The physiology of vitamin D: Current concepts

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The vitamin D endocrine system, besides playing pivotal roles in calcium homeostasis & bone mineral metabolism, is now recognized to subserve a wide range of fundamental biological functions in cell differentiation, inhibition of cell growth as well as immuno modulation. Vitamin D is a prohormone which is converted into its active hormonal form 1, 25 (OH) D, 1, 25 (OH) D activates its cellular receptor (VDR) which activate target genes to engender its biological actions. This review provides a summary of recent understanding of the complex actions of the vitamin D hormone 1, 25 (OH) D which is a final product of 1α hydroxylation in the proximal tubular cells of kidneys. Emerging evidence also indicates both 1, 25 (OH) D independent as well as depended action of vitamin D receptor (VDR). Thus, the vitamin D system action may involve more than one single receptor and legand. The presence of 1α hydroxylase in many target cells other than proximal renal tubular cells indicates autocrine and paraocrine functions for 1, 25 (OH) D in the control of cell proliferation and differentiation. Vitamin D and related molecules belong to a elaborate endocrine system that acts on target genomic receptors in several organ systems to control cell proliferation and differentiation.

Key words Bone mineral metabolism - calcium - vitamin D - vitamin D receptor

“Calcium supplementation, alone or in combination with vitamin D, is effective in the preventive treatment of osteoporotic fracture”.

The above front page promotion in a recent issue of the Lancet underscores the continuing importance of vitamin D supplements in global bone-mineral metabolic health promotion. The authors of the meta-analysis recommended 1200 mg calcium and 800 IU of vitamin D daily to people above 50 yr to prevent bone loss and fractures.

Currently vitamin D can be called the ‘sunlight hormone’ on the basis of the emerging knowledge of its all important role as a key regulatory substance that is elaborated in human ‘skin organ’ nudged by the primordial power of the sun. Known traditionally as anti rickettic factor or sunshine vitamin, presently it is no more identified a vitamin, but rather as a steroid hormone that regulates an astonishingly complex system of genomic functions that are relevant to such diverse and fundamental bio-activities as inhibition of cell growth and promotion of cell differentiation, immuno regulation and prevention of neoplastic transformation!

In this review some current concepts have been summarized in the evolving body of knowledge on genomic action of 1, 25 (OH) D, (the hormone) and also on the impact of its ‘deficit’ on the clinical biology of human bone-mineral health in our subcontinent.
Molecular evolution & action of vitamin D hormone

The ultraviolet (UV) spectrum of sunlight (wave length 290-310 nm) impinges on human skin and facilitates the conversion of 7-dehydrocholesterol present in the subcutaneous fat to pro-vitamin D, which undergo thermal isomerization to vitamin D3 and vitamin D2. Vitamin D3 and D2 are not naturally present in the food consumed by the vast majority of people of the Indian sub-continent. Besides, the pigmented skin bestowed on us by solar energy to protect us from its cancerous insults, simultaneously deprive us of our dermal ability to synthesize vitamin D with the help of the same celestial power.

Liver, kidney and vitamin D

Hydroxylation of 7, 8 dehydrocholesterol to 25(OH)D3 in the liver and subsequently to 1, 25 (OH)2 D3 in the proximal tubular cells of the kidneys, and several other vitamin D responsive organ systems are well known facts of vitamin D physiology. 1α-hydroxylase enzyme activity is tightly regulated in view of the important biological role of 1, 25 (OH)2 D3 in calcium homeostasis. Thus dietary calcium (Ca++) regulates the enzyme directly through changes in serum calcium and indirectly by altering parathyroid hormone (PTH) levels. The stimulation of 1α-hydroxylase by hypocalcaemia is markedly blunted by parathyroidectomy. However 1α-hydroxylase activity as well as its mRNA activity can be altered by direct action of calcium in cell lines of human renal proximal tubules2. Besides, PTH, stimulated by calcium deficiency, also stimulates 1α-hydroxylase as well as its mRNA in proximal renal tubular cells. However PTH stimulation of 1α-hydroxylase mRNA has been shown to be mediated by CAMP3, which stimulates 1α-hydroxylase gene transcription.

