Elmer Verner McCollum (1879-1967) was the first to discover the antirachitic vitamin which he named as vitamin D in the early part of 20th century (1919). He had earlier discovered, vitamin A and B and was using the letters of the alphabet to name these factors. When Fink named them “vitamin”, McCollum opposed the move and hence the name vitamins. He did extensive work on the effects and deficiency of various nutrients on tissues and is credited with the discovery of trace minerals also. His parting words when he died in 1967 “I have had an exceptionally pleasant life and I am thankful”. Isolation and characterization of the chemical nature of the vitamin took another decade and Angus, Otto Diels and Kurt Alder were awarded the Nobel Prize in 1950 for their work, which eluded McCollum. The work of De Luca and Michael Follick pioneered the work on the elucidation of activation pathways of Vitamin D3 and its hormone like nature in the 1970s.

**FORMATION OF THE ACTIVE HORMONE**

Cholecalciferol or Vitamin D3 is formed in the skin by the action of UVB rays (290-315 nm) on 7-dehydrocholesterol. Provitamin D3 is first converted to previtamin D3 and then D3. Cholecalciferol undergoes 2 successive hydroxylation reactions. Vitamin D2 or ergosterol, the plant derived prohormone is also activated by a similar pathway. Therefore human beings get the vitamin either from exposure to sunlight or diet natural or fortified.

The 25-hydroxylase is a cytochrome P450 dependent hydroxylase, the product of the gene CYP27A1 which occurs in the liver. 25-hydroxy-D3 is biologically inert but represents the major storage and circulating form of the vitamin. 25-hydroxy-D3 circulates bound to D binding protein (DBP), taken up by renal cells where it is hydroxylated by a 1 alpha hydroxylase, another cytochrome P450 dependent enzyme, the product of CYP27B1 gene. The 1 alpha hydroxylase is tightly regulated by calcium homeostasis. Hypocalcemia and resultant secretion of PTH, upregulates and induces the CYP27B1 gene. When vitamin D status is low, the gene is upregulated. But when vitamin D status is high or adequate, it is inactivated by 24 hydroxylase coded by CYP24R1. A similar process of inactivation or conversion of cholecalciferol to inactive metabolites occurs in skin which prevents the formation of excess D3 on overexposure to sunlight. Advancing age, presence of melanin and use of sunscreen lotions decrease the formation of the vitamin.

**MECHANISM OF ACTION**

1,25-DHCC is transported bound to DBP to target cells where it translocates to the nucleus and binds to vitamin D receptor (VDR). The ligand receptor complex forms a heterodimeric complex with retinoid X receptor (RXR) and the ligand receptor complex binds to specific VDRE (vitamin D response element), located at the promoter region of target genes. The vitamin D receptor also known as NR111, (Nuclear receptor superfamily 1, group1, member1) is a member of the nuclear receptor subfamily of transcription factors encoded by the VDR gene.
The rate of gene transcription is co-regulated by SK11P. Five genes having VDRE or which are transcriptionally stimulated have been identified in target tissues, intestine, kidney, parathyroid gland and bone. The traditional calcemic effects were maintenance of calcium and phosphorus homeostasis and maintaining bone content.

1,25-DHCC is recognized by its receptor on osteoclasts causing an increase in the expression of the receptor activator nuclear factor kB ligand (RANKL). The receptor on pre-osteoclasts, RANK binds RANKL. Induction of pre-osteoclasts to become mature osteoclasts occurs. Mature osteoclasts remove calcium and phosphorus from bone. This would maintain the calcium and phosphorus levels in blood. Adequate calcium and phosphorus promote mineralization of the skeleton.

The Calcemic Effects of 1,25-DHCC are

i. Increased calcium absorption from intestine
ii. Increased reabsorption of calcium and phosphorus by renal tubules
iii. Increase in mineralization leading to trabecular and cortical bone density
iv. Feedback regulation by PTH secretion.

The vitamin D receptors can regulate gene expression by 3 mechanisms

i. Positive regulation by binding to VDREs present on the promoters of response genes
ii. Negative regulation by binding to inhibitory VDREs
iii. Inhibition of expression of genes by antagonizing the action of transcription factors like NF-AT and NF-kB.
iv. In addition to these increase in fibroblast growth factor 23, decreases 1 alpha hydroxylase activity reducing the levels of 1,25 DHCC.

