Review Article

One Hundred Years after Vitamin D Discovery: Is There Clinical Evidence for Supplementation Doses?

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Abstract

In the last decade, an increasing awareness was directed to the role of Vitamin D in nonskeletal and preventive roles for chronic diseases in different fields. Vitamin D deficiency was reported in many countries worldwide and is considered as a pandemic. However, no consensus exists about whether and how supplementation of Vitamin D may be beneficial as a preventive or adjuvant therapy. Thereby, this review aimed to deliver an overview about the administrated doses of Vitamin D in randomized controlled clinical studies, in order to evaluate the currently available clinical evidence. In addition, focus was placed on the recent advances on Vitamin D nonskeletal actions. The results sometimes showed a great discrepancy between the recommended Vitamin D dose by different guideline authorities, which are from 400 to 4000 IU/day, and the used doses in recent randomized controlled clinical studies, which were up to 100,000 IU/day. Different studies showed the positive effect of Vitamin D in supporting the immune system and preventing different chronic and infectious diseases. These findings reflect the need to rethink existing reference ranges and intake recommendations. Based on the analyzed range of clinically applied doses, we recommend a Vitamin D supplementation based on three different ranges, which include <40 ng/ml, >40 <80 ng/ml, and >80 ng/ml with oral Vitamin D intake of 10,000 IU/day, 5000 IU/day, and 1000 IU/day, respectively. A 25-hydroxyvitamin D blood serum monitoring is furthermore recommenced every 3 months to re-adjust the Vitamin D dose based on the above-mentioned concept. Ongoing clinical studies will have to further prove this concept for different patient groups.

Keywords: Chronic disease, COVID-19, immune system, infectious disease, influenza, supplementation, supplements, Vitamin D, vitamins

Introduction

A healthy immune system is the basis of general health and body defense for many diseases. Lifestyle, individual behaviors, and living environment directly influence our health. In particular, good nutrition is key to maintain general health.[1] Imbalance and malnutrition can compromise the immune system and increase the risk of preventable chronic diseases.[1] In last decades, the prevalence of chronic diseases such as diabetes mellitus, obesity, cardiovascular diseases, and hypertension increased significantly, especially in industrial countries. One of the most important reasons for this change is the rapid change of the society's lifestyle.[2]

The role of Vitamin D in health and diseases gradually gained increasing interest in different fields. Shortly after Vitamin D was discovered in 1922, the Nobel Prize in Chemistry was awarded to Adolf Windhaus in 1928 for his studies on sterols and their connection with vitamins,[3] which increased the research interest in this field.

Naturally, the endogenous synthesis of Vitamin D takes place in the skin and is limited to the time period of sun exposure. The ultraviolet B waves transform 7-dehydrocholesterol

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to cholecalciferol (Vitamin D3). To reach its active form, Vitamin D undergoes further transformations in the liver (to calcidiol 25-hydroxyvitamin D3 [25-OHD3]), which is the most sufficient metabolite of Vitamin D. The next transformation step takes place in the kidney, where calcidiol (25-OHD3) is transformed to the active form of Vitamin D, calcitriol (1,25-dihydroxycholecalciferol). Calcitriol serves as a transcription factor of genes for target proteins and thereby belongs to a wide range of hormones. Therefore, Vitamin D is considered as a prehormone rather than a vitamin[4,5] [Figure 1].

Soon after its discovery, the relation between Vitamin D and parathyroid hormone, as well as its role in the regulation of the mineral and bone hemostasis, was recognized.[6-8] In this process, the active form of Vitamin D, i.e., calcitriol, targets different mechanisms to maintain the calcium level. In addition, it activates bone formation and regeneration by supporting cell differentiation and increasing calcium and phosphate serum concentration.[9] One of the early well-understood diseases that result following Vitamin D deficiency is rickets in children (and osteomalacia in adults), which is characterized by severe mineralization disorder.[10] Thereby, many studies focused mainly on the role of Vitamin D in bone health. Moreover, it gained expanding importance for the treatment of patients suffering from osteoporosis. Consequently, the history of its discovery made Vitamin D most popular for skeletal health.

However, in the last decade, numerous studies showed the crucial role of Vitamin D for general health and its wide range of function throughout the body.[9] Various studies reported on the influence of Vitamin D to prevent chronic diseases and to reduce the prevalence of cardiovascular diseases and metabolic diseases such as diabetes mellitus.[11] In addition, many studies analyzed its impact on the immune system and showed its capacity to reduce inflammation and support regeneration in different preclinical and clinical studies.[12] In this context, Vitamin D also showed a beneficial impact on the prevention of infectious bacterial and viral diseases such as influenza[13] and acute respiratory tract infection.[14] In addition to the classical role of Vitamin D for bone health, its immunological features further highlight the importance of its adequate intake to maintain the required need of the body.

