VITAMIN D REVISITED: METABOLISM AND ACTIONS

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Abstract: Vitamin D, a prohormone, is initially converted to 25(OH) D_3 in the liver. Further hydroxylation in the kidney forms 1,25 $(OD)_2$ D_3, the active metabolite of the vitamin D. Traditionally, vitamin D has been considered to be concerned with calcium homeostasis. However, the discovery of vitamin D receptors (VDR) in diverse tissues such as gonads, stomach, epidermis, pituitary gland, pancreas, breast, parathyroid gland, thymus, T-lymphocyte, cardiac muscle, skeletal muscle and placenta etc. gave rise to speculation that vitamin D may have more diverse physiological role than hitherto believed to be. In this review, information on metabolism, VDR receptors and mode of action of 1, 25 $(OH)_2$ D_3 has been updated. Besides "traditional actions", the "newer actions" of vitamin D on parathyroid gland, endocrine pancreas, keratinocytes, immune system and reproduction including pregnancy have been discussed.

Key words: Vitamin D, 1, 25 $(OH)_2$ D, VDR, Keratinocytes, Parathyroid gland, Insulin secretion, immune system, Pregnancy

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A. Introduction

Like most of the vitamins, the discovery of vitamin D was a consequence of the knowledge of its deficiency disorders. The first clinical description of rickets appeared in 1650[1] after the widespread appearance of rickets in northern Europe due to industrialization. The incidence of rickets reached serious proportions with the development of urbanized industrial population. Smoky skies coupled with relatively indoor life necessitated by this environment drastically reduced the solar exposure of the people, thereby curtailing the chief source of vitamin D. Cod-liver oil was recognized as a therapeutic measure for curing rickets in 1811[1]. By the beginning of 20th century, the relation between dietary deficiency and many diseases such as beriberi and scurvy was demonstrated and the term vitamin was introduced by Funk in 1912[2]. In a series of publications, Mellanby[3,4,5] demonstrated that rickets is a deficiency disease and it could be cured by cod-liver oil- or butter fat but attributed the cure to "fat soluble A." Huldscginsky in, 1920, provided the experimental proof of the curative effects of u.v. radiation on rickets[1]. In 1925, McCollum had the honor of naming the fourth discovered vitamin as vitamin D6. In 1924, Steenbock showed that phytosterol and ergosterol became rich in vitamin D after U.V. radiation [7,8]. Subsequently, vitamin D was crystallized from irradiated ergosterol and the compound was named calciferol[9]. In 1935, Windaus et al.[10] determined the chemical structure of calciferol, and 7- dehydrochlesterol was shown to be a provitamin D. After this, except for official adoption of the name vitamin D_2, for ergocalciferol and vitamin D_3, for cholecalciferol[11], the research activity on vitamin D almost came to stand still. Only in late sixties, the role of the liver and the kidney in vitamin D metabolism was elucidated. Since then, there has been a spurt of research activity on vitamin D and every year hundreds of papers appear in literature on various aspects of vitamin D metabolism.

B. VITAMIN D METABOLISM

(i) Cutaneous Production of Vitamin D

Diet is a very poor source of vitamin D. Vitamin D does not occur in vegetable kingdom. In non-vegetarian diet, egg and fish liver oil are the only important sources of vitamin D. Cow's milk is a poor source. However, cutaneous synthesis of vitamin is an important source of vitamin D. Most of the vitamin D synthesis occurs in the actively growing layers of the epidermis (strata spongiosum and basale) by exposure to sunlight[12]. Radiation

energies between 290 and 320 nm are most effective[13]. 7-dehydrocholesterol present in the epidermis acts as a provitamin D. Ultraviolet radiation produces a cleavage of B ring thereby forming previtamin D, (9,10 -secosteroid). Previtamin D undergoes a temperature dependent isomerization to form vitamin D_3 (also called cholecalciferol), taking 2-3 days for completion of the process . The unique thermally regulated synthesis of vitamin D_3 ensures a gradual release of the vitamin from the epidermis into circulation. This concept is confirmed by the observation that subjects exposed to whole body U.V. radiation have a significant increase in the circulating concentrations of vitamin D_3, about 6-9 hours after the exposure that reaches a peak 24-48 hours after the exposure, before gradually returning to baseline by 7 days[14]. Once vitamin D is formed, vitamin D-binding protein in the dermal capillary circulation helps to translocate the vitamin from blood-less epidermal tissue into circulation.