Dietary PO4 restriction also has been shown to stimulate renal 1α-hydroxylase activity as well as its messenger (mRNA). This action of PO4 is independent of PTH and is believed to be through a systemic hormone which is yet to be identified. However, it is conjectured that it may be one of the several newly discovered phosphaturic factors known collectively as phosphatonin. Phosphatonin are currently thought to be regulators of circulating 1, 25 (OH)2D3, through their action on 1α-hydroxylase gene4.

In organs and tissues other than kidney where 1α-hydroxylase activity related 1, 25 (OH)2D3 production is shown to occur, it acts locally in autocrine or paracrine fashion. In such extra-renal organs 1,25 (OH)2D3 synthesis and degradation are under control of local factors like cytokines and growth factors5.

The strong bio-efficacy of 1, 25 (OH)2D3 is controlled by countervailing its biological and clinical action by an equally powerful steroidal hormone 24, 25 (OH)2D3. 24 hydroxylation of vitamin D is brought about by the enzyme 24 hydroxylase, whose gene expression is enhanced by increasing PO4 level and decreasing PTH levels6. Thus the 24 hydroxylase gene is regulated in a reciprocal manner to the gene expression of 1α hydroxylase7.

Vitamin D transport

Vitamin D is poorly soluble in aqueous media and highly soluble in lipids. Therefore it is transported in blood with the help of a binding carrier protein called vitamin D binding protein (DBP). DBP binds vitamin D related molecules with the following sequence of affinity: 25 (OH) D = 24, 25(OH)2D > 1,25(OH)2D > Vitamin D8. Plasma concentration of DBP is 20 times higher than the total amount of vitamin D metabolites and 99 per cent of them are protein bound. Protein bound vitamin D metabolites have limited access to target cells and hence increased half-life in circulation. The resultant protection from exposure to cell metabolic process, makes vitamin D less susceptible to hepatic metabolism and exertion through biliary channels. Thus, vitamin D and metabolites have high half life in circulation. Only free fractions of vitamin D are metabolized and tissue availability of vitamin D is determined by the free fraction like most other hormones. Thus DBP protects the tissues from toxic levels of vitamin D. Therefore when DBP concentration is reduced, as happens in chronic liver diseases, nephrotic syndrome and malnutrition, the susceptibility to vitamin D intoxication is high. The reverse is true in conditions like pregnancy and oestrogene therapy, where the DBP concentrations increase.

Free 1, 25 (OH)2D concentration remains constant even as DBP levels change as a result of the tight self-regulation of vitamin D metabolism. DBP linked vitamin D is actively transported by receptor mediated uptake in the brush border of the renal proximal convoluted tubular cells and not by diffusion through its baso-lateral surface9. Because of this, DBP deficient rats do not face vitamin D intoxication. Nor do DBP deficient rats face vitamin D deficiency because DBP independent uptake of vitamin D occurs through endocytosis – a megalin facilitated process. (Megalin is a part of a set of complex proteins that facilitate endocytosis.)
Inside the renal tubular cells, DBP is degraded and 25 (OH) D is released for metabolism by 1α or 24 hydroxylases. There are many intracellular D binding proteins (DBP) which bind vitamin D and regulate their intracellular metabolism. IDBPs are homologues of heat shock proteins (HSP) that bind both 25(OH) D compound, as well as oestrogens, to serve diverse roles. Overexpression of IDBP-3 in cells expressing the megalin increases the movement of 25 (OH) D into the mitochondria for hydroxylation. Indeed, IDBP-3 interacts directly with 1α-hydroxylase. On the other hand, overexpression of IDBP-1 enhances the movement of 1α-hydroxylase to VDR. Megalin has also been shown to bind the VDR to co-activate SKI interacting protein (SKIP), providing another means of regulating vitamin D action.

