets was first described in the medical literature in the 1600's. The treatment of rickets with cod liver oil first recorded in the 1700's. The discovery of vitamin D and a light activated mechanism was made during the 1920s when testing conducted with vitamin A found that irradiating rat liver with UV light promoted growth and bone calcification just like vitamin D [53]. In the late 1960's the photoreaction of the skin and a secosteroid were connected. Until the 1980's this was predominantly the extent of our knowledge of vitamin D. The 1980's highlighted vitamin D in the immune system, chronic disease, and variety of other medical conditions. When researchers found vitamin D binding receptor (VDR), it was determined that vitamin D had effects additional to calcium regulation and skeletal health. Since then, the VDR has been found on a variety of organs and cells, such as: the parathyroid gland, pancreas, colon, brain, intestines, kidney, lymphocytes, T cells, and keratinocytes [54-56]. As methods of measurement of vitamin D became simplified, it became evident that more people were prove to vitamin D deficieny. More and more disease states became associated with low vitamin D levels.

Since the discovery that vitamin D plays a role in non-skeletal diseases, there has been an abundance of research to establish the exact role of vitamin D within these diseases. Because many genetic and environmental confounding variables impact research, randomized controlled trials (RTCs), high quality systematic reviews, and meta-analyses are the most effective study methods to determine vitamin D influence on clinical outcomes. In addition to these human studies, animal models have allowed researchers to understand how vitamin D plays specific roles within the physiological mechanisms of certain diseases.

The state of deficiency is then questioned as cause or effect. To date we remain at this state of knowledge, but new advances point to vitamin D being an anti-inflammatory agent. The anti-inflammatory function maybe acts as prevention rather than treatment of many deficiency-associated illnesses. Rickets stands alone as a condition directly cured by vitamin D treatment.

Bones and muscle

Normal bone formation and reformation is regulated by vitamin D, PTH, and calcitonin [57]. In addition, vitamin D regulates calcium uptake within the muscle and the calcium absorption ultimately produces contraction and relaxation movements. 1,25-$(OH)_2D$ stimulates bone formation by first activating osteoblasts with a cAMP mechanism, which in turn produces osteoclastic differentiation inducing factors to promote bone growth [57]. As with the case in rickets, the resulting hypocalcaemia from low vitamin D levels induces secondary hyperthyroidism to maintain blood serum calcium levels. It has been suggested that vitamin D supplementation for skeletal diseases, such as osteoporosis, can decrease risk of falls and effectively prevent fractures by increasing bone density [58]. As a person ages, there is less calcium uptake into the bone, which allows their skeletal system to weaken and their risk of osteoporosis and falling increases. Meta- analyses have shown increased vitamin D dosage negatively correlated with amount of reported fractures. Using doses ranging from 482-770 IU, non-vertebral fractures were reduced by 20% and hip fractures were reduced by 18%. They noted that calcium supplementation alone did not decrease risk of fractures, supporting the notion that vitamin D plays a direct role in bone density maintenance. In another study from France, 3270 elderly women taking 1200 mg of supplemental calcium and 800 IU/d of cholecalciferol reduced their occurrences of hip fracture by 43% and non-vertebral fracture by 32% [59]. In this case, both

vitamin D and calcium were supplemented. Other reports show that supplementing both vitamin D and calcium produce significant anti-fracture outcomes. Systematic reviews and meta-analyses report that both vitamin D and calcium supplementation reduced risk by approximately 10-15% for hip fracture or total non- vertebral fractures [60]. These studies found that those significant reductions came from doses greater than 400 IU/d. Due to lack of statistical power of these studies, it has been hard to determine what confounding variables to consider part of the disease pathology. Generally, the evidence of vitamin D supplementation for anti-fracture benefits is greatly supported by data from the elderly population.

Autoimmune diseases

The role of vitamin D within the immune system was discovered when the presence of sarcoidosis indirectly caused an increase in cholecalciferol due to the increase of 1α-hydroxylase activity. VDR can be found on activated T lymphocytes, monocytes, macrophages, and dendritic cells [61]. From the discovery of different VDR locations, it was suggested that vitamin D could play a variety of roles in both innate and adaptive immune systems by having either an immunosuppressive or immunoregulatory effect.

The central role of vitamin D within the innate immune response includes the VDR. If VDR becomes dysfunctional, chronic infections can develop alongside an increase in autoimmune disease markers. These infections are prevented when vitamin D binds to VDR in monocytes and transcriptionally activates production of antimicrobial peptides, such as cathelicidin (CAMP) [62,63]. These antimicrobial peptides mediate the responses of vitamin D to eliminate pathogens within the body [62,64,65]. One of the main responses is prevention of antigen presentation. Vitamin D alters the antigen recognition binding site or decreases antigen binding affinity by disrupting the phospholipids on the cell wall [61]. Vitamin D also rids bacteria from the body by activating autophagy [66]. In addition, vitamin D plays a role in differentiation and function of dendritic cells [63].