The discovery that most tissues other than the previously listed target cells also have the vitamin D receptors and the enzyme machinery to synthesise the active 1,25-D3 have provided new insights into the actions and therapeutic implications of the vitamin.

The protective effect of vitamin in preventing cancer, ameliorating cardiometabolic risk and immunomodulatory effects in autoimmune diseases were re-emerged in the beginning of the 21st century. By 2007 many research papers on the beneficial effects of the vitamin in reducing the risk of cancer were published. Post menopausal women on calcium and vitamin D supplementation had increased levels of 25-OHD3 (40–60 ng/mL) and 60–70% reduction in the risk for cancer. Dr Edward Giovannucci, the chief proponent of the anticancer effects of D3, emphasized that too little sun increased the risk for cancer. The “Safe sun” is considered as sun bath for 15 minutes at a time, a few times a week, without sunscreen. The idea took much longer to be accepted since the fear of skin cancer on exposure to UVR was deep rooted in people.

Further work on the effects of D3 in reducing the cardiometabolic risk and type II diabetes mellitus, pointed to the changing lifestyles and increased incidence of obesity as the major factors producing a vitamin D deficient state.

Hypovitaminosis D in the 21st century

More than one billion people are pronounced as vitamin D deficient by current studies. The causes of vitamin D deficiency are

1. Decreased exposure to sunlight leading to decreased skin synthesis
   i. Covering by clothes
   ii. Use of sunscreen
   iii. Geographical location
   iv. Increased skin pigmentation-inverse relation (only fair skinned people can synthesis sufficient vitamin D3 in higher latitudes)
2. Decreased bioavailability due to malabsorption and obesity. Obesity causes sequestration of vitamin D in adipose tissue. The storage site for 25-OH-D3 is adipose tissue.
3. Increase in population of people aged over 65. A decrease in vitamin D production by 70% is noticed after the age of 65.
4. Decreased synthesis of 25-OH-D3 is seen in liver failure and increased excretion is noted in nephrotic syndrome due to loss of DBP.
5. Decreased synthesis of 1,25-DHCC in chronic kidney disease (CKD). In stages 2 and 3 of CKD, hyperphosphatemia increases the growth factor (FGF 23) that decreases 1 alpha hydroxylase activity. In stages 4 and 5, there is inability to produce the active vitamin.
6. Hereditary causes due to end organ resistance or mutations causing hypophosphatemia.

The long half life of 25-OH-D3 makes it the ideal marker of vitamin D status. Currently assayed by CLIA (chemiluminescent immunoassay). The increase in the number of assays of 25-OH-D3 has increased by 50% in 2009 compared to the previous year. 1,25-OH-D3 has a short half life and its level is maintained within normal limits by renal hydroxylation as long as kidney function is normal. Change in seasons, exposure to sunlight and dietary intake also can alter the levels. In Northern latitudes serum levels of 25-OH-D3 is lower by 20%. 30 minutes exposure to sun will increase the level. 40–50% of the circulating 25-OH-D3 is formed from skin.

Reference Range of 25-OH-D3 in Blood

Based on the finding that outdoor workers in tropics had values ranging from 40–65 ng/mL, the following values were suggested

<table>
<thead>
<tr>
<th>Reference range</th>
<th>30–75 ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&gt;30 ng/mL</td>
</tr>
<tr>
<td>Insufficient</td>
<td>20–29 ng/mL</td>
</tr>
<tr>
<td>Deficiency</td>
<td>10–19 ng/mL</td>
</tr>
<tr>
<td>Severe deficiency</td>
<td>&lt;10 ng/mL</td>
</tr>
</tbody>
</table>

WHO has suggested a serum level of 25-OH-D3 of < 20 ng/mL as deficient and less than 30 ng/mL as insufficient.

The National Health and Nutrition Survey conducted in USA found that dietary intake of vitamin D was as low as 200 IU/day. Accordingly 50–80% of the US population was found to be deficient with a mean value of 25 ng/mL.

It was noted that at serum levels below 30 ng/mL, PTH secretion was stimulated and active calcium absorption occurs presumably due to stimulation of calcitriol formation.

Recommended Daily Allowance

RDA has to be decided as 400 IU/day for adults and 600 IU/day in those over 60 years. Cancer prevention trials have suggested that the dose may be increased to 1000 IU/day in adults and 1500 IU/day to curb cancer risk. For every 100IU intake of the vitamin, the serum level increased by 1ng/mL.