Megalin levels are increased by 1, 25 (OH)2 D3, providing a feed–forward mechanism for 1, 25 (OH)2 D3 production. Partial nephrectomy may lead to gradual fall in megalin expression in the remnant kidney, beginning within two weeks – followed later by increase in 1α-hydroxylase in mRNA levels. This compensates for reduced megalin levels and decreased synthetic capacity due to loss of renal mass. This fact justifies the role of vitamin D supplementation in patients with early renal failure.

1, 25 (OH)2 D3 action

Genomic action of vitamin D: VDR is a member of the superfamily of nuclear receptors for steroid hormones, which can be categorized as a ligand activated transcription factor. Details of the macromolecular interactions between genomic elements and ligands that bring about transcription of vitamin D response genes are not relevant to the present context. Suffice it to realize the complex and subtle nature of the inter- and intra-macromolecular interactions that form the basis for the genomic action of vitamin D and related expression of vitamin D responsive genes.

Non genomic action of vitamin D: There exist rapid-response non-genomic actions of vitamin D, which are mediated through cell surface receptors. Vitamin D induction of phosphoinositide metabolism shift the cytosolic calcium levels, cyclic GMP levels, Mitogen-activated protein (MAP) kinase levels, protein kinase C (PKC) levels, opening of chloride channel, etc., which are examples of such rapid action of vitamin D without genomic involvement. In chondrocytes, such rapid action of vitamin D results in an increase in membrane-lipid turnover, prostaglandin production and protease activity that lead to bone matrix modification and calcification. At least two distinct types of receptors are presumably involved in these actions, known as 1, 25 D3 MARRS (membrane associated rapid response steroid binding proteins) isolated from chick intestinal basolateral membrane, which is a thiol dependent o xo-reductase ERp57. VDR is also thought to play an important role in engendering to rapid action of 1, 25 (OH)2 D3.

Regulation of VDR-1, 25 (OH)2 D3 action

1,25 (OH)2 D3 action is determined by the net balance between the rate of uptake of the ligand into the cell and rate of its inactivation within the cell. Experiments show that intracellular ligand (vitamin D) inactivation plays critical role in the in situ control of response to vitamin D. Also, the actual content (Cone) of (IDBP3) may modulate ligand-VDR association - dissociation rates and therefore ligand depended VDR activation in a cell specific manner depending on the relative concentrations of the reactants.

VDR gene in chromosome 12 is present in allelic variants. Substantial variations of the polymorphic allele occur in different races and ethnic groups. Their expression associates with decreased bone density, propensity to hyperparathyroidism, resistance to vitamin D therapy, susceptibility to infections, autoimmune disorders and cancers. Therefore, systematic assessment of VDR-gene polymorphism in population groups, should find clinical application in disease prevention as well as in predicting the response to vitamin D therapy.

Post-translational modification of VDR

Ligand binding to VDR promote serine phosphorylation of the receptor. VDR phosphorylates in different loci. Phosphorylation of different loci are mediated by various kinases including casein kinase II, (at serine 208), PKC (at serine 51) and PKA with diverse effects on the transcriptional activity. Nuclear action of VDR could also be modulated by other hormone systems acting at cell surface to activate protein kinase cascades as demonstrated by inhibition of osteocalcin gene expression through 1,25 (OH)2 D3 also can cause rapid and sustained activation of PKC on bone cells that result in specific activation or inhibition of VDR.

Post-translational VDR modification is induced by substances from uremic plasma ultrafiltrate that reacts covalently with VDR at or near the DNA binding
domain and thus disrupting VDR-RXR binding of DNA. Kidney diseases related vitamin D resistance may be related to the above phenomenon.