In adaptive immunity, vitamin D acts as both immunoregulator and immunosuppressor of specific responses between B and T lymphocytes and macrophages. The dysfunction of adaptive immunity causes the onset of autoimmune diseases. Vitamin D targets $CD4^+$ cells, which suppresses both T cell proliferation and inflammatory inducing factors [67,68]. T cell suppression is important, because T cells drive autoimmune diseases such as multiple sclerosis, diabetes mellitus type I, and rheumatoid arthritis [69]. When Vitamin D 1,25 targets $CD4^+$ cells, it regulates Th1 cells so that interleukin (IL)-2 cannot be produced and T lymphocyte recruitment is suppressed. In addition, vitamin D activates proliferation of the immunosuppressive regulatory T cells that accumulate in sites of inflammation. These pathophysiological effects of vitamin D are theorized to prevent the onset of autoimmune diseases.

Multiple sclerosis: The basis of research about vitamin D and multiple sclerosis (MS) has come from mice studies. Researchers alter the mice's neurological pathways to induce a form of MS called Experimental Autoimmune Encephalomyelitis (EAE). These MS studies show vitamin D as a regulator of the immune system as well as a protector of the nervous system [70]. Regarding the immunological function in MS prevention, it is shown that vitamin D suppresses production of IL-12 which ultimately prevents T cell differentiation [67,69,70]. In the neurological aspect of MS prevention, Vitamin D targets myelin producing cells in the CNS [71]. This prevents inflammation as well as influences neurotrophic factors involved with enhancing synaptic transmission and calcium cell signalling. The benefits of vitamin D supplementation for MS patients are not exactly clear. T cells had to migrate to specific tissues to have an autoimmune response, thus targeting Th cells may be an attractive method to treat MS [72]. These results are supported by another report where vitamin D supplementation prevented EAE in mice by reversibly modulating Th cell localization in CNS. Research is still developing as to how vitamin D supplementation can be used to prevent MS.

The relationship between vitamin D and MS also has environmental components. It is generally observed that MS is more prevalent in higher latitudes than equatorial latitudes [69]. The primary environmental agent that many researchers hypothesize contribute to MS is the lack of sunlight that prevents the synthesis of vitamin D in the skin, Systematic reviews and meta-analyses reveal that there are no apparent clinical benefits to MS patients when using vitamin D supplements and should not be considered as a primary form of treatment [73]. Vitamin D should be considered as prevention of MS rather than treatment.

Insulin-Dependent diabetes mellitus: Type 1 Diabetes, is an autoimmune disorder where the immune system attacks pancreatic β cells that produce insulin for the body [74]. Vitamin D is possibly part of the disease pathology because there are VDR found on pancreatic β cells and because *in vitro* vitamin D can suppress immune-inflammatory agents that can kill the β cells [74,75]. There have been cases of IDDM that show low serum levels for both 25-hydroxyvitamin D and 1,25α-dihydroxy vitamin D as compared to healthy individuals [76,77]. In addition, these cases showed low PTH levels with sequential hypercalcemia that lead to alteration of bone mineral homeostasis; however, the physiological mechanism for the bone mineral loss remains unclear. Some reports have said that IDDM is associated with polymorphisms of CYP27B1, but this association has not been verified [76]. Although, previous mice studies contain supporting evidence of an association with diabetes and the CYP27B1 gene.

There is significant evidence of correlation between vitamin D and IDDM in epidemiological studies. Some research suggests that vitamin D supplementation given earlier in life is positively associated with decreased risk of developing IDDM [76,78]. A study from Finland, a country with one of the highest incidences of IDDM in the world, created a birth cohort in the 1960s of 10,000 children and gave them 2000 IU/d of vitamin D during the first year of life [74]. After 31 years, they re-evaluated the experimental group and found that they had decreased their chances of developing IDDM by 80% [74].

