NFI BULLETIN
Bulletin of the Nutrition Foundation of India
Volume 22 Number 1
January 2001

Vitamin D Deficiency In North India

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During the last three decades there has been remarkable advancement in the understanding of the biology of vitamin D and its role in human health and disease. In this article, current concepts in the biology of vitamin D are reviewed and our own recent work briefly reported.

BIOLOGY OF VITAMIN D

Vitamin D – a pro-hormone: Vitamin D is currently regarded as a steroid hormone. It is synthesised by the human skin on exposure to ultraviolet light. It is a pro-hormone converted to its active form through hepatic and renal metabolism. The production of its most potent metabolic derivative, 1, 25, $(OH)_2$ D_3 in the kidney is tightly regulated. Just as other steroid hormones, its biological action is mediated through a specific, high-affinity nuclear receptor present in target tissues. The 1, 25, $(OH)_2$ vitamin D_3 receptor has been demonstrated in a large number of target tissues, including intestinal epithelium, hemoeopoietic tissues, immune system, pituitary, pancreas and brain. Its important role in the growth and functional differential, through paracrine and autocrine mechanisms of several tissues, is being progressively unravelled. Besides, evidence is accumulating to show that target tissues themselves can metabolically convert vitamin D_3 to active normal form through tightly regulated and tissue-specific metabolism. For instance, it has been shown that functionally activated macrophages convert 25 (OH), D_3, to 1, 25 $(OH)_2$ D_3. In target cells, such as intestinal epithelium and osteoblasts, it induces genomic expression of functional proteins. Thus, new knowledge on vitamin D supports the view that it is more truly a hormone than a vitamin.

Metabolism of vitamin D: Vitamin D and its biologically active hydroxyl derivatives are synthesised from 7-dehydrocholesterol in skin. The key step in the chemical transformation of 7-dehydrocholesterol is the opening up of the B ring of the cyclopentanoperhydrophenantherene structure to yield pre-vitamin D_3, which isomerises to form the 9, 10 secosteroid known as vitamin D. The opening of the B ring of 7-dehydrocholesterol is a non-enzymatic chemical process brought about by the exposure of stratum malphigii of epidermis to 290-310 nm ultraviolet light from direct sunlight. The various conformers of vitamin D_3 formed by this process are in equilibrium in the skin. Holick *et al*[1] have shown that latitude-specific geographic habitat of people is an important determinant of ultraviolet light mediated vitamin D secosteroid synthesis in the skin.

Thus, in temperate northern latitudes, the incident slanting sunrays are not very effective in bringing about the dermal transformation of 7-dehydrocholesterol to pre-vitamin D_3 because of greater absorption of the ultraviolet rays during the longer course of slanting sunrays through the earth's atmosphere. In old age, dermal vitamin D synthesis declines because of age-related decline in 7-hydrocholesterol content of skin. Melanin is a strong absorbent of ultraviolet rays and, hence, a powerful inhibitor of ultraviolet rays mediated dermal synthesis of vitamin D. Besides, in tropical climates, because of the ambient heat and intensity of sunlight, a major proportion of dehydrocholesterol in the skin is converted to inactive Lumisterol and Tachysterol.

Vitamin D_3 forms in the skin photochemically and is transported into the circulation by the vitamin D-binding protein. In the liver mitochondria, the circulating vitamin D_3 is hydroxylated at the C-25 position to form 25 (OH) D_3. In the kidney, the 25 (OH) D_3 is further hydroxylated at 1 or 24 carbon atoms to form 1 α, 25 $(OH)_2$ D_3 or 24, 25 $(OH)_2$ D_3. Proximal renal tubule is the principal site for these key hydroxylation processes. However, other tissues such as bone, placenta, and intestines have been shown to have this ability.

Besides the 1 α and 24-hydroxylated products, over 35 different additional metabolic products of

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vitamin D have been isolated. The processes that govern the formation of such a large number of metabolites in the body and their biological significance are currently not known. This area is one of intensive investigation to look for metabolites of different functional specificity, in view of the wide and varied distribution of vitamin D_3 receptors in different tissues of the body.

Hydroxylation of 25 (OH) vitamin D at 1 α and 24-positions are mediated by the enzymes 1 α hydroxylase and 24-hydroxylase. These enzymes are mixed function oxidases present in mitrochondria. The relevant mitochondrial redox proteins comprise three components present in the inner mitrochondrial membrane named ferrodoxin, ferredoxin reductase, and cytochrome P-450. The linked reaction that ultimately transports electrons from NADPH to the terminal cytochrome P-450 component, resulting in the reduction of one molecule of oxygen (O_2) to one molecule of water and one hydroxyl function. The hydroxyl function so generated is transferred stereo-specifically to the respective carbon atoms of the secosterol to generate the respective hydroxylated derivatives.

