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Vitamin D – metabolism, physiological functions and pathological consequences of its deficiency

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ABSTRACT

Vitamin D3 is a prohormone produced in skin through ultraviolet irradiation of 7-dehydrocholesterol. It is biologically inert and must be metabolized to 25-hydroxyvitamin D3 in the liver and then to 1,25-dihydroxyvitamin D3 in the kidney to achieve an active form. The hormonal form of vitamin D3, i.e., 1,25-dihydroxyvitamin D3, acts through a nuclear receptor to carry out its many functions, including calcium absorption, phosphate absorption in the intestine, calcium mobilization in bone, and calcium reabsorption in the kidney. It also has several non-calcemic functions in the body. This overview provides a description of the synthesis, physiological roles and pathological consequences of vitamin D deficiency.

Keywords: *1,25-dihydroxyvitamin D3, VDR.*

1. INTRODUCTION

At global level, there are not many foods rich in vitamin D, while supplementation programs are either optional, or not applied in a constant manner, inadequate or inexistent. Therefore, vitamin D is synthetized in the human body mainly at the level of cutaneous tissue, as a result of solar exposure. However, there are many factors that influence the amount of UV radiations to which a person can be exposed to, such as: the time of the day when the exposure takes place, latitude, altitude, clothing, the use of solar protection cosmetic products, skin color or age [1].

Assessment of the vitamin D level in a population can represent a difficult process. Despite the fact that 1,25 dihydroxy vitamin D3 [1,25(OH)2D] represents the biologically active form of vitamin D, most of its effects correlates better with the circulating 25(OH)D levels. A possible explanation is represented by a broader (extra renal) distribution of the 1α-hydroxylase enzyme, responsible for the intracellular synthesis of 1,25 (OH) 2 D in many other target tissues, resulting in local increased concentrations, with specific implications on the respective tissue and cells [2].

It is therefore preferable to study the concentration of 25 (OH) vitamin D and not that of 1,25 (OH) D. The deficiency of vitamin D leads to an increase in parathyroid hormone (PTH) levels, which on its turn increases the activity of 1α-hydroxylase, which influences the serum level of 1,25 (OH) D. In addition, 1,25 (OH) D has a half-life of only 5 hours, while the half-life of 25 (OH) D is of 2-3 weeks, considered to be in balance with vitamin D deposits from the organism [3]. The purpose of the present review was to reveal some aspects regarding the physiological roles of vitamin D into the organism and the pathological consequences of its deficiency.

2. SYNTHESIS AND METABOLISM

Vitamin D is assimilated from food (10-20%) or results from skin synthesis under the influence of solar radiation (80-90%) [4]. The 1,25(OH)2D is the major biologically active metabolite of vitamin D from sterol family. The vitamin D precursor (cholecalciferol or vitamin D3) is either ingested from the diet or synthesized in the skin from 7-dehydrocholesterol (provitamin D3) as a result of exposure to the sun. The form of vitamin D derived from plants is vitamin D2 or ergosterol. None of the two forms of vitamin D has significant biological activity. Both should be metabolized to active forms. This activation occurs in two stages, initially in the liver then in the kidneys. Cholecalciferol is transported to the liver linked to a specific α1 globulin. In the liver, vitamin D undergoes a hydroxylation process that results with the formation of 25-hydroxy vitamin D (calcidiol), a metabolite with limited biological activity. Since the liver does not interfere significantly at this stage, the circulating level of 25-hydroxy vitamin D reflects the level of vitamin D either ingested or synthesized in the skin. 25-hydroxy vitamin D is then transported by a kidney-binding protein where it is hydroxylated for the second time by 1-α hydroxylase in the proximal renal tube to form a 1,25-dihydroxyvitamin D3 (calcitriol). The renal hydroxylation of the 25-hydroxy

vitamin D represents a major event from vitamin D metabolism, being regulated by serum phosphate, calcium and circulating PTH. The PTH and decrease of phosphorus concentration in the serum act independently on increasing the synthesis of 1,25(OH)2D by inducing 1-α hydroxylase activity, PTH being the strongest stimulus [5].

The low calcium concentration stimulates the parathyroid glands to increase the amount of synthesized PTH, which in turn increases the production of 1,25(OH)2D in the proximal kidney tubules. Conversely, an increase in blood calcium suppresses PTH secretion, which reduces the production of 1,25(OH)2D. The only extra renal production sites of 1,25(OH)2D are the placental and granulomatous tissues. In humans the half-life of 1,25(OH)2D is 5 hours, being excreted as a residual metabolite via urine and feces.

There are many other metabolites of vitamin D produced in the kidney, most of them being biologically inert. The most notable are 24, 25-dihydroxy vitamin D3 produced by 24-α hydroxylase, which is activated when the level of PTH is low or when the phosphate level is high [5].

