

Review

Hormonal actions of vitamin D and its role beyond just being a vitamin: A review article

Khurshid Ahmad Khan^{1,2*}, Professor Javed Akram¹ and Marium Fazal¹

¹Allama Iqbal Medical College and Jinnah Hospital Lahore, University of Health Sciences Lahore, Pakistan.

²Higher Education Commission of Pakistan.

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Over the last decade there has been considerable evolution of our understanding of vitamin D metabolism and its biological activity. The discovery that most tissues and cells in the body have vitamin D receptors and that several possess the enzymatic machinery to convert the primary circulating form of vitamin D to its active form, 1,25-dihydroxyvitamin D, has provided new insights into the function of this vitamin. Aside from calcium homeostasis vitamin D has been demonstrated to exert a wider range of biological activities including regulation of cellular differentiation and proliferation, immune functions, reproduction, and of special significance is to note its role in reducing the risk of many chronic illnesses, including common cancers, autoimmune diseases, infectious diseases, and cardiovascular disease. Although it is fascinating that its *in vitro* biological activities were previously known in the past 2 decades, the physiological context of these is showing proof in many human studies now. Undiagnosed vitamin D deficiency is very common and 25-hydroxyvitamin D is the barometer for vitamin D status. Magnitude of this vitamin deficiency is so massive that unless some substantial measures are taken for its correction with supplementation, appropriate sun exposure and public education we may go back to where we were in 19th century, in terms of skeletal health and other newly discovered problems with vitamin D deficiency, when seeing patients with advanced rickets and osteomalacia was not an uncommon sight at all.

Key words: 25(OH) D, 25-hydroxy vitamin D; 1,25(OH)₂D, 1,25-dihydroxy vitamin D.

INTRODUCTION

Vitamin D is a group of fat-soluble secosteroids, the two major physiologically relevant forms of which are vitamin D₂ (ergocalciferol) and vitamin D₃ (cholecalciferol). These are collectively known as calciferols. Vitamin D system plays a primary role in the maintenance of calcium homeostasis. The stringent regulation of extra-cellular

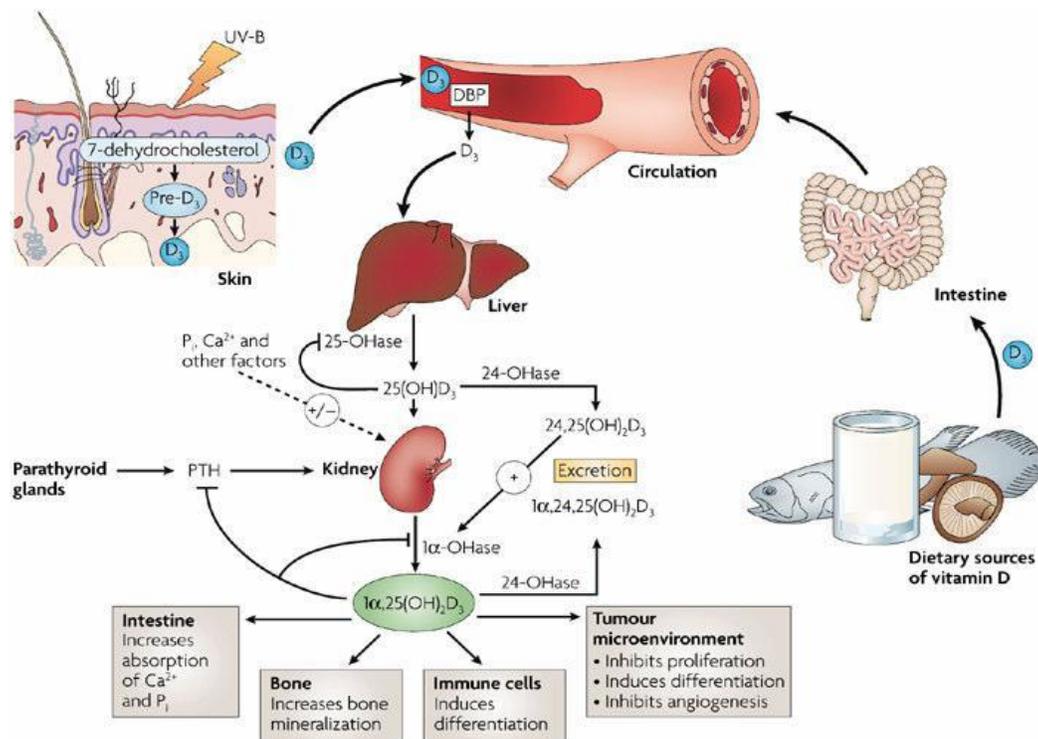
fluid (ECF) calcium concentration within narrow limits is primarily maintained by vitamin D (Horlick, 2008; Quarles, 2008).