Regulation of vitamin D metabolism: Of the several factors that regulate 25 (OH) vitamin D_3 metabolism in the kidney, parathyroid hormone (PTH) and 1, 25 $(OH)_2$ D_3 are the most important. Conversion of 25 (OH) D_3 to 1, 25-dihydroxy D_3 is down-regulated by the product of the reaction itself, in a step of auto-regulation. PTH, on the other hand, upregulates 1, 25 $(OH)_2$ D_3 formation. In the case of hydroxylation of vitamin D_3 at 24 position, the relevant enzyme activity is inhibited by PTH and stimulated by 1, 25 $(OH)_2$ D_3. The predominant hydroxylated product of 25 (OH) D_3 formed in the kidney at a given time is determined by vitamin D and calcium status of the individual. Thus, in vitamin D deficient states, 1-hydroxylase activities is high and 24-hydroxylase activity is low. Consequently, vitamin D deficient states will be associated with elevated 1, 25 $(OH)_2$ D_3 and PTH levels and decreased 24, 25 $(OH)_2$ vitamin D_3 levels. The reverse will hold good in vitamin D replete states. There is thus a reciprocal relationship between PTH and 1, 25 $(OH)_2$ D_3 action on 1-hydroxylase and 24-hydroxylase enzyme activities.

Calcitonin, estrogen and pituitary hormones are also shown to have some regulatory influence on vitamin D metabolism in the kidney. Thus, calcitonin stimulate 1, 25 $(OH)_2$ D_3 formation, perhaps indirectly through the parathyroid gland. Studies on avian models demonstrate enhanced 1-hydroxylase activity in response to *in vivo* administration of oestradiol. This affect may well be due to the effect of estrogen to increase the level of DBP. Estrogen has also been shown to increase the number of PTH receptors in kidneys, an effect that could lead to elevated 1-hydroxylase activity, in the absence of changes in circulatory PTH concentrations. A role for GH or PRL in vitamin D metabolism in humans is not clearly demonstrated, though in experimental rat models hypophysectomy result in lowered 1, 25 (OH) D_3 and increased 24, 25 $(OH)_2$ D_3.

Biological actions of vitamin D: Intestine, bone and kidneys are the classical organs shown to be responding to vitamin D action. However, during the last 30 years, an increasing number of tissues (at present the list incorporates 37 tissues) have been shown to express vitamin D receptors. These facts are consistent with the emerging concept that the biologically active vitamin D derivative (1, 25 $(OH)_2$ D_3 calcitriol) is a pleuripotent hormone with far-ranging functions encompassing almost all organ-systems of the body. It has been shown to subserve a key role in such vital biological functions as cell proliferation and differentiation, besides playing an important role in bone-mineral metabolism through its action on intestine, bone and kidneys. Such is the versatility of the calcitriol molecule – it is envisaged that its analogs may provide therapeutic tools that could subserve such diverse functions as immune modulator, inducer of cell differentiation and as specific calcitropic agents. Indeed, the entire gamut of metabolic derivatives (total estimated to be over 37) of vitamin D is at present denoted collectively as the vitamin D endocrine system.

VITAMIN D DEFICIENCY IN NORTH INDIA

It has been long recognised that emigrants from South Asia to the developed countries of the North, such as the UK, are very susceptible to vitamin D deficiency and related osteomalacia. In 1973, S.W. Stranbury and colleagues examined the question as to why South Asian emigrants are susceptible to clinical vitamin D deficiency by studying the prevalence of osteomalacia among native Punjabis living in India[2]. In the township of Ludhiana, they conducted a field survey for chemical osteomalacia by measuring blood levels of calcium phosphorous and alkaline phosphatase in a cohort of normal subjects. The study showed that only one of the 114 subjects examined showed laboratory features suggestive of osteomalacia. This study published in the *Lancet* has been quoted widely to support the belief that the people living in sunny South Asian countries have no vitamin D deficiency. On the other hand, in a review of the problem of rickets or osteomalacia, by Bhattacharyya[3], the wide prevalence of rickets in South Asia was comprehensively documented.

TABLE 1
Direct Sunlight Exposure and 25 (OH) Vitamin D Status

Study groups	Maximal exposure (min /day) sunlight	25 (OH) D (nmol/L)	PTH (intact) (ng/L)	Total Serum Ca (mmol/L)
Soldiers	370 ± 3[a]	47.17 ± 11.73[a]	17.6 ± 4.8b[b]	2.35 ± 0.17[a]
Physicians and nurses	25 ± 5[b]	7.98 ± 3.49[c]	38.8 ± 18.2[a]	2.17 ± 0.10[b]
Depigmented subjects	5 ± 5[c]	18.2 ± 11.23[b]	35.5 ± 12.6[a]	2.22 ± 0.10[a]

Data is given as mean ± SD; Values in the same column with different superscript letters are significantly different, $P<0.05$.