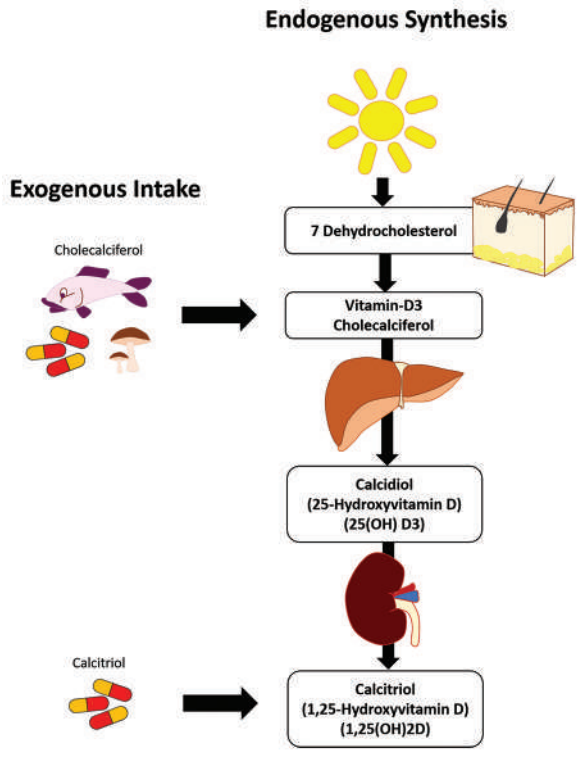

Figure 1: Schematic illustration of the endogenous vitamin synthesis and the exogenous Vitamin D activation

Unfortunately, the endogenous synthesis of Vitamin D is limited by sun exposure, which is not sufficiently available throughout the year in many countries. Moreover, the exogenous intake by food is limited and rather unknown for most of the world population. These factors resulted in a high prevalence of Vitamin D deficiency in many different countries in the past years.[15] To date, the Vitamin D deficiency pandemic was frequently reported but was not well recognized in many countries.[16] The first recommendation to administrate Vitamin D supplementation started around 1940.[17] Eighty years later, there is still no consistent consensus about the Vitamin D supplementation and intake.[17] One of the reasons is the historic development and understanding of its role. Thereby, supplementation recommendation focused mainly on the necessary intake to maintain bone health. Although increasing evidence of the multiple functions of Vitamin D to prevent many diseases was reported, these guidelines were barely changed. An additional factor is the poor evidence for the healthy serum concentration range and maximum concentration range, which has to be maintained when considering all reported functions of Vitamin D. This aspect was inconsistent through the literature because of the unstandardized measurement assays of Vitamin D according to the testing laboratory.[18] In addition, there are many concerns and fears regarding Vitamin D toxicity. Altogether, there is a high clinical need to understand the role of Vitamin D and highlight recent researchers to establish supplementation protocols to maintain general health. Therefore, the aim of this narrative review is to deliver an overview about the administrated doses of Vitamin D in randomized controlled clinical studies to evaluate the currently available clinical evidence. In addition, this review focuses on the recent advances on Vitamin D nonskeletal actions.

Measurement of Vitamin D Serum Concentrations and Definition of Hypovitaminosis

Vitamin D (25-hydroxyvitamin D [25(OH) D]) is a highly lipophilic molecule. When circulating in the blood, about 80% of it is bound to its carrier protein (Vitamin D-binding protein [DBP]). Another 10%–15% of Vitamin D is transported by the carrier protein albumin. Only a minor part of Vitamin

D circulates freely in the blood and provides thereby a higher availability to the cells. The 25(OH) D serum concentration is the most reliable indicator to evaluate the Vitamin D status. However, measurement of free 25(OH) D is technically rather difficult because of its low concentration and the limitation of available test methods.[19] This aspect is still a topic of discussion in the research. In routine clinical practice, the total 25(OH) D serum concentration is measured to assess the Vitamin D level.[15] Similar to other vitamins and blood components, Vitamin D values are mostly expressed either in nanomole per liter (nmol/l) or in nanogram per milliliter (ng/ml) according to the used unit system. Basically, 1 nmol/l equals 0.4 ng/ml (conversion factor: 2.5). The reference ranges of Vitamin D are inconsistent according to different recommendation. However, the most recommended range that is considered as adequate and healthy is 40–60 ng/ml (100–150 nmol/l).[20]

