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Invited critical review

25-Hydroxyvitamin D: Analysis and clinical application

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ABSTRACT

25-Hydroxyvitamin D (25-OHD) is one of the most popular tests requested by clinicians nowadays because in addition to bone diseases, many non-skeletal disorders have been suggested to be linked to vitamin D deficiency or insufficiency. Methodologies used in clinical laboratories include competitive vitamin D protein binding assays (CPBA), immunoassays, high performance liquid chromatography (HPLC), and liquid chromatography–tandem mass spectrometry (LC–MS/MS). In this review article, we introduce the basic metabolism and physiology of vitamin D, key issues in the methods for 25-OHD measurement currently used in most clinical laboratories, and clinical applications of 25-OHD testing. We conclude that although the methodologies for 25-OHD testing have improved significantly, considerable bias between different methods and laboratories still exists. Therefore, standardization of the method is critical. The optimal 25-OHD levels should be determined based on the standardized method. Also, more studies are needed to further determine the relationship between vitamin D deficiency or insufficiency and non-skeletal diseases as well as daily vitamin D dose requirement for reducing the risk of non-skeletal diseases.

Contents

1. Metabolism and physiology of vitamin D

Vitamin D is a fat-soluble vitamin with a structure similar to steroids and cholesterol. The two common forms of vitamin D are vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). The structural difference between vitamin D_2 and vitamin D_3 is in their side chains. Unlike vitamin D_3, the side chain of vitamin D_2 contains a double bond between carbons 22 and 23, and a methyl group on carbon 24. The molecular weights of D_2 and D_3 are 396.65 Da and 384.64 Da, respectively. Vitamin D_3 is naturally produced in the skin of vertebrates through the conversion of 7-dehydrocholesterol (provitamin D) by ultraviolet light. It is estimated that a single exposure to sun for 20 min produces 15,000 to 20,000 IU of vitamin D_3 [1] and the synthesis of vitamin D_3 in the skin is suppressed with prolonged exposure of ultraviolet light. Therefore, sunlight overexposure will not result in vitamin D_3 intoxication [2]. Also, in the blood stream, vitamin D_3 derived from the skin lasts at least twice the time of ingested vitamin D_2 [3]. Vitamin D_2 is of plant, invertebrates, and fungi origin and mainly obtained from foods and supplements.

Vitamin D is transported to the liver by vitamin D-binding protein (DBP). In the liver, vitamin D_2 and vitamin D_3 are metabolized to

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25-hydroxyvitamin D_2 (25-OHD_2) and 25-hydroxyvitamin D_3 (25-OHD_3) respectively by vitamin D 25α-hydroxylase. Then 25-OHD is further hydroxylated to an active or hormonal form of vitamin D, 1,25-dihydroxyvitamin D (1,25-$(OH)_2D$) by 25-OHD 1α-hydroxylase in the kidney or placenta. The conversion of vitamin D to 25-OHD is not well controlled, conversely the conversion of 25-OHD to 1,25-$(OH)_2D$ is tightly regulated by the parathyroid hormone (PTH) and levels of calcium and phosphorous [1]. Decrease in serum calcium concentration stimulates the secretion of PTH which in turn increases the renal activity of 25-OHD 1α-hydroxylase for the production of 1,25-$(OH)_2D$. Low phosphate concentration also stimulates the conversion of 25-OHD to 1,25-$(OH)_2D$, however this is independent of PTH. 1,25-$(OH)_2D$ surges the calcium and phosphate concentration in blood by acting on the intestine to stimulate the calcium and phosphate absorption from intestine [4], which assists in bone mineralization. By increasing osteoclastic resorption, 1,25-$(OH)_2D$ causes bone mineral resorption and in kidneys it decreases both calcium and phosphate excretion by acting on renal tubular reabsorption [4]. Another important hormone that regulates calcium homeostasis is calcitonin. Calcitonin inhibits bone resorption and decreases calcium tubular reabsorption resulting in lower serum calcium, indirectly influencing the synthesis of 1,25-$(OH)_2D$ [5]. The synthesis of 1,25$(OH)_2D$ is also inhibited by circulating FGF23 produced by osteocytes [6].

2. Vitamin D deficiency/insufficiency and diseases

Historically, vitamin D deficiency is recognized by the presence of rickets or osteomalacia [7]. Nowadays, assessment of vitamin D status is based on the measurement of serum 25-OHD. In adults, PTH level is suppressed to a nadir value when serum 25-OHD concentration reaches to 30 ng/mL. Therefore, a cutoff value 30 ng/mL is being used for optimal vitamin D status by most clinical laboratories. However, many patients with a 25-OHD concentration lower than the cutoff value have no evidence of disease. Some researchers suggest that vitamin D status could be classified into optimal (≥20 ng/mL), insufficient (11–20 ng/mL) and deficient (≤10 ng/mL) based on serum 25-OHD concentration [1]. However, the definition of vitamin D deficiency or insufficiency is still open to debate. For instance, some researchers have defined vitamin D insufficiency as 25-OHD of 21–29 ng/mL [7]. Recently, the Institute of Medicine (IOM) defined vitamin D deficiency as 25-OHD less than 20 ng/mL [8].