2. PHYSIOLOGICAL ROLE OF VITAMIN D

The main physiological role of vitamin D is to maintain constant levels of calcium ions in the extracellular environment. Extracellular calcium is vital for the functioning of many metabolic processes and neuromuscular activities. Vitamin D influences the calcium level mainly by controlling its absorption in the intestine, by its direct action on bone tissue and by the effect on the secretion of the PTH. Vitamin D3 or cholecalciferol, after its synthesis in the skin, and vitamin D2 or D3 assimilated from food undergo a first hydroxylation process in the liver to form 25-hydroxyvitamin D, the main form found in circulating blood. 25(OH)D subsequently undergoes a novel enzymatic hydroxylation mediated by 1α-hydroxylase, resulting in the active form, the double hydroxylated vitamin D, 1,25(OH)2D or calcitriol. This form acts through specific vitamin D receptors to regulate not only calcium metabolism but also differentiation and division of different cell types [6]. The 1,25(OH)2D attached to a binding protein is transported to the gut where the free form is taken up by the cells and transported to a specific nuclear receptor. Although the receptor binds to several forms of vitamin D, the affinity for [1,25(OH)2D is approximately 1000 times higher compared to 25-hydroxyvitamin D3, indicating that the former is much biologically active than the second. As a result of this intestinal interaction, the calcium binding protein is synthesized and osteocalcin and alkaline phosphatase are produced in the bones. In the intestine, the role of 1,25(OH)2D is to stimulate the transport calcium and phosphate from the lumen of the small intestine into the circulation by stimulating the expression of calcium transport proteins, resulting in an increase in plasma levels of calcium and phosphate. It also increases bone resorption and the effect of PTH in nephron to accelerate reabsorption of calcium in the renal tubes.

The 1,25(OH)2D is a strong differentiating agent for osteoclast precursors, causing them to maturate and to form multi-nucleated cells that are capable of bone resorption.

This pathway enables [1,25(OH)2D to provide a calcium and phosphate supplement necessary at the bone surface to form normal mineralized bone.

Therefore, deficiency of vitamin D has implications for bone demineralization, secondary hyperparathyroidism (caused by persistent hypocalcaemia), acute loss of bone cortex and is associated with the pathogenesis of osteoporosis and bone fractures [6].

It is suggested that in addition to its central role in bone mineralization, in calcium homeostasis or in the many pathologies resulting from the disruption of these processes, vitamin D is also involved in muscular processes, in the pathology of psoriasis, certain types of cancer, cardiovascular diseases, Alzheimer disease, multiple sclerosis, diabetes etc. [6]. The correlation of different pathologies with low levels of vitamin D has led to the hypothesis that the administration of vitamin D supplements could be useful for the treatment of these diseases.

3. PATHOLOGICAL CONSEQUENCES OF VITAMIN D DEFICIENCY

3.1. Vitamin D and obesity. A link between obesity and vitamin D deficiency was revealed, but the determination of the mechanism behind these observations could not be elucidated. There are studies suggesting that a high body mass index (BMI) leads to a decrease in vitamin D level, whereas a reverse effect of low vitamin D on the BMI is unlikely. Obesity is a factor favoring vitamin D deficiency, but there is no evidence that low levels of vitamin D may be the cause of obesity [7], nor that administration of supplements with vitamin D could treat this condition. However, there has been shown that 1,25(OH)2D is active in adipose tissue and is involved in various processes such as modulation of inflammation or adipogenesis [8]. Therefore, patients with a BMI higher than 30 kg/m2 require higher or more frequent doses of vitamin D [9].

3.2. Vitamin D and diabetes. It is known that insulin secretion requires calcium, which suggests that indirectly, vitamin D could contribute to maintaining insulin secretion.

The conditions that link vitamin D deficiency with hyperglycemia include type 1 and type 2 diabetes. Among the individuals suffering from these conditions, the incidence of vitamin D deficiency is higher than that in the healthy population. Pancreatic β cells express vitamin D receptor (VDR), vitamin D binding proteins and some allelic variants of the genes involved in vitamin D metabolism [10].