A serum concentration below 30 ng/ml (75 nmol/l) is meanwhile considered as a deficiency in the literature.[16,17,20,21] Many studies reported the Vitamin D status of different populations and recognized the worldwide Vitamin D deficiency pandemic.[16,22] Observational studies reported that the prevalence of 25(OH) D level below 20 ng/ml (50 nmol/l) reaches 24% in the USA, 37% in Canada, and 40% in Europe.[16,20] These levels are observed all over the world, for example, more than 20% of the Indian population had a 25(OH)D level below 12 ng/ml (30 nmol/l).[16] The level of 25(OH)D serum concentration depends on the general health status, lifestyle, body mass, and age. Recent estimation in Germany (Robert Koch Institute) reported that 58% of 18–79-year-old participants have a serum concentration below 20 ng/ml (50 nmol/l).[23] The serum concentration of Chinese postmenopausal women during wintertime was around 14 ng/ml (35 nmol/l). In Italy, the summertime mean 25(OH) D concentrations reached about 33 ng/ml (82.5 nmol/l) and 20 ng/ml for men in winter (50 nmol/l).[20]

At our center, a pilot study was performed for volunteer medical staff of the oral, maxillofacial, and facial plastic surgery, Goethe University, Germany. Twenty-four medical staff were tested, from which 85.7% had a Vitamin D level under 30 ng/ml and 45.8% had a Vitamin D level under 10 ng/ml [Figure 2].

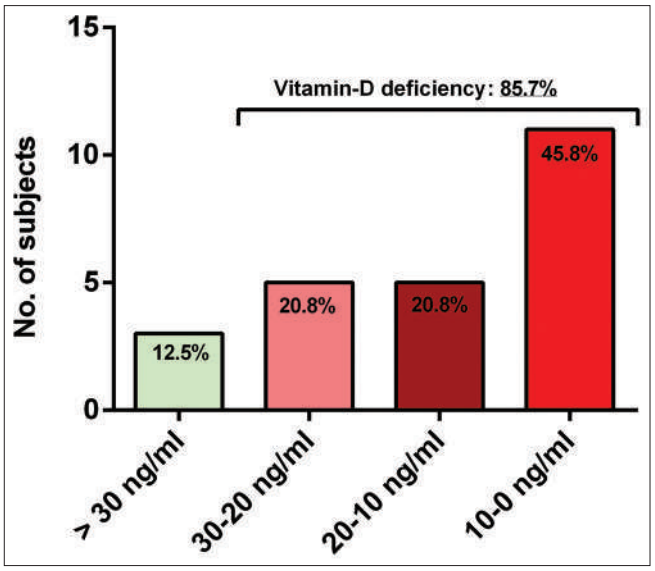

Figure 2: Vitamin D levels of 24 volunteers of the medical staff working in the clinic for oral maxillofacial and plastic facial surgery, University Hospital Frankfurt, Goethe University, Germany

Current Guidelines of Vitamin D Supplementation

In addition to the endogenous synthesis of Vitamin D, there are natural exogenous sources of Vitamin D in different types of food. The amount of Vitamin D content can be expressed using two different units according to the used system. One used unit is the mass unit as microgram (μg). Alternatively, the international unit (IU) is the most used unit to express the amount and dose of Vitamin D supplementation. In general, 40 IUs equal 1 μg.

Food can provide Vitamin D supplementation as a natural source. Animal products provide mainly cholecalciferol (Vitamin D3), while plant and fungal sources contain mainly ergocalciferol (Vitamin D2).[4] The richest natural source of Vitamin D is fish. For example, fresh eel provides 1200 IU/100 g, fresh wild salmon contains up to 1000 IU/100 g, and baked salmon delivers 540 IU/100 g.[4] However, in most cases, the required Vitamin D supplementation is not achieved by natural nutrient sources.[16] Therefore, supplementation is highly required in almost every case. However, current guidelines for supplementations are inconsistent and mainly based on the estimated need for bone health. Recommended doses range from 400 IU/day to 4,000 IU/day. The European Food Safety Authority recommends an intake of 600 IU/day for healthy adults.[24] Similarly, the UK Scientific Advisory Committee on Nutrition advised an intake of 400 IU/day for all age groups.[25] The Institute of Medicine (US) recommended 600 IU/day for individuals under 70 years old and 800 IU/day for individuals over 70 years old.[26] The updated reference values of the German Nutrition Society (2012) recommended different intake values for each age group. For infants 400 IU/day, for all other groups, 800 IU/day Vitamin D intake was advised.[23] The Endocrine Society (USA) recommended 1000–4000 IU/day.[27] Some authors recommended a daily intake of 5000 IU/day to maintain the healthy Vitamin D level of around 40 ng/ml.[28] The GrassrootsHealth Institute gathered data about daily Vitamin D dose of 10,000 IU/day and did not report adverse events.[20,29,30]