Rheumatoid arthritis: Rheumatoid arthritis is defined as chronic inflammation of the skeletal joints. Like IDDM, vitamin D is thought to be an immunoregulatory agent in RA. The observation of VDR in cells associated with rheumatoid inflammation, such as macrophages and synovial fibroblasts suggests that VDR may regulate inflammatory activity in the affected tissues [1]. The evidence from randomized controlled studies does not support the biological plausibility of a vitamin D function in the pathophysiology of RA. A woman's health initiative trial supplemented 400 IU of vitamin D for female patients with RA for 5.1 years and found no reduction of RA incidence [60]. With contradicting biological and statistical data, there is no reliable

evidence to make conclusions that there is a relationship between vitamin D supplementation and treatment of arthritis. Studies such as this again point to preventative rather than a curative effect of vitamin D in RA.

Other non-skeletal diseases

Sickle cell disease: Patients with Sickle Cell Disease (SCD) are at high risk for vitamin D deficiency, because of multiple factors that may include darker skin pigmentation, decreased physicality, and poor nutrition [33]. It is thought that vitamin D supplementation could alleviate symptoms of SCD because its proposed antimicrobial properties may prevent vaso-occlusive events commonly found in SCD [79]. A SCD study from Lee et al. [79] found that vitamin D deficiency significantly associated with pain (p=0.0121). The study concluded that the possible pain regulation by vitamin D supplementation could occur by suppressing inflammatory cytokines in the immune system. Notably, this study also found that African American individuals within their study population had higher amounts of PTH and lower amounts of DBP, which makes them more vitamin D deficient than those SCD patients of lighter skin tone. Vitamin D supplementation may result in better pain control when systemic levels are corrected.

Psychiatric disorders: Vitamin D is a neurosteroid that can cross the blood brain barrier There is evidence that vitamin D is also synthesized in the fetal and adult brain, suggesting that vitamin D is involved in either brain development or neurological processes [54]. The impact of vitamin D on psychiatric disorders is unclear. Current research supports that vitamin D deficiency is a risk factor in the development of psychiatric diseases [54]. Other research suggest that vitamin D sufficiency may be neuro protective for some species of animals.

Schizophrenia: One of the causes of schizophrenia is abnormal prenatal brain development. Animal studies show that vitamin D deficiency during brain development can be a risk factor for psychiatric disorders. Animal studies have shown that fetal rats with levels of 25- hydroxyvitamin D that were >90% lower than those of the controls developed abnormal behaviours. In addition, the fetal rats had longer cortexes and enlarged ventricles, which are the same observations in human brains with schizophrenia [80].

Epidemiological studies with schizophrenia showed a consistent relationship with the seasonal birth effect, where those born in the winter/spring are more likely to develop schizophrenia than those born in the summer/autumn [81,82]. The most reasonable explanation is that the summer/autumn months have longer days with more sunlight, which allows for more biosynthesis of vitamin D. Humble provides a case study of a 26 year old female from the Middle East that immigrated to Sweden [83]. Before this immigration, she had suffered only 1-2 brief psychotic episodes. When she arrived in Sweden, she developed a severe psychosis with voice hallucinations. She was admitted to the hospital and they found her 25-hydroxyvitamin D levels to be 5 ng/mL and her PTH levels were high. After treatment with 1600 IU of vitamin D_3 and 1000 mg of calcium for three months, her psychiatric state dramatically improved. After her treatment, her 25-hydroxyvitamin D levels had increased to 29.7 ng/mL and her PTH was normalized. The patient had lived with mild psychosis in a country with intense sunlight and went into a severe psychotic state when moving to Sweden, where the climate has less sun exposure. Although there is no case for causality, and the effects of the calcium supplementation were not evaluated in the case study, it has opened the door for further research in areas involving schizophrenia and seasonality.

Depression: The biological mechanism of action for vitamin D for depression is unknown. Some literature suggests a possible relationship between low vitamin D and catecholamine dysfunction, yet there has been no further research on this relationship [83]. Another well supported mechanism proposes vitamin D within the CNS and decreasing levels of pro- inflammatory cytokines [80]. Pro-inflammatory cytokines, which can be released by stress, alter the same neurotransmitters and neuropeptides that are compromised in major depressive disorder [80]. 1,25α- dihydroxyvitamin D_2 or D_3 regulates the Th1/Th2 pathway by suppressing cytokine production and inducing anti-inflammatory cytokines. 1,25α- dihydroxyvitamin D_3 is synthesized by microglial cells , which are primary mediators of pro-inflammatory immune responses in the brain [84-86]. While there is an adequate amount of biological evidence to suggest causation, it is difficult to conclude a direct relationship [83].

Observational studies, systematic reviews, and meta-analyses are not sufficiently robust to use as causation for depression. They pose a high risk of bias, insufficient randomization of subject population, and large losses of patient follow-ups. Most studies on depression that have found supporting evidence are in relation to seasonal affective disorder. While epidemiological studies have shown positive correlations, not enough evidence is provided to conclude a direct relationship between SAD and vitamin D, nor can a direct conclusion be made that vitamin D can significantly improve mental health [1,60].