Melanin pigment present in the epidermis interferes with the synthesis of vitamin D by absorbing u.v. radiation. The view of Loomis[15] that skin pigmentation is evolved for the control of vitamin D synthesis in the skin is supported by excessive cutaneous melanin seen in populations exposed to greater u.v. radiation. Moreover, it has been observed that when surgically excised skin from Blacks and Caucasians were exposed to solar radiation, greater amount of vitamin D was produced in the latter[16]. However, now it is clear that melanin is only one of the many factors that regulate photosynthesis of vitamin D in the skin. Cutaneous production of vitamin D seems to be under an autoregulatory control. Excessive exposure of even Caucasian skin to sunlight does not cause vitamin D intoxication. Continuous exposure to UV radiation depletes cutaneous provitamin D, but does not increase production of previtamin D. Holick et al.[17] have reported the effect of exposure of skin for different durations to sunlight. During the first 10-15 minutes of exposure, approximately 15% of provitamin D changed to previtamin D. After one hour of exposure, 40 % provitamin D was depleted but only 15 % increase in previtamin D was observed. The remaining 25% of photolyzed provitamin D was accounted for by the presence of inactive isomers, tachysterol and lumisterol (Fig. 1). Further solar exposure depleted the stores of provitamin D in the epidermis, but the concentration of previtamin D_3 or vitamin D_3 did not increase. Vitamin D_3 being heat-sensitive, may also be photodegraded to 5,6-trans-vitamin D_3 and suprasterol[17].

Because of the complex mechanism of vitamin D_3 production in the epidermis, the amount of solar exposure required for providing vitamin D adequate for the body's requirements varies in different individuals and under different conditions. The photosynthesis of vitamin D_3 depends upon (i) the surface area of the skin exposed to sunlight, (ii) the time of the day of exposure (UV radiation is most intense between 11 AM and 2 PM, (iii) the amount of melanin pigment present in the epidermis, (iv) latitude (UV radiation is most intense at the equator), (v) season (in winter less UV radiation reaches the surface of the earth, (vi) Environmental pollution such as smoke, fog and dust prevents UV radiation from reaching the earth. However, prolonged exposure to sunlight does not necessarily mean greater production of vitamin D since as mentioned earlier, solar radiation can isomerize previtamin D_3 to inactive, isomers, tachysterol and lumisterol as well as produce photodegradation of vitamin D_3[17].

Vitamin D-binding protein has no affinity for tachysterol or lumisterol and hence translocation of these isomers into circulation does not occur. These products are sloughed off during natural turnover of skin. Patients with uremia seem to be unable to produce vitamin D in the skin. It is believed that one or more substances present in the skin of a patient with chronic renal failure act like melanin and absorb UV radiation [18].

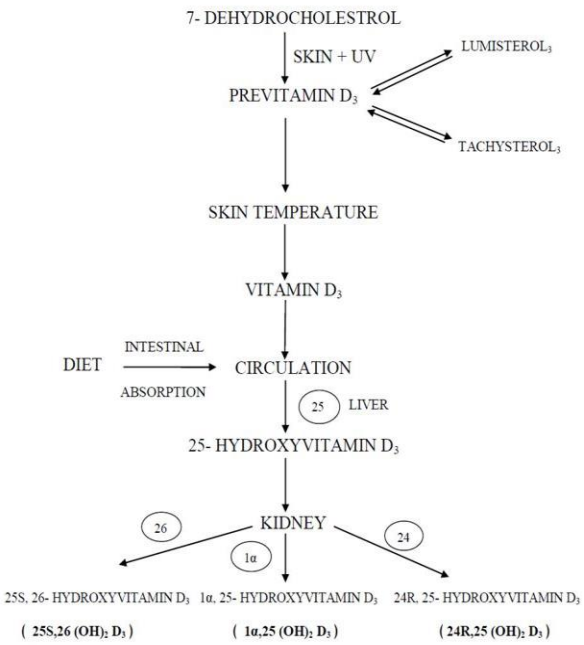

Fig.1. Vitamin D metabolism.