**Vitamin D endocrine system: Role of 1, 25 (OH)\(_2\)D\(_3\) in calcium homeostasis**

The vitamin D endocrine system helps maintain extracellular Ca\(^{++}\) levels through its action in kidneys, bones, parathyroids and intestine. 1, 25 (OH)\(_2\)D\(_3\) produced in the kidney (proximal tubules) induces intestinal calcium absorption, controls bone-remodelling and suppresses PTH function (hormone production and cell growth)\(^{11}\). These effects of vitamin D hormone help attain calcium homeostasis.

**Bioactions of vitamin D**

**Intestine**

1, 25 (OH)\(_2\)D\(_3\) enhances the efficacy of small intestine to absorb calcium and phosphorus. Both 1, 25 (OH)\(_2\)D\(_3\) and VDR are required for optimal intestinal absorption of calcium. 1, 25 (OH)\(_2\)D\(_3\) induces active cellular calcium uptake and transport mechanisms. Calcium uptake requires epithelial calcium channel TRPV6 and to a lesser extent epithelial calcium channel TRPV5 calbindin D transport calcium across the cell and plasma membrane Ca\(^{++}\)channels, prealbumin and Na+, Ca++ exchanger mediate the final delivery of Calcium to the blood stream\(^{12}\). Initial calcium uptake is the rate limiting step in intestinal Ca++ absorption, which is highly dependent on vitamin D\(^{12}\). The expression of TRPV5 and TRPV6 channels is reduced in the VDR null mice. In contrast, the messenger RNA level of both channels are upregulated on calcitrol supplementation in wild type mice. The higher potency of calcitriol compared with the analogue 19-nor-1,25 (OH)\(_2\)D\(_2\) in upregulating the expression of the channels could account for the reduced calcaemic action of the latter in the intestine\(^{12}\). Intestinal TRPV\(_5\) and TRPV\(_6\) expression confers calcium influx with properties identical to those observed in native distal renal tubular cells including high Ca++ selectivity and negative feedback regulation to prevent Ca++ overload during transepithelial Ca++ transport\(^{13}\). Calcium transporting protein TRPV6, calbindin D9K and (PMCA lb) are increased by high dietary Ca++ in the duodenum of \(\alpha\) hydroxale null mice, thus demonstrating a 1, 25 (OH)\(_2\)D\(_3\) independent upregulation.

1, 25 (OH)\(_2\)D\(_3\) also increases active PO4 transport through stimulation of the expression of the Na-P\(_i\) co-transporter\(^{15}\) and changes the composition of the enterocyte plasma membrane that increases the fluidity and PO4 uptake\(^{16}\).

**Skeleton**

Vitamin D is essential for the development and maintenance of mineralized skeleton. However, administration of a rescue diet (high Ca, high P & high lactose diet) can make vitamin D non-essential for the maintenance of mineralized skeleton. So also in type II vitamin D deficient rickets, abnormalities of bone mineralization were completely resolved by calcium infusion\(^{17}\). However, only a combination of high calcium 1,25(OH)\(_2\)D\(_3\) could normalize chondrolyte growth.

Growth plate development requires co-ordinated calcium and 1, 25 (OH)\(_2\)D\(_3\) actions and VDR, whereas optimal osteoblastic bone formation and osteoclastic bone resorption demand both 1,25(OH)\(_2\)D\(_3\) and VDR. However, chondrocyte growth and development of growth plate are both dependent on 1, 25 (OH)\(_2\)D\(_3\). Moreover it has been demonstrated that the 1,25(OH)\(_2\)D\(_3\) VDR system is critical in PTH induced osteoclastogenesis.

1,25(OH)\(_2\)D\(_3\) regulates osteoclastogenesis in reciprocal regulation of receptor activation of NF-kB (RANK) ligand (RANKL) and osteoprotegerin(OPG). 1,25(OH)\(_2\)D\(_3\) increased the expression of RANKL on the surface of osteoblasts. RANK interactions with its receptor RANKL promotes maturation of osteoclast progenitor cells and mature osteoclasts, the bone resorbing cells. 1,25(OH)\(_2\)D\(_3\) VDR complex also represses the expression of OPG, a decoy receptor that binds RANKL and prevents RANK mediated osteoclastogenesis.