In addition, the highest tolerable dose to reach and maintain an adequate Vitamin D level without toxicity or side effects remains variable. The European Food Safety Agency stated that doses up to 10,000 IU/day are safe for patients without comorbidities, whereas the European Food Safety Authority considered the highest safe daily intake to be 4000 IU/day.[24]

Vitamin D Supplementations and Doses of Reviewed Clinical Studies

In addition to the recommendations of the different world societies and authorities, randomized controlled clinical trials (RCTs) were performed in different clinical fields to establish supplementation protocols and evaluate the impact of Vitamin D in healthy people and patients [Table 1]. Schoolchildren in Japan were supplemented with 1200 IU/day for 1 year as a preventive therapy for influenza.[31] The results showed a significantly reduced incidence of influenza for children in the treatment arm taking 1200 IU/day of Vitamin D.[31] The VITAL study analyzed the influence of Vitamin D on the prevention of cancer and cardiovascular

Table 1: Overview of the administered doses and serum concentrations in selected clinical trials

Category	Administered dose	Treatment period	Baseline concentration	Reached concentration	Clinical outcome	Side effects
Schoolchildren[31]	1200 IU/day	12 months	Not reported	Not reported	Vitamin D3 supplementation during the winter reduces the incidence of influenza A	No
Cancer and cardiovascular diseases[32]	2000 IU/day	12 months	29.8 ng/ml	41.8 ng/ml	High-dose Vitamin D for 5 years among initially healthy adults in the United States did not reduce the incidence of cancer or major cardiovascular events	No
Diabetes mellitus[33]	4000 IU/day	12 months	28.0 ng/ml	52.3 ng/ml	Lower risk of diabetes in the Vitamin D group than in the placebo group. But no significant difference	No
	4000 IU/day	24 months	28.0 ng/ml	54.3 ng/ml		No
Ventilated intensive care unit patients[34]	50,000 IU/day	5 days	23.2 ng/ml	45±20 ng/ml	Significant decrease in hospital length of stay over time in the 250,000 IU and the 500,000 IU Vitamin D3 group, compared to the placebo group	No
	100,000 IU/day	5 days	20.0 ng/ml	55±14 ng/ml		No
Patients of intensive care unit[35]	540,000 IU	One shot	11.15 ng/ml	28.08 ng/ml	Hospital mortality was significantly lower with 28 deaths among 98 patients (28.6% [95% CI, 19.9%-38.6%]) for Vitamin D3 compared with 47 deaths among 102 patients (46.1% [95% CI, 36.2%-56.2%]) for placebo (HR, 0.56 [95% CI, 0.35-0.90]), *P* for interaction=0.04)	No
	90,000/month	5 months	11.15 ng/ml	46.0 ng/ml		
Vitamin D-deficient individuals[36]	25,000 IU every fortnight	2 months	7.6 ng/ml	19 ng/ml	Body weight is a critical factor for estimating the supplementation dose	No
	25,000 IU/week	1.5 months	8 ng/ml	25 ng/ml		No
	25,000 IU/week	2 months	8.4 ng/ml	35.6 ng/ml		No
Healthy Vitamin D-deficient individuals[37]	1000 IU/day	5 months	28.8 ng/ml	33.6 ng/ml	Healthy men seem to use 3000-5000 IU cholecalciferol/day, apparently meeting >80% of their winter cholecalciferol need	No
	5000 IU/day		27 ng/ml	64 ng/ml		No
	10,000/day		26 ng/ml	89.6 ng/ml		No
Breast cancer patients with bone metastases[38]	7000 IU/day	4 months	<20 ng/ml	Not reported	Breast cancer patients with deficient/ insufficient 25-OH Vitamin D levels had significantly lower lumbar bone mineral density (*P*=0.03)	No
Long-term hospitalized individuals[20,39]	5000 IU/day	12 months	24 ng/ml	68 ng/ml	Applied dose is safe	No
	10,000 IU/day	12 months	25 ng/ml	96 ng/ml		No
Healthy volunteers[40]	100,000 IU/ month	36 months	24.4 ng/ml	54 ng/ml	Monthly supplementation with 100,000 IU Vitamin D3 did not affect the incidence rate of kidney stone events, or hypercalcemia	No
Multiple sclerosis[41]	20,000 IU/day	12 months	21.6 ng/ml	44 ng/ml	Vitamin D3 add-on treatment to interferon β-1b reduces MRI disease activity in MS	No
Multiple sclerosis[42]	50,000 IU/week	6 months	15.3 ng/ml	33.7 ng/ml	Adding high-dose Vitamin D3 supplementation during pregnancy to routine care of women with MS had a significant effect on the serum 25(OH) D levels, EDSS, and number of relapse events during pregnancy and within 6 months after delivery	No
Asthma, rheumatoid arthritis, rickets, and tuberculosis in the 1930s and 1940s[20,43]	60,000-600,000 IU/day	Not reported	Not reported	Not reported	Applied dose leads to hypercalcemia	Reports of hypercalcemia associated with the use of supraphysiological doses of Vitamin D surfaced