(ii) Hepatic Metabolism of vitamin D

Vitamin D_3, synthesized in the skin, enters the circulation bound to vitamin D-binding protein. Dietary vitamin D_2 or D_3 enters the circulation through lymphatic system. Subsequently, both vitamins D_2 and D_3 are metabolized similarly.

In the liver, vitamin D is metabolized by vitamin D-25-hydroxylase to form 25-hydroxyvitamin D [25(OH) D] (now also known as calcidiol). The enzyme is located in the mitochondrial and microsomal fractions of the hepatocytes [19]. Although there are few reports of the presence of extrahepatic vitamin D-25-hydroxylase in the chick and the rat [20], the liver seems to be the only site of 25(OH) D synthesis in humans. The reserve capacity of vitamin D-25-hydroxylase in the liver is substantial. Severe parenchymal damage is required to lower the level of plasma 25(OH)D [21]. The enzyme vitamin D-25-hydroxylase does not seem to be tightly regulated since circulating levels of 25(OH) D vary with the amount of dietary intake of vitamin D or with the degree of solar exposure[22]. Decreased plasma 25(OH)D levels are observed in patients with nephrotic syndrome having proteinuria greater than 4g/day, due to renal loss of vitamin D tagged to vitamin D-binding protein [23].

(iii) Renal Metabolism of Vitamin D

As early as 1833, Lucas[24] recognized the association between chronic renal disease and bony lesions resembling rickets. Observations of similarity in bony lesions in patients of nutritional rickets and those with chronic renal failure, led Liu and Chu [25] to propose that uremia interferes with the action of vitamin D. It was only in 1970 that Fraser& Kodicek demonstrated the intimate relation between the kidney and vitamin D metabolism [26]. These workers demonstrated that homogenates of chick kidney could metabolize 25(OH) D to a biological active metabolite. It was also shown that physiological concentrations of 25(OH) D could not stimulate intestinal calcium transport in anephric rat[27]. Fraser & Kodicek[26] identified the active metabolite as 1,25-dihydroxycholecalficerol [1,25 $(OH)_2$ D_3] (now also known as calcitriol). The renal 25 (OH)-1- alpha-hydroxylase is located in the proximal convoluted tubules[28] . It is now accepted that 1, 25-dihydroxy metabolites of vitamin D_2 or D_3 are the biologically active forms of vitamin D_2 and D_3 respectively. These metabolites are 10 times more active than vitamin D_2 or D_3 in healing rickets or stimulating intestinal calcium absorption [29].

The activity of renal 25-OH-1-alpha hydroxylase appears to be tightly regulated since plasma 1, 25 (OH) 2 D_3 concentration remains constant over a wide range of substrate 25 $(OH)D_3$. Parathormone (PTH) seems to play a crucial role in the synthesis of calcitriol since it was found that hypocalcemic vitamin D deficient rats could metabolize calcidiol to calcitriol more effectively than normocalcemic vitamin D replete rats[30]. But, when vitamin D-deficient hypocalcemic rats were thyroparathyroidectomized, the difference was lost[31]. However, according to Holick et al.[22], PTH may not be absolutely essential for the synthesis of 1, 25 $(OH)_2$ D, since patients with hypoparathyroidism often have low-normal concentrations of calcitriol. Under certain physiological conditions, factors other than PTH may regulate 1, 25 $(OH)_2$ D synthesis. In pregnancy,

and lactation, growth hormone, estrogens and prolactin seem to enhance renal production of 1, 25 $(OH)_2$ D directly or indirectly [32,33] .