In this context, it appears that the most critical endocrine role of vitamin D is to enhance the intestinal absorption of dietary calcium and phosphate. The discovery that most tissues and cells in the body have a vitamin D receptor and that several possess the enzymatic machinery to convert the primary circulating form of vitamin D, 25-hydroxyvitamin D, to its active form, 1,25-dihydroxyvitamin D, has provided new insights into the function of this vitamin (Bikle, 2009, 2008).

Aside from calcium homeostasis vitamin D has been demonstrated to exert a wider range of biological activities including regulation of cellular differentiation and proliferation, immune functions, reproduction, and of

*Corresponding author. E-mail: Dockhan@Live.com. Tel: 092-3002026000.

Abbreviations: 25(OH) D, 25-hydroxy vitamin D; 1,25(OH)₂D, 1,25-dihydroxy vitamin D; UVB, ultraviolet B; MED, minimal erythematous dose; FGF23, fibroblast growth factor; VDRE, vitamin D receptor elements; DM, diabetes mellitus; PTH, parathyroid hormone.



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Figure 1. Adapted from nature reviews cancer 7, 684-700 (September 2007).

special significance to note its role in reducing the risk of many chronic illnesses, including common cancers, autoimmune diseases, infectious diseases, and cardiovascular disease (Horlick, 2008; Anderson et al., 2003).

Currently vitamin D is being recognized more and more as a hormone rather than just merely a vitamin. It plays its vital role in every aspect, be it cardiovascular disease, endocrinology, infectious disease or oncology. Although it is fascinating that it, *in vitro* biological activities were previously known in the past 2 decades but the physiological context of these is showing proof in many human studies now.

SYNTHESIS, PHARMACOKINETICS AND METABOLISM

Humans get vitamin D from exposure to sunlight, from their diet, and from dietary supplements. A diet high in oily fish prevents vitamin D deficiency. Solar ultraviolet B radiation (wavelength, 290 to 315 nm) penetrates the skin and converts 7-dehydrocholesterol to previtamin D3, which is rapidly converted to vitamin D3. Vitamin D2 is

produced by ultraviolet radiation on ergo sterol in a variety of plant materials and yeast. Therefore it is taken orally by the human body. Vitamin D3 however is produced from 7-dehydrocholesterol in the skin. Ultraviolet radiation, when falls on this molecule, converts it into a previtamin D3 which under the action of heat isomerizes in skin to form vitamin D3 or cholcalceferol (Figure 1). With continued exposure to ultraviolet radiation, pre D3 is converted to the biologically inactive lumisterol and tachysterol and also there is photo conversion of D3 itself to suprasterols and 5, 6 transvitamin D3 (Bikle, 2009, 2008). These are the inactive metabolites of vitamin pre D3 and D3 and are the reason why toxic amounts of vitamin D cannot be produced by sun exposure.