Rickets, osteomalacia, and osteoporosis are common manifestations of vitamin D deficiency, as vitamin D plays an important role in bone growth and bone remodeling by osteoblasts and osteoclasts. Causes of vitamin D deficiency/insufficiency include reduced skin synthesis of vitamin D, decreased bioavailability (malabsorption, obesity), increased catabolism, breast-feeding, decreased synthesis of 25-OHD in liver failure, increased urinary loss of 25-OHD in nephrotic syndrome, decreased synthesis of 1,25-$(OH)_2D$ in chronic kidney disease, and other heritable and acquired disorders [9]. In addition to bone, intestine, kidneys, and parathyroid, vitamin D receptor (VDR) is found in most tissues and cells [10], which lead to the hypothesis that vitamin D may have a wide range of biological functions and its deficiency may be associated with increased risk of a range of diseases, such as cardiovascular diseases, cancer, and diabetes, in addition to the well-known vitamin D deficiency related diseases including rickets, osteomalacia, osteoporosis, and secondary hyperparathyroidism (Table 1). The exact mechanisms underlying the associations between non-skeletal diseases and vitamin D deficiency/insufficiency are not well defined, but it is suggested that activation of VDRs expressed in the brain, muscles, adipose tissue, pancreas, colon, breast, and immune cells is responsible for so-called non-classical effects of vitamin D [11]. For example, Di Rosa et al. have found that 1,25-$(OH)_2D$ influences macrophage chemotaxis and differently modulates the expression of IL-1β, IL-6, TNF-α and toll like receptors (TLRs) in the two different stages of monocytes/macrophage maturation [12]. These findings may explain why vitamin D deficiency is associated with some infections such as HIV. On the other hand, 1,25-$(OH)_2D$ suppresses proliferation and immunoglobulin production and retards the differentiation of B cell precursors into plasma cells. Also, 1,25-$(OH)_2D$ inhibits T cell proliferation. These immunosuppressive effects of 1,25-$(OH)_2D$ suggest that vitamin D deficiency may result in autoimmune diseases. In addition, studies show that 1,25-$(OH)_2D$ regulates renin expression and angiotensin II production [13,14] that regulate blood pressure. In addition, vitamin D reduces the expression of adhesion molecules, inhibits endothelium-dependent vasoconstriction, and hinders vascular smooth muscle cell proliferation and macrophage activation [15]. The above functions of vitamin D indicate the association between vitamin D deficiency and cardiovascular diseases. The exact role of vitamin D in the treatment and prevention of cancer remains unclear. The accepted basis for the promise of 1,25-$(OH)_2D$ in the prevention and treatment of malignancy includes its anti-proliferative, prodifferentiating effects on most cell types [16], For instance, 1,25-$(OH)_2D$ stimulates cell cycle inhibitor p21 and p27 expressions, inhibits the transcriptional activity of β-catenin, and promotes the repair of DNA damage [17–19]. Additionally, the hormonal secretion especially insulin is also influenced by vitamin D and the presence of VDR on pancreatic β cells. So it is believed that low vitamin D contributes to the pathogenesis of diabetes [20,21]. Furthermore, there is considerable evidence indicating that with higher vitamin D concentration, the cardiovascular mortality is lowered [22,23]. Overall, as VDR is widely present on many tissues, upon its activation it regulates calcium metabolism, cellular growth, proliferation and apoptosis, and other immunological functions [24,25].

3. Measurement of vitamin D

Serum concentration of 25-OHD is used to evaluate vitamin D status due to the following reasons: 1) 25-OHD has a relatively longer half-life (15 days) compared to the active form, 1,25-$(OH)_2D$ (15 h) [26]; 2) in

Table 1
Vitamin D deficiency/insufficiency and diseases.

Diseases	Vitamin D status	References
Asthma	Vitamin D insufficiency is associated with higher odds of severe asthma exacerbation.	[62]
Autoimmune diseases	Vitamin D can prevent or improve symptoms of multiple sclerosis.	[63,64]
Bone diseases	Serum concentrations of 25-OHD are usually <10 ng/mL in patients with rickets or osteomalacia.	[1]
	There is a correlation between 25-OHD concentration and the occurrence of osteoporosis.	[65]
Cardiovascular diseases	The 25-OHD concentration of patients with heart failure is about 34% lower than controls with same age and sex;	[66]
	Higher 25-OHD concentration is associated with lower cardiovascular mortality;	[67,68]
	The research data for hypertension is inconsistent.	[69,70]
Cancers	Low vitamin D intake, less sunlight exposure increases the risk of cancer.	[71–73]
Depression	Low levels of vitamin D are associated with depression.	[74]
Diabetes mellitus	Taking of vitamin D supplements in childhood may be associated with a reduction risk of type 1 diabetes.	[75]
	Studies show that vitamin D supplements fail to show benefits for onset of type 2 DM.	[76,77]
Infection	Vitamin D insufficiency has been linked to the increased risk of active tuberculosis, upper respiratory tract infection and influenza.	[78,79]
Obesity	Inverse association of 25-OHD level and body mass index (BMI).	[80]