(iv) Alternate Renal Metabolic Pathways for 25 (OH) D

When vitamin D nutrition and circulating plasma concentrations of calcium and phosphorus are normal, 25 (OH) D is metabolized into a variety of products (Fig. 1), by hydroxylation at C 24, 25 and 26 to form 1,25 $(OH)_2$ D, 24,25 $(OH)_2$ D and 25,26 $(OH)_2$ D [22]. The plasma concentrations of each of 24, 25 $(OH)_2$ D and 25,26 $(OH)_2$D are 50-100 times the concentration of 1,25 (OH) D. The metabolites other than 1, 25 $(OH)_2$ D have no biological activity. Production of 25 (OH) D is uncontrolled. Its plasma concentrations vary directly with the dietary intake/ cutaneous production of vitamin D. When plasma concentration of 1, 25 $(OH)_2$D is adequate, remaining 25(OH) D is converted to 24,25 $(OH)_2$D or 25,26 $(OH)_2$D. Renal 25 (OH)-1-alpha-hydroxylase converts two inert metabolites mentioned above to 1,24,25 trihydroxy cholecalciferol (1,24,25 $(OH)_3$ D) and 1,25,26 trihydroxy cholecalciferol (1,25,26 $(OH)_3$D). The trihydroxy metabolites again have no biological activity.

(v) Extrarenal Metabolism of 25(OH)D

Initially, kidney was believed to be the only site of 1, 25 $(OH)_2$ D synthesis. Twenty-four hours after injection of ^{3}H- 25(OH)D, ^{3}H-1,25 $(OH)_2$ D could be detected in the blood and tissues of vitamin D deficient rats but not in vitamin D deficient rats that had undergone bilateral nephrectomy before receiving radioactive 25 (OH)D [34]. Later it was discovered that bilateral nephrectomy reduced but did not abolish the conversion of 25(OH) D to 1,25 $(OH)_2$ D [35]. In vitro studies have confirmed that placenta is the 1, 25 $(OH)_2$ D synthesis in pregnancy[36]. In addition, in vitro, a wide variety of cultured cells from normal human bone, and osteosarcoma have a capacity to convert 25 (OH) D to 1, 25 $(OH)_2$ D[37]. These observations also help to explain why hypercalcemia occurs in some patients of sarcoidosis, tuberculosis, silicosis, Hodgkin's disease and non-Hodgkin lymphoma. Such patients have recently been shown to have elevated plasma levels of 1, 25 (OH)2 D [38,39]. To date, 25-OH-1-α-hydroxylase has been reported in many cells and tissues including prostate, breast, colon, lung, pancreatic β cells, monocytes, and parathyroid cells. However, the extrarenally produced 1, 25$(OH)_2$D primarily serves as an autocrine/paracrine factor with cell-specific functions [40](Fig. 2).

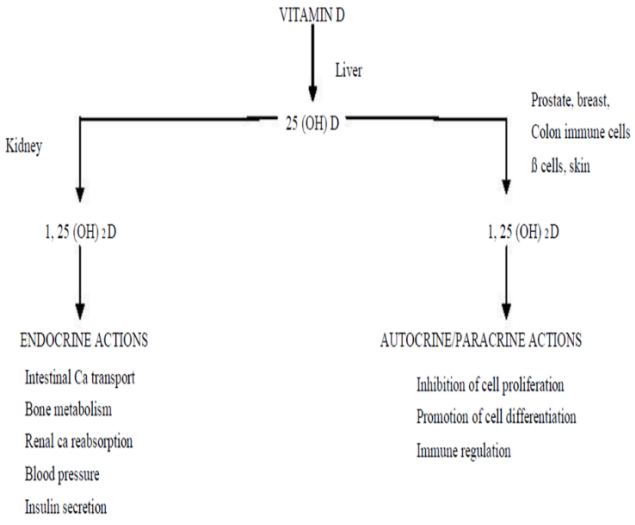

Fig.2. Renal and Extrarenal production of 1,25-dihydroxyvitamin D. (After Dusso et al[40]).