The ultraviolet radiation is the radiation on the lower end of the invisible spectrum of light and frequency of 290 to 315 of ultraviolet B radiation is essential for this conversion (Horlick, 2008). This radiation best reaches the earth between 10:00 and 15:00 h. The air pollutants act as a second barrier after the ozone to these UVB photons and decrease their availability by 60%. Melanin in the skin acts to absorb the UVB radiation and is therefore a competitor to 7-dehydrocholesterol. The

minimal erythema dose (MED) by UVB radiation in Asians (49 to 133 mJ/cm²), is more than Caucasians (31 to 48 mJ/cm²) (Ilahi et al., 2008). Sunscreen use with sun protection factor above 8 blocks UVB penetration. Advancing age adversely affect vitamin D status of human body because of slowed action of enzymes. Ideally 5 to 30 min in the sun, twice a week, with exposed face, arms, legs or back is adequate for normal levels of vitamin D to be achieved (NHANES). However with difference in the aforementioned factors of skin color, BMI and age are the requirements do change from person to person.

Vitamin D₃ is transported from skin via blood to the liver, where it is converted by mitochondrial hydroxylase enzyme CYP27A1, to 25 hydroxyvitamin D. This is an important circulating form of vitamin D due to its stability in the blood and greater water solubility and also a long half life and is therefore its measurement in the blood stream is an acceptable indicator of vitamin D status of a person. 25(OH) D is then converted by another mitochondrial enzyme CYP27B1 (1-alpha hydroxylase) in the proximal tubule of the kidney to 1,25(OH)₂ cholcalceferol which is the active form of vitamin D. 1-alpha hydroxylase is stimulated by PTH and inhibited by high blood levels of calcium and phosphate. This enzyme also exists in various other cells of the body like colon, prostate, breast, brain, vascular smooth muscles, macrophages and beta cells of pancreas (Bikle, 2009, 2008; Horlick, 2008; Anderson et al., 2003).

REGULATION AND MECHANISM OF VITAMIN D ACTION AT MOLECULAR LEVEL

Vitamin D status in the body is regulated by parathyroid hormone and serum calcium levels. Parathyroid hormone increases production of vitamin D by increasing activity of CYP27B1 and decreasing activity of CYP24. Fibroblast growth factor (FGF23) produced by osteocyte in response to 1,25(OH)₂ vitamin D causes negative feedback and reduces the renal production of vitamin D (Quarles, 2008). Increased calcium level in blood decreases the production of 1,25 (OH)₂ D via its action on CYP27B1. 1,25(OH)₂ vitamin D acts as its own suppressor through suppression of the enzymes that synthesizes it.

The biological activity of 1,25D is mediated by a high affinity receptor, VDR, which acts as a ligand-activated transcription factor. VDR was found originally in organs involved in calcium homeostasis, including the intestine, bone, kidney, and the parathyroid glands. The isolation of cDNA clones for the avian, human, mouse, and rat VDR has led to VDR being detected in many other non-classical tissues and cell types (Erben et al., 2002). The action of 1,25 (OH)₂ D in these tissues has been associated with a diverse range of biological functions

such as modulation of immune function, inhibition of cell growth, and induction of cell differentiation (Omdahl et al., 2002). There is evidence that for many, if not all, vitamin D responsive genes, unliganded VDR/retinoid X receptor (RXR) heterodimer binds to VDRE of the promoter of the target gene, recruiting a co-repressor complex resulting in repression of basal gene transcription (Omdahl et al., 2002). Upon VDR binding of 1,25(OH)₂ D, the co-repressor is displaced allowing recruitment of a co-activator complex of proteins that interacts with the RNA polymerase machinery and stimulates gene transcription (Omdahl et al., 2002). In short, the interaction of the 1,25(OH)₂ D-bound VDR-RXR complex with nuclear proteins forms a "pre-initiation complex", regulating the rate of transcription of the target gene. This so-called genomic action of 1,25(OH)₂ D can be preceded by more rapid non-genomic actions, occurring in minutes. These involve cytoplasmic membrane-associated events such as activation by 1,25(OH)₂ D of the mitogen-activated protein kinases (MAP kinases), protein kinase C and other cell signalling pathways (Song et al., 1998).