Interactions between osteoblasts and osteoclasts integrate bone remodelling. The binding of RANKL to RANK induces a signaling cascade that results in differentiation of maturation of osteoclasts. 1,25(OH)\(_2\)D\(_3\) as well as PTH and prostaglandins stimulate RANKL expression,\(^{18}\) but 1,25(OH)\(_2\)D\(_3\) also inhibits OPG production with corresponding increase in osteoclastogenesis and osteclast activity.

**1,25(OH)\(_2\)D\(_3\) VDR & parathyroids**

The 1,25(OH)\(_2\)D\(_3\) VDR system appears necessary for maximal PTH induced osteoclast production. The vitamin D endocrine system is a potent modulator of parathyroid function. Vitamin D deficiency results in parathyroid hyperplasia and increased PTH synthesis and secretion. However, 1, 25 (OH)\(_2\)D\(_3\) administration inhibits PTH synthesis and parathyroid cell growth, making 1,25 D\(_3\) therapy effective in treating secondary
hyperparathyroidism of chronic kidney disease. In addition to direct transrepression of PTH gene by the 1, 25 (OH)2 D3-VDR complex, the vitamin also regulates both parathyroid levels VDR and the response of the parathyroid gland to calcium. 1, 25 (OH)2 D3 induced increase in parathyroid VDR results from increases in its mRNA levels, possibly secondary to increase in serum calcium as well as through ligand dependent protection of proteosomal VDR degradation. In rats with kidney failure, a strong direct correlation has been shown to exist between serum 1, 25 (OH)2 D3 levels and parathyroid VDR protein content. Further, prophylactic 1, 25 (OH)2 D3 prevents the decrease in parathyroid VDR expression that accompanies the progression of kidney disease.

The PTH gene is directly transrepressed by 1,25(OH)2 D3-VDR complex. Besides, 1,25(OH)2 D3 regulates both the parathyroid levels of VDR and the response of the parathyroids to calcium. 1,25(OH)2 D3 induced increases in parathyroid VDR and its mRNA.

1, 25(OH)2 D3 has several renoprotective effects. They include attenuation of the development of glomerulosclerosis and retarding the progression of albuminuria.

Non classical actions of vitamin D

Wide range of evidences from genetic, nutritional and epidemiological studies link vitamin D endocrine system with diseases like hypertension, myopathic disorders, proneness to infection, autoimmune disorders and cancer. Thus clonal proliferation of lukaemic cells has been shown to be inhibited and their differentiation promoted by 1, 25 (OH)D3. Strong epidemiological evidence show linkages of prostate, breast & colon cancer with vitamin D deficiency. Cell biological studies show that 1,25 (OH)2 D3 - VDR system arrests cancerous cell cycle at G1- GO transition through multiple mechanisms. 1,25 (OH)2 D3 production decreases in the progression of malignancy in prostate cancer cells. 1, 25 (OH)D3 induced apoptosis contribute significantly to growth suppression properties of sterols in hyperproliferative disorders. In breast cancer cells, 1, 25 (OH)D3 induces apoptosis through reciprocal modulation in Bcl2 and Bax content. It also increases intracellular calcium which activates the calcium dependent proapoptotic proteases, microcalpain and capsase 12. It also enhances antitumoral properties of ionizing radiation. Thus, in general vitamin D has anti-cancer properties that are of potential clinical utility.

Other nongenomic action of 1, 25 (OH)D3 include, antiproliferative properties on keratinocytes in skin diseases like psoriasis, immuno suppressive properties on autoimmune destruction of Langerhans cells to prevent type I diabetes mellitus, etc. Such immune suppressive effects of 1, 25 (OH)2 D3 may prove valuable in the treatment of autoimmune disorders such as psoriasis, melanoma and scleroderma and type I diabetes mellitus.