C. MECHANISMS OF ACTION OF 1, 25 $(OH)_2$ D

Vitamin D receptor (VDR) was discovered in 1968[41]. In 1980s, VDR was found to be widely distributed in different tissues of the body such as gonads, stomach, epidermis, pituitary gland, pancreas, breast, parathyroid gland, thymus, cardiac muscle, skeletal muscle, placenta etc. [42,43]. Initially no physiologic significance was attached to such reports; only intestine, bone and kidney continued to be recognized as the target tissues of 1, 25 $(25)_2$ D. Subsequently, reports indicated calcium-binding protein synthesis in many of these tissues, including brain [43]. In vitro, 1, 25 $(OH)_2$ D was found to inhibit proliferation of human fibroblast cells and keratinocytes, increase TSH synthesis, inhibit PTH synthesis [44,45].

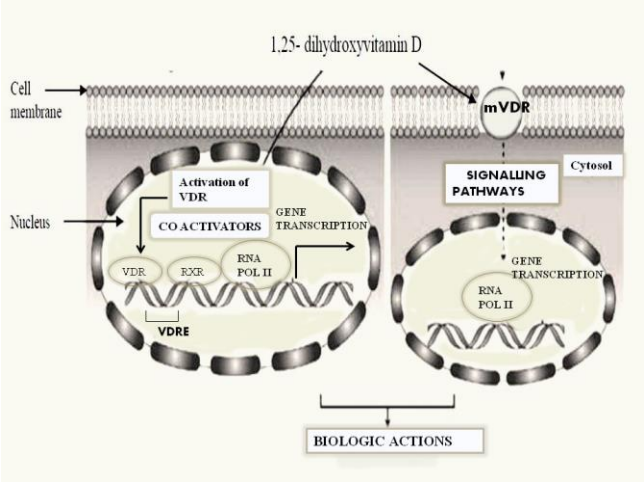

Fig. 3. Mechanism of genomic (left) and non-genomic (right) actions of vitamin D_3. (Adapted from Sergio et al. [46]).

(i) **VDR and transcription regulation**

The genomic action of 1, 25$(OH)_2$ D is well described. Most of the pleiotropic and long-term actions of 1, 25$(OH)_2D_3$ are mediated through genomic actions. 1, 25$(OH)_2D_3$, in concert with vitamin D binding protein (DBP), is transported to the nucleus, where it binds to the vitamin D receptor (VDR). The VDR then complexes with retinoid X receptor (RXR), forming a heterodimer, which then binds the vitamin D-responsive element (VDRE) located in the promoter region of the gene. This association recruits either co-activators or co-suppressor molecules (depending on the tissue type). This triple complex then binds to the transcription machinery [46](Fig. 3) .

(ii) **Non-Genomic action of 1,25 (OH) D**

A variety of hormones, those serve as ligands for nuclear hormone receptors, also exert biological actions that do not require gene regulation. They seem to act through cell membrane receptor rather than nuclear receptors. 1, 25 $(OH)_2$ D has been shown to have rapid effects in selected cells through membrane receptors. The proposed mechanism of non-genomic action of 1, 25 $(OH)_2$ D is shown in Fig. 3.

D. BIOLOGICAL ACTIONS OF VITAMIN D

(i) Classical actions

The classical actions of vitamin D on the intestine, bone and kidney are concerned with calcium homeostasis.

(a) Intestine.

In the enterocytes of the small intestine, the genomic action of 1,25 $(OH)_2$ D results in greater production of not only calcium binding protein, calbindin, but also alkaline phosphatase and a brush border protein [43,47].The net result is greater absorption of dietary calcium and phosphates. The mechanisms by which 1,25$(OH)_2$D regulates transcellular calcium transport are best understood in the intestine. Here 1, 25$(OH)_2$ D stimulates calcium entry across the brush border membrane into the cell, transport of calcium through the cell, and removal of calcium from the cell at the basolateral membrane. Entry at the brush border membrane occurs down a steep electrochemical gradient. The molecular mechanism of 1, 25 $(OH)_2$ D as a stimulator of intestinal phosphate absorption remains unknown, despite many efforts by the investigators [48], but a cytosolic calcium binding protein calbindin-D9K seems to be involved [49].