CI: Confidence interval, MRI: Magnetic resonance imaging, 25(OH)D: 25-hydroxyvitamin D, MS: Multiple sclerosis, HR: Hazard ratio, EDSS: Expanded Disability status scale

diseases. In this study, a total of 25,871 participants were included, and the Vitamin D group received a daily dose of 2000 IU/day for 5 years, but unfortunately, Vitamin D deficiency was not an inclusion criterion. No adverse events were reported in this rather long-term study.[32] In the D2D study, patients with prediabetes received 4000 IU/day for 2 years to raise their Vitamin D level from 28 ng/ml to 54 ng/ml. Here, also, no toxicity or adverse events were reported.[33] A relatively high-dose treatment was applied for ventilated intensive care patients in a further RCT. They received 50,000 IU/day in one group and 100,000 IU/day in another group for a total time of 5 days. The Vitamin D deficiency was eliminated, and the patients reached a level of 45 ng/ml and 55 ng/ml, respectively. There was a significant decrease in hospital length of stay over time in the 250,000 IU and the 500,000 IU Vitamin D3 group, compared to the placebo group (25 ± 14 and 18 ± 11 days compared to 36 ± 19 days, respectively; $P = 0.03$). This study did not report any adverse events in relation to Vitamin D.[34] Another study used a one-time supplementation of 540,000 IU to increase the Vitamin D deficiency of critically ill patients of the intensive care unit. Thereafter, the patients received a monthly dose of 90,000 IU per month to maintain the Vitamin D level. The primary outcome was hospital length of stay. The median (interquartile range [IQR]) length of hospital stay was not significantly different between groups (20.1 days [IQR, 11.1–33.3] for Vitamin D3 vs. 19.3 days [IQR, 11.1–34.9] for placebo; $P = 0.98$). Hospital mortality was significantly lower with 28 deaths among 98 patients (28.6% [95% confidence interval (CI), 19.9%–38.6%]) for Vitamin D3 compared with 47 deaths among 102 patients (46.1% [95% CI, 36.2%–56.2%]) for placebo (hazard ratio [HR], 0.56 [95% CI, 0.35–0.90], P for interaction = 0.04) but not 6-month mortality (34.7% [95% CI, 25.4%–45.0%] for Vitamin D3 vs. 50.0% [95% CI, 39.9%–60.1%] for placebo; HR, 0.60 [95% CI, 0.39–0.93], P for interaction = 0.12).[35] A further study analyzed different treatment doses and treatment periods for Vitamin D-deficient volunteers. One group received 25,000 IU every fortnight (calculated daily dose: 6250 IU/day) for 2 months, the second group received 25,000 IU/week (calculated daily dose: 3771 IU/day) for 1.5 months, and the third group received 25,000 IU/week (calculated daily dose: 3771 IU/day) for 2 months. It was shown that weekly intake raised the Vitamin D level higher than intake every fortnight. Also here, no adverse events were reported.[36] Aiming to establish a Vitamin D supplementation protocol, another study treated patients with Vitamin D deficiency with either 1000 IU/day, 5000 IU/day, or 10,000 IU/day over 5 months and reached the best Vitamin D increase in the 10,000 IU/l group without reporting any toxicity or side effects.[37] Breast cancer patients with bone metastasis and bisphosphonate treatments received 7000 IU/day for 4 months without any complications.[38] A further RCT considered a monthly high-dose supplementation of 100,000 IU/month (calculated daily dose: around 3000 IU/day) for several years and followed especially kidney-related adverse events. Eighteen urolithiasis events were recorded, 7 in the Vitamin D arm and 11 from the placebo arm. In addition, there was no case of hypercalcemia in the Vitamin D arm. The authors concluded that this high dose did not increase the risk of kidney stones.[40]

Historic high-dose Vitamin D studies, conducted in the 1930s and 1940s, used daily doses between 60,000 IU/day and 600,000 IU/day to treat different pathologies such as rickets, asthma, rheumatoid arthritis, and tuberculosis. These studies reported hypercalcemia associated with high doses of Vitamin D that was possibly associated with idiopathic infantile hypercalcemia, for example, as a CYP24A1 loss of function mutation.[20,43]