Reproductive health: Inadequate vitamin D levels have been linked to male and female infertility. In males, vitamin D levels correlate with total testosterone levels, sperm motility and semen quality [43,44]. Infertility is one of the rare conditions where higher levels are associated with adverse effects and levels should be maintained between 20 and 50 ng/dl. Vitamin D levels in females are reported to correlate very strongly with functional ovarian follicles and percentage of pregnancies [87].

The clinical observation of pregnant women with preeclampsia is that they have low urinary calcium excretion, as compared to elevated in healthy pregnant women [1]. The mechanism of how vitamin D is involved with preeclampsia is relatively unknown and remains controversial. One large study involving nearly 3000 nulliparous women taking 400-800 IU of D_3 daily in addition to a healthy diet, resulted in a 27% decrease in pre- eclampsia [88]. In the US population, there is also correlation between Vitamin D levels above 20 ng/L and decreased occurrence of pre-eclampsia. This finding has not been documented in populations in the US [89]. While there is evidence of association of increased vitamin D supplementation and decreased risk of preeclampsia, the role of vitamin D within this condition has yet to be further explored.

Cancer: Vitamin D is thought to have a role in cancer prevention, given the fact that vitamin D deficiency and insufficiency prevalence within the cancer population is doubled that of the general population [90,91]. The biological basis of vitamin D in context of cancer comes from the regulated activity of 1α-hydroxylase to fight infection and mediate anti- inflammatory reactions. 1,25α-dihydroxyvitamin D_3 has been shown to suppress growth of cancer cells and promote cell

differentiation by regulation of multiple pathways within the body. Vitamin D deficiency and insufficiency are closely associated with breast, bowel and prostate cancers. Increased survival in prostate cancer has been documented after correcting vitamin D levels after diagnosis. Decreased survival and low vitamin D level is also associated with hematological malignancies [92]. Following bone marrow transplantation, survival has increased 12% by raising vitamin D levels to a therapeutic level (unpublished data G Wallace). There remains poor understanding of the physiological role of vitamin D in cancer treatment. Because of the role of vitamin D as an anti- inflammatory agent, the question remains with cause or effect. Knowing the benign mature of oral vitamin D, more attention should be paid to vitamin D supplementation.

Discussion

Medical research involving vitamin D and human diseases is extensive, but the relationships between low vitamin D status and symptoms of these diseases are still being evaluated. When looking at clinical evidence to analyse the relationship between vitamin D levels and these linked diseases, it is important to note that association is not an indicator of causation, and the only evidence that can be used comes from small, usually non- randomized trials. Cause or effect remains a key question to be answered in vitamin D research.

It is the opinion of these authors that the recommended vitamin D dosing in the U.S. is too low at the current standard. Vitamin D levels should be obtained for any individual at risk for low vitamin D levels and anyone exhibiting symptoms described above. These levels should be monitored regularly and supplementation modified to obtain optimal levels. *In lieu* of obtaining levels, vitamin D should be prescribed at moderate doses. Dosing of vitamin D must be tailored to individual patient needs and the medical conditions that each patient is facing at the current time [93]. Diet, season, skin color, medication and latitude all must be included in the prescribing of vitamin D. Toxicity from vitamin D remains a remote possibility. The literature supports that vitamin D is a very safe supplement. With over 25,000 accidental acute ingestions reported, there was only one fatality in a patient with chronic over ingestion. The side effects, although seldom seen in the US, are easy to monitor with 25-hydroxyvitamin D levels and serum calcium levels. The authors also feel it is essential to prescribe vitamin D with the implied intent of the current literature. Vitamin D adequacy is more about prevention of disease rather than cure of symptoms. We recommend maintaining the 25-hydroxyvitamin D_2 or D_3 levels in the mid therapeutic range (30-100 ng/mL) at all stages of life. Adult daily intake of vitamin D in the 3000 to 5000 IU per day is likely to be helpful in maintaining sufficient levels of vitamin D and unlikely to be harmful. Pediatric daily intake of vitamin D in the range of 1000-2000 IU per day is likely to be helpful in maintaining levels in most children. Levels of 25-hydroxyvitamin D can be checked once or twice annually in most patients to adjust dosing. Calcium levels can be monitored for evidence of hypervitaminosis D as well as physical signs.

Vitamin D is an essential nutrient and has extraordinary potential in health maintenance. Large, long term, multi-instructional studies are needed to expose all the benefits of vitamin D and it health benefits.

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