(b) Bone

Bone and muscle accumulate about 60% of injected dose of vitamin D [50]. Though gross skeletal abnormalities have been observed in vitamin D deficient animals, no direct effect of 1, 25 $(OH)_2$ D on the process of ossification has been observed. 1, 25 $(OH)_2$ D does not seem to be essential for ossification of bone. When plasma calcium and phosphate levels were maintained at normal range in vitamin D deficient rats by dietary manipulation, the skeletal histology was found to be normal [51]. However, in cultured rat osteosarcoma cells, 1, 25 $(OH)_2$ D stimulates the synthesis of osteocalcin , the bone derived protein, in a dose dependent manner [52]. In patients with postmenopausal osteoporosis, 1,

25 $(OH)_2$ D administration has been shown to increase circulating osteocalcin levels [53].

Mobilization of calcium from the bone is another well-known function of vitamin D, especially when administered in pharmacologic doses. At physiologic concentrations, 1, 25 $(OH)_2$ D acts in concert with parathormone to stimulate osteoclastic activity [54]. At pharmacologic concentrations, it was found to stimulate osteoclastic activity by inducing stem cells to differentiate into osteoclast cells [55]. Exposure to human peripheral monocytes that possess receptors for 1, 25 $(OH)_2$ D results in their differentiation into multinucleated giant cells capable of mobilizing calcium from bone chips [56].

(C) Kidneys

The most important endocrine effect of 1, 25$(OH)_2$ D_3 in the kidney is a tight control of its own homeostasis through simultaneous suppression of 1-α-hydroxylase and stimulation of 24-hydroxylase.

In the kidneys, 1, 25 $(OH)_2$ D increases reabsorption of calcium in the distal tubules through a cytosolic transport protein calbindin-D28K seems to be important [49]. However, 1, 25$(OH)_2$ D_3 involvement in the renal handling of calcium and phosphate continues to be controversial due to the simultaneous effects of 1, 25$(OH)_2D_3$ on plasma PTH and on intestinal calcium and phosphate absorption, which affect the filter load of both ions .

(ii) Non-classic actions of vitamin D

1. Role of Vitamin D Hormone in the Parathyroid Gland

Perhaps the most well-established non-classic function of 1, 25$(OH)_2$ D_3 is in the parathyroid gland. Specific localization of 1, 25$(OH)_2$ D_3 in the parathyroid gland [42] and the presence of VDR[57] strongly suggested that 1,25$(OH)_2$ D_3 may have a direct action through its receptor in the parathyroid glands. Moreover, PTH secretion by isolated parathyroid glands or cells could be suppressed by the direct administration of 1, 25$(OH)_2$ D_3[58]. Likewise, the vitamin D appears to be involved with the development of both primary and secondary hyperparathyroidism [59]. The specific mechanism by which vitamin D interacts with the parathyroid gland to bring about observed effects is not yet fully understood. But, knowledge has with clear implication on prospects of possible medical treatment of hyperparathyroidism [60].

2. Role of Vitamin D Hormone in Skin

Hosomi et al.[61] provided evidence for the first time that 1, 25$(OH)_2$ D_3 induces keratinocyte differentiation.. Exactly how important this differentiation effect of 1, 25$(OH)_2$ D_3 is in vivo is difficult to assess. Certainly, vitamin D-deficient animals do not have a problem with keratinocyte differentiation. Thus hyperproliferation of the keratinocyte and failure to differentiate is not found in vitamin D-deficient animals [62]. The differentiation of the keratinocyte is associated with an inhibition of proliferation. This inhibition of proliferation has been utilized in the treatment of hyperproliferative diseases of skin as, for example, psoriasis. Both 1, 25$(OH)_2D_3$ and analogs can be used as a significant therapy against psoriasis with as many as 70% patients responding to this treatment [63]. However, exactly how 1, 25-$(OH)_2$ D_3 induces differentiation of the keratinocyte and inhibits proliferation remains to be investigated. It has been proposed that the keratinocyte functions in a paracrine fashion in which 1, 25 $(OH)_2$ D3 is produced by the keratinocyte itself to stimulate differentiation of the keratinocyte [64].