PHYSIOLOGICAL EFFECTS OF VITAMIN D

Bone and mineral metabolism

The well known effects of vitamin D are in relation to bone and mineral metabolism. Role of vitamin D for bone and mineral metabolism are through its effects on calcium and phosphorus levels in blood and its feed back actions on parathyroid hormone and fibroblast growth factor 23 (FGF23). It increases intestinal absorption of calcium and phosphate through vitamin D receptor (VDR) mediated increase in transcription of genes involved in calcium transport. The cytosolic calcium binding protein, calbindin -D9k, thought to be particularly important in calcium transport is a key target of vitamin D action. In kidneys, it increases the renal calcium transport protein calbindin-D28k and also promotes PTH mediated calcium transport (Anderson et al., 2003).

The availability of calcium in blood enables bone to utilize it for mineralization. This would give the impression that adequate calcium levels achieved otherwise may thus render vitamin D unimportant for the bone. However, *in vitro* studies have shown that vitamin D is important for regulation of osteoblast gene transcription and differentiation (Anderson et al., 2003) . Osteoclast formation is also affected by the presence of vitamin D, by alteration in expression of certain molecules required (Blair and Athanasou, 2004) for osteoclastic activity.

Parathyroid hormone (PTH), which functions to increase the calcium and decrease phosphorus levels in the blood is inhibited by 1,25(OH)₂D. Vitamin D also up regulates the calcium sensing receptors on the parathyroid and is an additional mechanism by which it controls PTH.

FGF23 which increases renal excretion of phosphorus is produced primarily by the osteocytes and osteoblasts in response to stimulation by vitamin D (Quarles, 2008).

Insulin secretion

Vitamin D stimulates insulin secretion. This is via direct action on pancreatic beta cells and indirectly by normalizing calcium levels extracellularly. The evidence of the aforementioned has been confirmed by finding VDREs on the insulin promoter gene, the presence of VDRs on the pancreatic beta cells and 1-alpha hydroxylase in the beta cells of pancreas (Pittas et al., 2007).

Regulation of immune functions

1,25(OH)₂D promotes innate immunity by enabling the activated macrophage to produce cathelicidin, which is a potent antimicrobial peptide. This vitamin is also known to suppress adaptive immunity by inhibiting the maturation of dendritic cells, reducing their capacity to present antigen to CD4 cells. It also further inhibits the proliferation and differentiation of CD4 cells into Th1 and Th17 cells and promotes the production of Th2 cells (Bikle, 2009, 2008).

Regulation of proliferation and differentiation of cells

It has been shown that there are many cells, for example, keratinocytes in the skin and cancer cells that express VDRs on their surface and 1-alpha hydroxylase enzyme in them. This enables a check on their proliferation, increasing differentiation and apoptosis (Holick, 2008).

VITAMIN D DEFICIENCY AND ITS PREVALENCE

The vagueness of symptoms of vitamin D deficiency makes this condition highly under diagnosed. Individuals may experience symptoms ranging from mere fatigue and myalgias to potentially life threatening conditions mimicking spinal muscular atrophy (Thys-Jacobs et al., 2007). In the pediatric group the term rickets and for adults osteomalacia formally describes this condition. Major effects of vitamin D deficiency are inadequate mineralization of bone and consequently bowed limbs, rickety rosary in children and looser's zones in adults. Vitamin D insufficiency and deficiency, as measured by low levels of 25-OH vitamin D, are common particularly among adolescents and the elderly.

The best laboratory indicator of vitamin D adequacy is the serum 25-OH vitamin D concentration. Although there

is no consensus on optimal levels of 25-hydroxy vitamin D as measured in serum, vitamin D deficiency is defined by most experts as a 25-hydroxyvitamin D level of less than 20 ng/ml (50 nmol/L) (Malabanan et al., 1998). A level of 25-hydroxyvitamin D of 21 to 29 ng/ml (52 to 72 nmol/L) can be considered to indicate a relative insufficiency of vitamin D, and a level of 30 ng/ml or greater can be considered to indicate sufficient vitamin D (U.S. Centers for Disease Control and Prevention, 2008). Vitamin D intoxication is observed when serum levels of 25-hydroxyvitamin D are greater than 150 ng/ml (374 nmol/L).