In type 1 diabetes, vitamin D administration in people with prediabetes may prevent or even reduce the autoimmune process, possibly by regulating the T-cell selection process, reducing the number of auto-destructive ones, and stimulating the formation of regulatory T cells. Although this immune modulation effect is still under discussion, the same reasoning may also be relevant for type 2 diabetes [10]. A normal maternal vitamin D level during pregnancy, and implicitly the fetus, reduces the risk of developing type 1 diabetes of the newborn. The risk increases when vitamin D deficiency occurs in the second trimester of pregnancy, when pancreatic β-cells of the fetus are formed (around week 12), and insulin secretion beginning (around the week 20 of pregnancy). For this reason, the mother should start taking supplements with vitamin D at the latest in the second trimester of pregnancy [11]. There is increasing evidence to highlight the role of vitamin D deficiency and the development of type 2 diabetes. Vitamin D improves the negative biochemical effects of type 2 diabetes, probably by increasing insulin secretion, by the positive effect it has on β-pancreatic cell function, and by decreasing the number of cytokines and insulin resistance [12]; this improvement in insulin sensitivity also occurs in people who do not suffer from glucose metabolism disorders.

3.3. Vitamin D and cardiovascular diseases. Several studies demonstrated that vitamin D deficiency has an essential role in the development of cardiovascular diseases. The expression of vitamin D receptor and enzymes that metabolize vitamin D has also been found to occur in the vascular system even in the heart. Numerous observational studies have been carried out confirming the association of low vitamin D levels with an increased incidence of cardiovascular disease and mortality. There are studies showing a non-linear decrease in mortality as the concentration of 25(OH)D increases [13]. Furthermore, the low levels of 25(OH) D have been reported as a risk factor, particularly of cardiac events that resulted in death [14]. Although there are many studies regarding the influence of vitamin D status on the risk of cardiovascular disease, a specific correlative mechanism has not yet been fully elucidated. Currently there is insufficient data to make general recommendations for vitamin D supplementation in order to prevent or treat cardiovascular disease [15].

3.4. Vitamin D and osteoporosis. Osteoporosis represents a common pathology, characterized by bone loss and bone damage, which increases bone fragility and fracture risk. Osteoporosis is often associated with a low calcium intake, but vitamin D deficiency also contributes to low calcium absorption [16]. Although rickets and osteomalacia are extreme consequences of the vitamin D deficiency, osteoporosis is an example of the long-term effect of calcium and vitamin D insufficiency. Appropriate vitamin D levels and deposits maintain bone strength and prevent osteoporosis in older adults, immobilized individuals experiencing exercise difficulties, postmenopausal women, and individuals who receive steroid treatments [17].

Normal bone tissue is in continuous remodeling. During the menopause, the balance of these processes changes, thus reaching the situation when the quantity of reabsorbed bone tissue is higher compared to the newly synthesized bone. Hormone therapy with estrogen and progesterone may be able to delay the onset of osteoporosis. There are professional groups and societies that support Hormone Replacement Therapy (HRT) as an option for women at risk of osteoporosis or fractures [18].

Most methods regarding vitamin D supplementation also include calcium administration, so it is difficult to isolate the effects of each nutrient. These supplements result in a slight increase in bone mineral density in postmenopausal women as well as in elderly men. It also helps reduce the incidence of fractures in institutionalized individuals, but the benefits are not so clear to the rest of the population. Supplemental therapy only with vitamin D appears to have no effect in reducing the risk of fractures, nor does it reduce the chances of falling for elderly people [19, 20].

3.5. Vitamin D and menopause. Decreases in estrogen functions observed during menopause result in an increase in bone metabolism, a decrease in bone mineral density and, implicitly, an increased risk of fracture. Moreover, weight gain, muscle mass depletion and visceral adipose growth affect most postmenopausal women, placing this group of patients among those at high risk of metabolic and cardiovascular disease.

Several studies have reported that with aging a decrease in vitamin D synthesis occurs in the skin, similar time exposure to sun radiation in older people producing up to 75% less vitamin D compared to young adults [21]. The prevalence of low vitamin D levels appears to be higher in menopausal women, especially among those suffering from osteoporosis or fractures [22]. Moreover, PTH levels are higher among the elderly, although they have serum levels

of 25-hydroxyvitamin D similar to those of young people, which can negatively affect bone metabolism [23]. In addition, the decline in estrogen concentrations after menopause decreases the activity of 1α-hydroxylase, which further implies a decrease in the synthesis of the active vitamin D form. These results suggest that a supplement with vitamin D, even at high doses, may be necessary in postmenopausal women to counteract PTH activity, possibly also exacerbated by low renal function.

Supplementation with vitamin D appears to be the most appropriate treatment option for postmenopausal patients and is recommended by more experts as a risk-free and inexpensive procedure. However, the role of vitamin D supplementation in the prevention and treatment of co-morbidity associated with aging and the consequences of menopause has not yet been fully elucidated.

There are well-documented guidelines and strategies for vitamin D supplementation that target the elderly (> 65 years) population in Central Europe which recommend supplementing with 800-2000 IU / day (20-50μg / day) throughout the year, due to the reduced vitamin D synthesis rate into the skin. In obesity, supplementation is recommended to be 1600-4000 IU / day (40-100 μg / day), depending on the severity of obesity, also throughout the course of the year [24].