Vitamin D Supports the Innate and Adaptive Immune System

Expanding interest has been directed to evaluate the impact of Vitamin D on the immune system. Several studies focused on its mechanisms of action on different cell types. Vitamin D receptor is expressed in a wide range of cells in the human body including cells of the immune system. The Vitamin D-specific receptor belongs to the group of nuclear receptors that serve as transcription factors. After binding to its receptor, Vitamin D regulates the expression of different genes and the synthesis of proteins. The wide distribution of Vitamin D receptor shows its importance and multifunctional role for the body.[9] Specifically, the immunomodulatory aspect was reported recently. In this context, Vitamin D is able to enhance the innate immune system and inhibit the adaptive immune system.[9] It is known that dendritic cells and macrophages as a part of the innate immune system as well as T- and B-lymphocytes as a part of the adaptive immune system express Vitamin D receptor. In this context, Vitamin D can regulate the inflammatory process.[44] Calcitriol, the active form of Vitamin D, regulated the initial inflammation by inhibiting monocyte proliferation and inducing monocyte differentiation to macrophages. Simultaneously, it enhances the defense capacity of macrophages and promotes their phagocytotic and antimicrobial capacity. In addition, it was shown that calcitriol has the capacity to reduce the proinflammatory response of antigen-presenting cells by reducing their capacity to express proinflammatory cytokines such as interleukin (IL)-1, IL-6, IL-12, and tumor necrosis factor-alpha.[5] Another highlighted effect of Vitamin D is the inhibition of B- and T-lymphocyte proliferation and the modulation of T-cell phenotype differentiation. Previous studies showed that Vitamin D inhibits the production of proinflammatory cytokines of Th1 lymphocytes and promotes the expression of anti-inflammatory cytokines of Th2 lymphocytes, which further explains its role as an immunomodulator. In addition, its influence on Treg cells was shown to be concentration-dependent.[9]

Several studies showed that Vitamin D exhibits the capacity to control gens that are responsible for proliferation. This fact underlines its importance in the cancer prevention and supportive therapy. Clinical studies reported the positive impact of Vitamin D to reduce the incidence and support

the treatment of different types of cancer including breast cancer, colon cancer, squamous cell carcinoma (SCC), and others.[11] Different studies suggested that Vitamin D has also antiproliferative and proapoptotic effects on basal cell carcinoma and SCC cancer cells but not healthy keratinocytes.[45]

Impact of Vitamin D on Chronic Diseases

An increasing prevalence of different chronic diseases such as hypertension, diabetes mellitus, cardiovascular diseases, and autoimmune diseases has been recorded in many countries in the last decade. The association of Vitamin D in the etiology, prevention, and treatment of chronic diseases has been suggested in many clinical studies. A recently published meta-analysis performed on 25 prospective cohort studies with around 10,000 cases showed that low Vitamin D level increased the risk of cardiovascular diseases by 44% (RR = 1.44, 95% CI: 1.24–1.69). It also increased the risk of cardiovascular disease mortality (RR = 1.54, 95% CI: 1.29–1.84) and incidence rates (RR = 1.18, 95% CI: 1–1.39).[46] Evaluation of the correlation between serum Vitamin D status and blood pressure was analyzed in a clinical study, and 8155 hypertensive patients with Vitamin D deficiency were enrolled. The patients were motivated to increase their Vitamin D level to a range of 40–60 ng/ml (100–150 nmol/l). They received individual doses of 1000–20,000 IU/day. After 12 months, 71% of the Vitamin D supplemented patients were no longer hypertensive.[47] Similar effects were observed in the prevention and treatment of diabetes mellitus. A placebo-controlled clinical study analyzed the effect of Vitamin D treatment on diabetes type II patients with mild-to-moderate depressive symptoms. The Vitamin D group received 4000 IU/day. Their Vitamin D level increased from 15.5 ± 8.8 ng/ml to 32.2 ± 8.9 ng/ml, and the depressive symptoms significantly decreased in comparison to the control group. In addition, the mean level of insulin was significantly higher in response to Vitamin D treatment compared to the control group.[48]

There is also evidence of a beneficial effect of Vitamin D on chronic obstructive pulmonary disease (COPD). A meta-analysis reported that Vitamin D deficiency was associated with increased risk of COPD (odds ratio [OR]: 1.77, 95% CI: 1.18, 2.64, $P = 0.006$) and with COPD severity (OR: 2.83, 95% CI: 2.00, 4.00, $P < 0.001$).[49] Further chronic diseases such as rheumatoid arthritis were also associated with Vitamin D deficiency. However, no consensus about the supplementation protocol is available, and thereby, the clinical outcomes are not reproducible.[50] In addition, high attention is directed to the role of Vitamin D in inflammatory autoimmune diseases such as multiple sclerosis.[5,51]