With the use of such definitions, it has been estimated that 1 billion people worldwide have vitamin D deficiency or insufficiency (Holick, 2006). According to several studies, 40 to 100% of U.S. and European elderly men and women still living in the community (not in nursing homes) are deficient in vitamin D. Children and young adults are also potentially at high risk for vitamin D deficiency. For example, 52% of Hispanic and black adolescents in a study in Boston and 48% of white preadolescent girls in a study in Maine had 25-hydroxyvitamin D levels below 20 ng/ml. In other studies, at the end of the winter, 42% of 15 to 49 year old black girls and women throughout the United States had 25-hydroxyvitamin D levels below 20 ng/ml (Nesby-O'Dell et al., 2002).

In Europe, where very few foods are fortified with vitamin D, children and adults would appear to be at especially high risk. People living near the equator who are exposed to sunlight without sunscreens have robust levels of 25-hydroxy vitamin D above 30 ng/ml. However, even in the sunniest areas, vitamin D deficiency is common when most of the skin is shielded from the sun. In studies in Saudi Arabia, the United Arab Emirates, Australia, Turkey, and Lebanon, 30 to 50% of children and adults had 25-hydroxyvitamin D levels under 20 ng/ml (El-Hajj et al., 2001). Also at risk are pregnant and lactating women who in one study were found to be vitamin D deficient by 73 and 80% of their infants were also found to be deficient in vitamin D at birth. (Lee et al., 2007).

MAGNITUDE OF VITAMIN D DEFICIENCY IN SOUTH ASIA

Magnitude of vitamin D deficiency in India and Pakistan is huge (Holick, 2007; Goswami et al., 2008). In one study on healthy urban and rural subjects in south India, there was significant hypovitaminosis D in urban population, with 62% being deficient (Zuberi et al., 2008). Because of this wide spread deficiency, deficient mothers are unable to transfer adequate vitamin D to their new born leading to poor infant health as well as high incidence of rickets. (Harinarayan et al., 2007). Although close to the equator

one would expect at least in school going children in south Asia to have adequate levels of vitamin D but in different studies about 30 to 50% of these children have been found to be deficient in vitamin D (Muthukrishnan et al., 2009; El-Hajj et al., 2001)

VITAMIN D DEFICIENCY AND DISEASES

Diabetes mellitus type 1 and 2

The presence of VDR receptor on beta cells of pancreas has led to the hypothesis that VDR receptor polymorphism may be related to the genetic susceptibility of type 1 DM or hypovitaminosis D might be an environmental factor for the development of this condition. When it comes to confirmation of the aforementioned findings with clinical studies, there have been conflicting data. While some small studies were able to corroborate this finding but others investigators had different results. One studies done in Florida on 415 individuals showed no significant association of type 1 DM with hypovitaminosis D (Bierchenk, 2009). Another study analyzing 1654 families for association of VDR polymorphism with type 1 DM showed no association as well (Kahles, 2009). In type 2 diabetes mellitus however, a meta-analysis of studies have shown an association of hypovitaminosis D with this (Pittas et al., 2007). The possible mechanisms for this action of vitamin D are by stimulating beta cells directly and via its effect on calcium levels in beta cells of pancreas which leads to increase in insulin secretion (Alvarex, 2010). Vitamin D also increases glucose uptake by peripheral cells and improves insulin sensitivity (Alvarex, 2010). However still more evidence is needed on human subjects before this action of vitamin D can be unequivocally established.