3. Role of Vitamin D in the Immune System

The presence of the VDR in activated T lymphocytes was reported by Provvedini et al.[65]. These results suggest a role for 1, 25-$(OH)_2D_3$ in the immune system, but the role is just now beginning to be defined. VDR have been reported in thymus, a repository of immature lymphocytes, as well. Vitamin D deficiency markedly reduces the ability of mouse to develop delayed hypersensitivity reaction [66]. These results suggest that T-helper cell lymphocyte is vitamin D responsive, but both immunostimulation and immunosuppression can be

found in in vivo conditions. Currently, there is no evidence that B-lymphocyte-mediated immunity is influenced by 1, 25 (OH)2 D [48].

The most dramatic results obtained to date in the immune system are those found in experimental autoimmune encephalomyelitis (EAE) that can be induced in mice. Administration of 1, 25 $(OH)_2$ D3 suppressed the development of the disease in experimental animals [67] (Fig 4). Current results strongly suggest that 1, 25 $(OH)_2$ D3 or its analogues function by stimulation TH-2 T-helper cells to produce transforming growth factor-β1 and IL-4.

Of some interest is the idea that immunomodulation action of vitamin D might be useful in the management of transplant rejection [48]. The possible use of vitamin D in the treatment of autoimmune diseases such as diabetes mellitus, rheumatoid arthritis is being investigated.

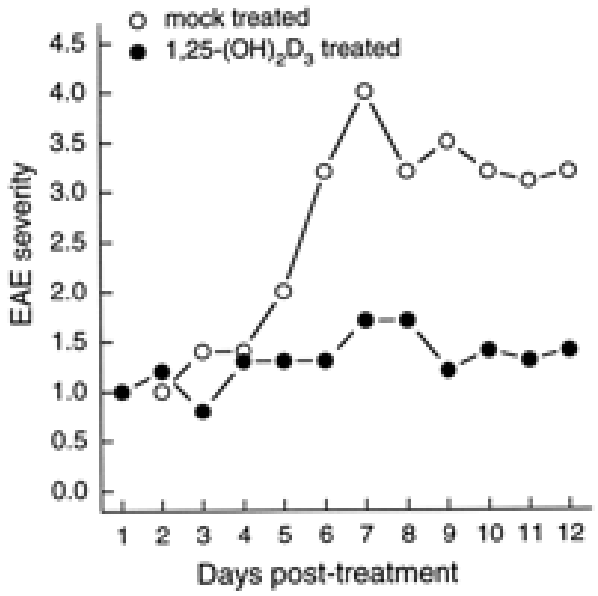

Fig. 4. Effect of administration of 1,25 (OH) 2 D on the development of induced experimental autoimmune encephalitis in rats (After Cantorna et al. [67]).

4. Role of vitamin D in Insulin Secretion.

The presence of a VDR in the cells of islets of Langerhans is now well accepted, but it is unclear as to what, if any, role vitamin D plays in the functioning of the islet cells. Initial results revealed that vitamin D-deficient rats were unable to respond to a glucose challenge by secreting appropriate amounts of insulin, which could be corrected by the administration of 1, $25(OH)_2D_3$ [68]. Other studies suggested that the effect of 1, 25 $(OH)_2$ D was mediated by the action of vitamin D in raising plasma calcium concentration[69]. Moreover, onset of experimental diabetes can be delayed by administration of 1, 25 $(OH)_2$ D[70]. Thus, a role of vitamin D on islet insulin release is most likely; either direct or indirectly through its effect on plasma calcium concentrations. Therefore, the relationship between vitamin D and diabetes is certainly worthy of further investigations.

5. Role of Vitamin D in Reproduction

Initially, during the course of producing vitamin D-deficient rats, came the observation that female reproduction is markedly diminished in vitamin D deficiency [71]. A reduction in fertility of 80% was found and could not be corrected by correcting the hypocalcemia[72]. This defect, therefore, is quite clearly one related to an absence of the vitamin D molecule. The infertility brought about by vitamin D deficiency in the female rat can be easily corrected by the administration of 1, 25-$(OH)_2D_3$ [72]. All such reports suggested that the ovary is a target of vitamin D action. More over the observations that ovarian cells contain VDR[73] and 1, $25(OH)_2D_3$ accumulates in the ovarian cells [42] lends further support to the view.