Role of Vitamin D in Infectious Diseases

The immunomodulatory function of Vitamin D and its impact on the immune system reflects its potential role in the defense of infectious diseases. Meanwhile, growing interest was directed to the influence of Vitamin D level on the prevalence and incidence of infectious diseases. Its potential anti-infective capacity made it a favorable candidate as an adjuvant therapy in numerous infective diseases.[13,52] A systematic review highlighted the correlation between Vitamin D deficiency and the status of chronic hepatitis B patients. In addition, it was shown that a low Vitamin D level is associated with a high hepatitis B viral load.[21] Another study has shown that genetic variants in the Vitamin D metabolic pathway are involved in the hepatitis C virus infection.[53] Recent research also suggested that Vitamin D may inhibit herpes infection in oral epithelial cells by regulating the gene expression of defense molecules such as LL-37.[54] Some studies also reported the above mentioned preventive impact of Vitamin D supplementation on the reduction of influenza infections in childhood.[31] Moreover, several studies reported the positive impact of Vitamin D on human immunodeficiency virus-1-infected patients.[55] Other studies suggested that Vitamin D-regulated microRNAs may have a protective impact on dengue virus infection.[56,57] Another study showed that patients who developed pneumonia exhibited a much lower Vitamin D level than the healthy group.[58] Interestingly, Vitamin D receptor polymorphism was shown in a recent meta-analysis to correlate with the risk of viral infections.[59] Based on these data, a recently published review discussed the potential role of Vitamin D in the current COVID-19 pandemic, as this virus also belongs to the family of enveloped viruses.[20] The European Food Safety Agency concluded that daily doses of Vitamin D up to 10,000 IU are safe.[24]

How to Supplement Vitamin D for Adults

Different doses were reported in the literature to increase the Vitamin D level to an adequate and health status. Notably, many authors recommended a higher daily dose than the corresponding authority guidelines. Most of the guidelines were committed to bone health. However, many studies proved or suggested an important role of Vitamin D in nonskeletal health. Based on the results of this review, we suggest a daily supplementation with adequate dose rather than interval supplementation with a high dose.[60] A clinical study showed that after increasing the Vitamin D level to the required range, a daily dose of 2000 IU was insufficient to maintain the required Vitamin D level over a long time.[61]

We recommend an individualized daily Vitamin D dose according to the patients' need. A Vitamin D level of <40 ng/ml requires a daily intake of 10,000 IU to rapidly increase the levels to 40–80 ng/ml. This dose was thoroughly investigated and shown to be safe.[20,24,29,30] To maintain a healthy Vitamin D level (40–80 ng/ml), we recommend a daily dose of 5000 IU. In cases, where the recommended range of 40 and 80 ng/ml is exceeded, the daily Vitamin D intake should be reduced to 1000 IU. Finally, the 25(OH) D should be monitored every 3 months to assess the individual differences in metabolism of Vitamin D and allow for further individual adjustment of the dose according to the patients' need [Figure 3].

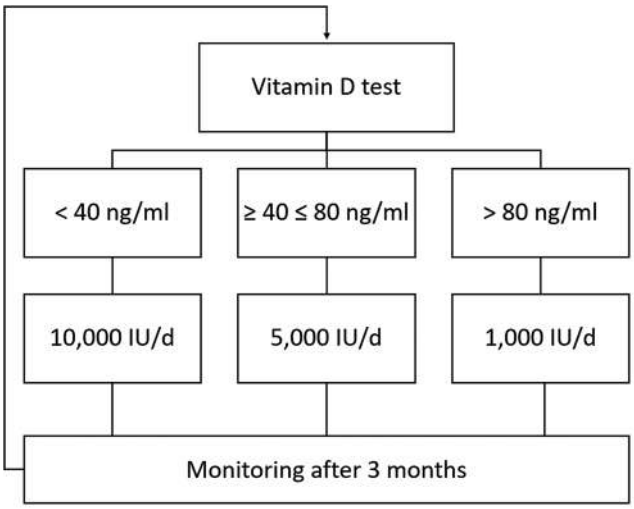

Figure 3: Supplementation protocol of Vitamin D in healthy adults to maintain an adequate Vitamin D status