The upper limit to avoid toxicity was fixed as 2000 IU/day. The maximum tolerable limit is 4000 IU/day and toxicity occurs with serum levels as high as 150 ng/mL. Since melanin reduces production of vitamin D3 by skin on exposure to sunlight, the RDA may be higher in black people compared to the white race. The evidence available so far does not indicate the optimum level of 25OHD3 required to turn on the VDR gene for its non classical effects.

A meta analysis of several trials in 2007, where 1200 mg of calcium and 800 IU/day of vitamin D3 supplementation resulted in a reduced risk of cancer. The Cochrane meta analysis of 2009 however showed only a marginal benefit in elderly in terms of gait speed and body sway.

Values <20 ng/mL were correlated with increased risk for metabolic, neoplastic and immune disorders like Type 1 DM, CVD risk and cancer risk.
Scientific basis for the present theories

1. 1,25-OH-D3 can modulate the expression of a large number of genes, about 5–10% of the genome.
2. Sequestration of 25-OH-D3 in the adipose tissue may produce deficiency in obese people.
3. Autocrine and paracrine actions may explain the actions on several tissues.

AUTOCRINE AND PARACRINE ACTIONS OF VITAMIN D3

Innate Immunity

Our predecessors knew the importance of bright sunshine for tuberculosis patients under treatment. Those who were inhabitants of higher latitudes were advised to stay at lower latitudes to facilitate better recovery. The practice of treating Lupus vulgaris by light radiation is another example.

Present scientific knowledge has brought to light the basis of this practice. Pathogens binding to TLR (Toll like receptor) induces transcription of VDR and CYP27B expression. Circulating 25-OH-D3 bound to plasma DBP enters macrophages, gets converted to 1,25-OH-D3 by mitochondrial CYP27B. The active form binds to VDR receptor and acts as a transcription factor. The upregulation of proteins with innate antibacterial action (cathelicidin) occurs. In addition, the hormone has been found to have paracrine effects on monocytes and T or B lymphocytes. Studies have shown positive results of vitamin D supplementation in the progression and incidence of autoimmune diseases like type 1 diabetes mellitus, IBD, multiple sclerosis, etc. It is seen that 1,25-DHCC is a powerful immunomodulator.

Keratinocytes

The cancer protective effect of vitamin D is attributed to the effects on keratinocytes. The observed effects are (a) The ability to arrest abnormal growth, (b) decrease angiogenesis in tumor tissue and (c) induce cell death at the appropriate time are the observed effects. Keratinocytes have been found to express both 1 alpha hydroxylase and 24 hydroxylase, so that active hormone is produced. At the same time, inactivation of any excess is also ensured preventing toxicity. There are also reports that 1,25-DHCC can also modulate the expression of tumor suppressor genes.

Autocrine and paracrine actions on bone

The beneficial effects of administration of D3 and calcium to patients with hip fracture in terms of increase in cortical and trabecular bone as well as the low circulating levels of 25-OH-D3 in elderly people who developed fracture hip point to an autocrine and paracrine role for 1,25 DHCC in the bone. The 1,25-DHCC is formed in the bone by activation and expression of CYP27B.

Effects on RAS

The effects of vitamin D3 in hypertension, kidney diseases and diabetes mellitus are attributed to its action through down regulation of RAS. Vitamin D is also seen to increase insulin production and improve myocardial contractility.

The Indian Scenario

According to Indian workers, changing food habits and reduced outdoor life have significantly reduced the availability of the vitamin, leading to increased prevalence of hypovitaminosis D in India also. The suggestion at a national level is to fortify dairy products and increase awareness to the advantages of exposure to sunlight.
The Current Status

Dynamics of vitamin D storage and reentry into circulation remain poorly understood even in the obese. Optimal dosage required to avoid deficiency/insufficiency also is uncertain. Most of the retrospective and prospective trials indicate a serum level <20 ng/mL as deficient and increasing the risk for cancer. Similar trials have also indicated a decreased incidence of Type 1 diabetes in children who had vitamin D supplementation. The results of further studies like the ongoing trial “VITAL” (Vitamin D3 and omega 3 fatty acids) in primary prevention of cancer and cardiovascular risk with 2000 IU/day of vitamin D3 supplementation may give more definite results.

To quote Hector De Luca, “It is starting to look impressive, but certainly it is not cause and effect”