In the case of male reproduction, vitamin D deficiency also reduces the effectiveness of the male [74]. A significant reduction found in sperm count in vitamin D rats [75] could be reversed by vitamin D repletion [76] . However, this diminished male fertility can be corrected by merely providing

additional calcium, raising plasma calcium concentration which in turn restores fertility [72]. In chicks, vitamin D seems to be essential for proper egg hatchability [77] and for normal embryo development [78].

Severe vitamin D deficiency in human pregnancy is known since long to produce congenital rickets [79,80]. The apparent benefits of vitamin D supplementation on intrauterine and neonatal growth of the fetus were initially demonstrated in Asian women by Brooke et al. [81] and by Marya et al.[82-84]. Brooke et al. administered 1000 IU of vitamin D, per day to Asian immigrants in the U.K. during the third trimester of pregnancy and observed a significant decrease in the incidence of low birth weight babies. Although, there was no significant difference between the mean birth weight in the supplemented and non-supplemented groups but a follow up study revealed significantly greater weight and height of babies from the supplemented group at the age of 9 months and 12 months, even though neither the mothers nor the babies received any vitamin D supplements postnatal [81]. Studies by Marya et al. [82-84] were conducted in Hindu women of Haryana (India). Administration of 600,000 units of vitamin D, in 7th and 8th months of pregnancy led to birth of infants with significantly greater birth weight and increase in certain other anthropometric measurements such as length, head circumference and skinfold thickness . Administration of 1200 IU of vitamin D, per day, throughout the third trimester also improved the fetal birth weight but to a lesser extent . The clinical studies suggest that administration of moderately high doses of vitamin D during pregnancy not only improves the intrauterine growth of the fetus but continues to confer the beneficial effect on the growth of the baby during the first year of life also.

Experimental studies conducted by Marya et al. [85-89] in the rat confirmed the improvement in neonatal growth of pups whose mothers received vitamin D supplementation during pregnancy. In these experimental studies, female rats fed on adequate amounts of vitamin D, calcium and phosphates were injected 300 IU, or 7500 IU vitamin D 3 as a single intramuscular injection on 10-12 day of pregnancy. The birth weight of the pups of supplemented groups was not different from controls but on d10, d20 d28, the pups weighed significantly greater than those in control group. At d 28, the pups in the supplemented groups showed significantly greater weights of liver, brain, gastrocnemius muscle and tibia. Neonatal growth was also found to be significantly better in pups whose mothers received vitamin D in early lactation, although vitamin D supplements directly to pups in early lactation did not produce any beneficial effect [90].

The clinical and experimental studies led to a large number of studies on the effects of vitamin D supplementation during pregnancy on maternal and fetal outcomes. The rationality of such studies was the reports that women all over the world suffered from vitamin D deficiency during pregnancy deficiency [91, 92]. Maternal vitamin D deficiency has been found to affect postnatal head and linear growth [93]. Hollis et al. [94] conducted a double blind trial on the possible benefits of vitamin D supplementation during pregnancy. It is concluded that vitamin D supplementation of 4000 IU/d for pregnant women is safe and most effective in achieving sufficiency in all women and their neonates regardless of race, whereas the currently suggested requirement is comparatively ineffective at achieving adequate circulating 25(OH)D concentrations, especially in African Americans.

De-Regil et al. [95] conducted a Cochrane review of six randomized controlled trials in 1023 women. The results showed that the provision of vitamin D supplements during pregnancy improves the women's vitamin D levels, as measured by 25-hydroxyvitamin D levels, at term. However, the clinical significance of this finding is yet to be determined as there is no evidence that vitamin D supplementation prevents pre-eclampsia, gestational diabetes or impaired glucose tolerance. Data from three trials involving 463 women show a trend for women who receive vitamin D

supplementation during pregnancy to less frequently have a baby with a birth weight below 2500 grams than those women receiving no treatment or placebo, although the statistical significance was border line. The number of trials and outcomes reported are too limited, and in general are of low quality, to draw conclusions on the usefulness and safety of this intervention as a part of routine antenatal care. Further rigorous randomized trials are required to evaluate the role of vitamin D supplementation in pregnancy [95].

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