Concluding Remarks and Future Outlook

Recently, Vitamin D has gained an increasing attention in different fields. The present review outlined the nonskeletal actions of Vitamin D and provided an overview about the current supplementation recommendations of different authorities as well as currently used supplementation doses in clinical studies. Most of the guidelines recommend daily doses between 400 and 4000 IU/day. This high variability is depending on the clinical background, treated population, and country. However, clinical studies applied doses of up to 100,000 IU/day without any adverse events of toxicity. These results underline the huge discrepancy between the growing evidence in the clinical field and the recommended dietary intake. One reason for the discrepancy is that the functions of Vitamin D outside bone health were not well recognized for a long time. Thereby, recommendations and authorities focused mainly on the needed dose for skeletal health. Unfortunately, despite the growing evidence of the role of Vitamin D in the immune system and the prevention of different diseases, most of the recommendations did not change. An additional interesting result of the present review is the discrepancy in the reference range for health status and hypovitaminosis, due to variable measurement techniques. A lot of effort has been made in the past years to establish standardized measurement assays with adequate references. These assays are critically needed to allow for reproducible and comparable values worldwide. Some studies previously showed that retrospective evaluation of Vitamin D values measured with specific assays showed totally different results when using standardized references.[15] Thereby, a unifying evaluation of the Vitamin D status will allow a more thorough definition of Vitamin D deficiency.

Although Vitamin D toxicity is rare, there is a high clinical concern regarding the dose safety. Physiologically, there is a negative feedback system for the endogenous Vitamin D synthesis by the skin, whereas no such regulation mechanism is available for the exogenous intake. Therefore, it was frequently reported in the past that Vitamin D overdose may result in hypercalcemia or kidney stones. However, no safe maximum dose was yet defined. Moreover, there is no evidence linking Vitamin D intake with long-term adverse health outcomes.[17] Clinical studies showed that a daily dose of 20,000 IU/day over 12 months was safe and did not show any adverse events.[41] Some reports of Vitamin D intoxication were reported in the literature.[43,62,63] The main reasons for these reports were the misunderstanding of Vitamin D supplementation units and confusion between micrograms and international units. For example, a manufacturer did not clearly state the dose of the Vitamin D product. Patients who took 2 teaspoons daily supposing a supplementation of 2000 IU had received more than 1 million IU daily for more than a year.[62,63] Another case was the miscalculation in Vitamin D-supplemented milk (250,000 IU in 8 oz milk), and some consumers had serum calcium levels around 16 mg/dl and Vitamin D levels of up to 550 ng/ml.[64] These accidentally occurred intoxications are substantially higher than here reported high-dose supplementation clinical protocols. These observations provide well-documented evidence of the range in which Vitamin D intoxications are evidenced. However, when administering Vitamin D supplementation, it has to be noted that there are no predisposing comorbidities that affect the Vitamin D metabolism or liver/kidney insufficiency in the body. In this context, a personalized dose may be beneficial for some patients according to their lifestyle, metabolism, and current Vitamin D level. For example, many patients with malabsorption syndromes require a much higher dose of Vitamin D supplementation to reach the adequate range than healthy persons.[1,15] Another factor shown to influence the Vitamin D level is body mass. A recent study showed that the Vitamin D level increases in individuals with a normal body mass index. which is significantly higher than those with a body mass index of >30 kg/m², when supplemented with the same daily dose.[65] Thereby, patients with a body mass index >30 kg/m² were shown to require three times greater daily intake than persons with a normal body mass index to reach the same Vitamin D level.[27] In addition, a regular monitoring of the mineral homeostasis and the Vitamin D value is advisable when using daily high dose, whereas doses of up to 4000 IU/day do not require short-time monitoring.

A further important finding of the present review is that there are different clinically applied protocols. Some authors considered the supplementation of a moderate daily dose, while others used a relatively high daily dose. When comparing these protocols, it appears that a daily dose can rather maintain the Vitamin D level in the desired range than a high monthly dose. This may be explained by the 25(OH)D half-life. It was previously shown that the 25(OH)D3 half-life depends on genetic variability of the DBP and is generally about 15 days.[66] Similarly, patients who received 25,000 IU every fortnight for 2 months had lower Vitamin D levels than the group who received 25,000 IU weekly.[36] Interestingly, a further study

treated 135 patients with Vitamin D level of ≥30 ng/ml with 50,000 IU weekly for 3 months to increase the Vitamin D level from 13.2 ± 3.3 ng/ml to 37.0 ± 4.7 ng/ml. Thereafter, the patients received a daily dose of 2000 IU to maintain their level. However, after 3 months, the Vitamin D level significantly dropped to 20.4 ± 5.4 ng/ml. These results show that 2,000 IU/day is not enough to maintain a healthy Vitamin D level even after a therapy.[61]

Altogether this review should draw the attention to the existing hypovitaminosis D pandemic and highlight the role of Vitamin D for the immune system and nonskeletal diseases. The results showed that there is a growing evidence in the literature that Vitamin D is an important factor that can be applied as adjuvant therapy for many diseases. In conclusion, it was shown that daily Vitamin D dose of 10,000 IU is safe for application in patients without comorbidities affecting Vitamin D metabolism. This daily dose is often required to reach a healthy Vitamin D level of 40–60 ng/ml.

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Conflicts of interest

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