Cardiovascular disease

It has been shown that individuals with hypertension when subjected to UVB radiations show reduction in their blood pressures as compared to those given UVA radiation. Also individuals with hypovitaminosis D have been shown to have higher chances of myocardial infarction (Horlick, 2008). It has been hypothesized that this limited proof maybe due to the effect of vitamin D on renin production, its ability to decrease vascular smooth muscle cell proliferation and its association with decreasing levels of low density lipoproteins and increasing level of high density lipoproteins (Horlick, 2008).

Autoimmune disease

Disease with an autoimmune etiology like multiple

sclerosis, rheumatoid arthritis and crohn's disease have been shown to have strong association with low levels of vitamin D. Different studies have assessed the direct association or the latitudinal association of these diseases with vitamin D deficiency. Mechanism is attributed to the effect of vitamin D on immune system as mentioned previously. However more data is needed to establish this link conclusively (Horlick, 2008).

Tuberculosis

Macrophages when become infected with mycobacterium tuberculosis, toll-like receptors cause the increased expression of VDR and 1-alpha hydroxylase enzyme. This leads to increased formation of $1,25(\text{OH})_2\text{D}$ which expresses defensin proteins and therefore helps in destroying mycobacteria (Bikle, 2009, 2008; Horlick, 2008). One recent study done in United Kingdom showed that even a single large dose of vitamin D enhanced the innate immune response against mycobacterium tuberculosis (Martineau et al., 2007). Recently even more data has come up establishing relationship between tuberculosis and vitamin D deficiency (Talat, 2010).

Cancers

Higher intake of vitamin D and calcium may be associated with lower risk of premenopausal breast cancer (Dixon et al., 2005). Although postmenopausal breast cancer is said to show no association with this vitamin (Abbas, 2008) but one case control study conducted in Germany did show positive strong association (Lin, 2007). Vitamin D has this anticancerous effect by modulating antiproliferative and prodifferentiating ability of human cells expressing VDR. Although there is a list of cancers showing relation to low levels of vitamin D, the most prominently addressed in research so far are cancers of the breast, colon and prostate (Bikle, 2009, 2008). In keratinocytes, this vitamin has shown to repair DNA damage induced by UVR and reduces apoptosis (Bikle, 2009, 2008; Wejse et al., 2007).

Post menstrual dysphoric disorder (PMDD)

This cyclical physically and emotionally distressing disorder has shown an unexplainable relation to vitamin D, it has been shown in a crosssectional and prospective study of women with and without PMDD, that women with PMDD showed failure of surge of $1,25(\text{OH})_2\text{D}$ in their system during the luteal phase of menstrual cycle, in contrast to healthy women who showed a prominent

increase of this vitamin at this phase (McCullough et al., 2009). This relationship and any further implications that has needs to be further evaluated.

Asthma and pulmonary functions

Men and women with a 25- hydroxyvitamin D level above 35 ng /ml (87 nmol/L) had a 176 ml increase in the forced expiratory volume in 1 s (Black and Scragg, 2005). Children of women living in an inner city who had vitamin D deficiency during pregnancy are at increased risk for wheezing illnesses (Camargo et al., 2007).

Psychiatric disorders

Vitamin D deficiency has been linked to an increased incidence of schizophrenia and depression (Gloth et al., 1999). Maintaining vitamin D sufficiency in utero and during early life, to satisfy the vitamin D receptor transcriptional activity in the brain, may be important for brain development as well as for maintenance of mental function later in life (Eyles et al., 2005).