At the All India Institute of Medical Sciences, New Delhi, cases of osteomalacia have been frequently seen in the endocrine and metabolic services unit. Thus, a recent study showed that more than 60 per cent of indoor admissions due to metabolic bone diseases were accounted for by osteomalacia and its complications. A significant proportion of these was patients treated with anti-TB drugs for putative TB spine, because of presenting symptoms of pain and stiffness of spine. Paradoxically, the anti-TB medication invariably worsened their condition, making many of them bed-ridden. In our experience, such patients get dramatically cured by appropriate treatment with vitamin D and calcium supplements. Similar experience has been reported from other hospitals of Delhi and other North Indian cities. Because of the widely held belief that osteomalacia does not occur in the tropics, clinicians started investigations to rule out rare and oesoteric genetic disorders, involving derangements of vitamin D metabolism[4], or of calcium malabsorption due to high phytate, oxalate and phosphate contents of Indian diet. The possibility of social customs such as *purdhah,* causing poor sunlight exposure among Muslim women, was also entertained. However, none of these could be established as the dominant cause of rickets and osteomalacia in North India.

Clinical experience with metabolic bone disease in North India has been a perpetual source of bewilderment to physicians. The clinical picture in many of the patients does not conform to descriptions available in literature based on experience in developed countries. Several of them do not resemble, neither morphologically nor radiologically, any well-characterised syndrome of bone-mineral metabolic disorders described in literature. A case in point has been the clinical picture of proven hyperparathyroidism in India[5]. Predominant bone disease, often associated with a typical radiological picture; normocalcemia in as much as half of them; unusually large size of resected adenoma are all common experiences of clinicians dealing with the problem in India.

In 1991-92 we studied a cohort of such patients with histologically proven parathyroid adenoma and showed that coexistent vitamin D deficiency was an important cause of these unique features of hyperparathyroidism in India[6]. A curious observation made during this study was that the group of normal control subjects incorporated in the study also showed markedly low 25 (OH) vitamin D_3 levels. This led to a detailed study[7] of vitamin D status of groups of normal healthy subjects who differed with respect to variables relevant to vitamin D and bone mineral metabolic status such as direct sunlight exposure, seasonal variation, skin pigmentation, dietary calcium and phytate content, and altered physiological states such as pregnancy and neonatal age.

The results showed that all study groups except the one with maximum direct sunlight exposure had sub-normal concentrations of 25 (OH) vitamin D_3 (Table 1). The vitamin D deficient groups tended to have imbalance in bone-mineral metabolic homeostasis when exposed to winter weather, low dietary calcium with high phytate content, significantly low calcium and high parathyroid hormone levels, indicating sub-clinical osteomalacia. In pregnant mothers, normal value of 1, 25 $(OH)_2$ D did not prevent disturbed bone-mineral metabolic homeostasis caused by the deficiency of 25 (OH) D. These observations, for the first time, provided scientific evidence of a wide prevalence of vitamin D deficiency and related sub-clinical dyshomeostasis of bone-mineral metabolism in apparently normal subjects exposed to situations that strain bone-mineral metabolism in North India.

Whether similar problems of vitamin D deficiency related sub-clinical bone-mineral metabolic dyshomeostasis occur among normal subjects living in the lower latitudes of the southern part of peninsular India, where sunlight is more intense, wintery climatic conditions do not prevail and dressing habits are different with enlarged scope for better sunlight exposure, are questions that remain to be investigated. However, at present, there is strong scientific evidence to refute the report of S.W. Stanbury and colleagues that vitamin D deficiency does not occur in India due to abundant sunlight.

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FOUNDATION NEWS

Annual Foundation Day Lecture

- Dr Prema Ramachandran, Advisor (Health), Planning Commision, on 'Nutrition in a knowledge-based society', on November 24.

Study Circle Lectures

- Dr R.L. Bijlani, Prof and Head, Department of Physiology, AIIMS, New Delhi, on 'Probiotics: Nutritional Considerations', on October 4.
- Dr R.P. Britt, Clinical Haematologist, UK, on 'Newer methods for haemoglobin estimation in the field', on December 13.

President's Engagements

- Attended the 32nd Annual Meeting of the Nutrition Society of India, held in Hyderabad on December 1 and 2.
- Presided over the 27th Kamala Puri Sabharwal Lecture at the Lady Irwin College on December 11.

The Foundation is grateful to FAO and WHO for matching grants towards the cost of this publication