Epidemiological observations suggest strong association between inadequate sunlight exposure related low 1, 25 (OH)D3 levels and high BP and/or high plasma rennin activity. It would appear that the 1, 25 (OH)2 D3 acts as a negative regulator of the renin-angiotensin endocrine system. In VDR null mice, marked increases in renin expression and plasma angiotensin II production caused hypertension, cardiac hypertrophy and increased water intake. In such animals intake of 1, 25 (OH)2 D3 suppressed renin production through a VDR mediated mechanism. In experimental animals, vitamin D deficiency is associated with an earlier and more aggressive form of diabetes, probably related to abnormalities in immune function and impaired glucose tolerance that can be relieved by calcitriol. 1, 25 (OH)2 D3 through a VDR mediated modulation of calbindin expression, appears to control intra-cellular calcium flux in islet cells, which in turn affects insulin secretion.

1, 25 (OH)2 D3 deficiency, in chronic renal failure results in abnormal insulin secretion, and blunted response of the pancreatic β cells, independent of alterations in VDR levels in pancreatic cells. Also, 1, 25 (OH)2 D3 administration corrects abnormal insulin secretion independently of changes in serum levels of calcium or PTH. The findings of 1 α-hydroxylase activity in pancreatic cells, raises the possibility of an autocrine control of insulin secretion by 1, 25 (OH)2 D3.

Evidences supporting direct action of 1, 25 (OH)2 D3 on skeletal muscles growth and differentiation exist. Thus skeletal muscle weakness and atrophy with electrophysiological abnormalities in muscle contraction and relaxation occur in chronic kidney disease related 1, 25 (OH)2 D3 deficiency. It also occurs in prolonged anticonvulsant treatment related low 1.25(OH)2D3 levels.

Vitamin D deficiency in India

Ever since the publication of Hodgkin and colleagues on uncommon prevalence of vitamin D
deficient rickets / osteomalacia among Punjabis in India, no studies appeared assessing the vitamin D status of subjects living in tropical and sub tropical latitudes of the India till 1995. Also there has been a prevailing impression among medical professionals that abundance of sunlight prevent vitamin D deficiency in India. However between 1995 and 2000\textsuperscript{27,28}, we published evidence to show that even doctors and nurses from northern latitudes of India have vitamin D deficiency. These observations are confirmed by other authors for Southern part of the country\textsuperscript{29}. These reports thus scientifically establish evidence for prevalence of vitamin D deficiency in the sub-continent, despite abundant sunlight. Meanwhile there is emerging evidence of wide prevalence of fluoride ingestion through drinking water in the sub-continent\textsuperscript{30}. There are also reports that scientifically show fluoride ingestion related renal tubular damage and related dysfunctions in the sub-continent\textsuperscript{31}. Thus it would appear that despite abundance of sunlight the people of the sub-continent are subjected to a variety of biologically (skin pigmentation) and environmentally (hydric fluorosis) induced dysfunctions that cause wide prevalence of bone mineral metabolic diseases, both sub-clinical and clinically overt, causing musculo-skeletal dysfunctions on the one hand and overt musculo-skeletal disorders on the other, due to vitamin D deficiency. Besides, practically the entire sub-continent has been mapped to have endemic fluorosis\textsuperscript{30}. Emerging scientific evidence also unfold variety of disorder affecting bones, muscles, kidneys and GI system to cause disorders of bone mineral metabolism, affecting millions living far and wide in the Indian sub-continent\textsuperscript{27,28,30}. Fortunately most of these are preventable and therefore appropriate measures like defluoridation of drinking water should be adopted countrywide expeditiously to prevent such disabling bone mineral metabolic diseases in India. Country-wide preventive intervention, in the form of milk and edible oil fortification with vitamin D would further enhance bone mineral metabolic health in India. Considering the magnitude and country-wide spread of such dysfunction and disorders, preventive measures of the type sighted are of urgent national need.

References


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