DEFINING VITAMIN D DEFICIENCY AND STRATEGIES FOR ITS SUPPLEMENTATION

Treatment of vitamin D deficiency is with oral or intramuscular supplementation taking into account the degree of severity. Vitamin D when given orally in an interventional study showed decline in its desirable blood levels by day seventy (Isaia et al., 2001). Recommendations from the Institute of Medicine for adequate daily intake of vitamin D are 200 IU for children and adults up to 50 years of age, 400 IU for adults 51 to 70 years of age, and 600 IU for adults 71 years of age or older (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes Food and Nutrition Board, Institute of Medicine, 1999). However, most experts agree that without adequate sun exposure, children and adults require approximately 800 to 1000 IU per day (Boonen et al., 2006). Children with vitamin D deficiency should be aggressively treated to prevent rickets. Since vitamin D2 is approximately 30% as effective as vitamin D3 in maintaining serum 25-hydroxyvitamin D levels so up to three times as much vitamin D2 may be required to maintain sufficient levels. A cost-effective method of correcting vitamin D deficiency and maintaining adequate levels is to give patients a 50,000 IU capsule of vitamin D2 once a week for 8 weeks, followed by 50,000 IU of vitamin D2 every 2 to 4 weeks thereafter (Malabanan et al., 1998). Alternatively, either 1000 IU of vitamin D3 per day (available in most pharmacies) or 3000 IU of vitamin D2 per day is effective. Strategies such as having

patients take 100,000 IU of vitamin D3 once every 3 months have been shown to be effective in maintaining 25-hydroxyvitamin D levels at 20 ng/ml or higher and are also effective in reducing the risk of fracture. Human milk contains little vitamin D (approximately 20 IU/L), and women who are vitamin D deficient provide even less to their breast-fed infants (Malabanan, 1998).

Lactating women given 4000 IU of vitamin D3 per day not only had an increase in the level of 25-hydroxyvitamin D to more than 30 ng /ml but were also able to transfer enough vitamin D3 into their milk to satisfy an infant's requirement (Hollis and Wagner, 2004). In Canada, to prevent vitamin D deficiency, current guidelines recommend that all infants and children receive 400 IU of vitamin D3 per day.

In patients with any stage of chronic kidney disease, 25- hydroxyvitamin D should be measured annually, and the level should be maintained at 30 ng/ml or higher, as recommended in the Kidney Disease Outcomes Quality Initiative guidelines from the National Kidney Foundation (K/DOQI, 2003).

Patients with stage 4 or 5 chronic kidney disease and an estimated glomerular filtration rate of less than 30 ml/min per 1.73 m² of body-surface area, as well as those requiring dialysis, are unable to make enough 1,25-dihydroxyvitamin D and need to take 1,25-dihydroxyvitamin D3 or one of its less calcemic analogues to maintain calcium metabolism and to decrease parathyroid hormone levels and the risk of renal bone disease (Holick, 2005).

Most important of all is not to forget the key source in vitamin D supplementation and that is ultraviolet exposure to sunlight or ultraviolet B radiation from a tanning bed or other ultraviolet B emitting devices, all of which are very effective.

CONCLUSION

Over the last decade there has been considerable evolution of our understanding of vitamin D metabolism and its biological activity. The discovery that most tissues and cells in the body have vitamin D receptors and that several possess the enzymatic machinery to convert the primary circulating form of vitamin D to its active form, 1,25-dihydroxyvitamin D, has provided new insights into the function of this vitamin. Aside from calcium homeostasis vitamin D has been demonstrated to exert a wider range of biological activities including regulation of cellular differentiation and proliferation, immune functions, reproduction, and of special significance is to note its role in reducing the risk of many chronic illnesses, including common cancers, autoimmune diseases, infectious diseases, and cardiovascular disease. Although it is fascinating that its *in vitro* biological activities were previously known in the past 2

decades but the physiological context of these is showing proof in many human studies now. Future studies should aim to decode underlying pathogenetic pathways of vitamin D action in newly discovered areas of vitamin D receptors. Undiagnosed vitamin D deficiency is very common and 25-hydroxyvitamin D is the barometer for vitamin D status. Magnitude of this vitamin deficiency is so massive that unless some substantial measures are taken for its correction with supplementation, appropriate sun exposure and public education we may go back to where we were in 19th century, in terms of skeletal health and other newly discovered problems with vitamin D deficiency, when seeing patients with advanced rickets and osteomalacia was not an uncommon sight at all.

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