3.6. Vitamin D and carcinogenesis. Recently, other effects of vitamin D, such as anti-proliferative, anti-inflammatory, pro-apoptotic, pro-differentiation, immune function modulation have been revealed. A particular attention has been paid to the potential of vitamin D analogs, in combination with other anti-tumor agents, in the treatment of some cancers. Moreover, it has been shown to have an inhibitory effect on the growth of tumor cells expressing VDR, like prostate, colon, breast, lung, and pancreas cancer cells [25]. Some epidemiological studies have suggested that the low levels of vitamin D are associated with an increased risk of breast cancer. A molecular epidemiological study identified several VDR genes, and finally showed that a polymorphism of the FokI and BsmI genes significantly correlated with the increased risk of ovarian and breast cancer [26]. The vital role of vitamin D has also been highlighted in the pathogenesis of ovarian cancer [27].

3.7. Vitamin D and skin disorders. Calcitriol (1,25-dihydroxyvitamin D3) has been used in topical applications in the treatment of certain skin pathologies, including psoriasis, a skin condition consisting of hyperproliferation of keratinocytes. Several studies have shown that topical application of ointments containing calcitriol (3 μg/g) is not a risk and can be effective against plaque psoriasis [28, 29]. Vitamin D analogs, such as calcipotriene or calcipotriol, have also been used to treat chronic plaques psoriasis, either by single administration or in combination with corticosteroids [30, 31].

Vitamin D Metabolism in Keratinocytes. The epidermal keratinocytes possess both the enzymes required to convert vitamin D to its active form, 1,25-dihydroxyvitamin D3 [32] and its receptor (VDR), a transcription factor that regulates gene expression. The active form of vitamin D acts like a steroid hormone. Inside the nucleus, VDR-associated with 1,25(OH)2D is heterodimerised with the retinoic acid X receptor (nuclear receptor). This complex binds small DNA sequences called vitamin D response elements (VDRE); this coupling initiates a cascade of molecular interactions that modulate transcription of certain genes [33]. In this way, 1,25(OH)2D acts locally and regulates the proliferation and differentiation of keratinocytes.

The biological activity of vitamin D in the skin. Epidermal control of proliferation and differentiation. The basal layer of the epidermis consists of a layer of round and undifferentiated keratinocytes located above the dermis. The cells in this basal layer proliferate constantly to produce new cells that will later enter the epidermal layer. From the moment keratinocytes leave the basal layer, differentiation processes (cell specialization for different functions), namely keratinization or cornification [34], is initiated.

Photo-protection. The skin may be affected by UV. Depending on the dose, ultraviolet rays can cause DNA damage, inflammatory responses, skin cell apoptosis, skin aging, and malignant melanoma. There have been investigated if 1,25-dihydroxivitamin D3 applied locally to the skin before or immediately after irradiation and concluded that vitamin D had photo-protective effects.

Documented effects of vitamin D in the skin include a decrease in DNA damage, apoptosis, an increase in cell survival rate and a decrease of erythema. The mechanisms of these effects are not very well known, but in one of the studies conducted on mice, 1,25(OH)2D induced the expression of metallothionein (a protein that protects against free radicals and oxidative stress) in the basal layer [35]. It was also demonstrated that other non-genomic actions of vitamin D contribute to photo-protection [36].

Other functions. In the skin, vitamin D receptor (VDR) acts independent of its association with 1,25(OH)2D. For example, VDR is important in regulating cell growth of mature hair follicles [37, 38]. It has been demonstrated that certain VDR mutations lead to a defective regulation of gene expression manifested by aberrations of the follicular cycle and hair loss in mice and humans [38]. VDR also acts as a tumor suppressor in the skin [39].

It can be concluded that 1,25(OH)2D and its VDR receptor have different biological functions, including

regulation of proliferation and differentiation of keratinocytes, hair follicle cycle and tumor suppression. Some studies on rodents and cell cultures have shown that the active form of vitamin D has a photo-protective effect. Moreover, vitamin D modulates inflammatory processes and may be involved in wound healing. However, further studies are needed to understand the exact role of vitamin D and VDR in maintaining skin health.

3.8. Deficiency of vitamin D in the pediatric population.

The necessary amount of vitamin D cannot be assured by human milk alone [40]. Nutritional deficiency of vitamin D is usually the result of inadequate nutrition, poor absorption, and increased need for intake or increased excretion rate. A vitamin D deficiency can occur when the usual intake is less than recommended for a certain period of time, exposure to the sun is limited, or there is a disability of the kidneys to turn 25(OH) vitamin D into its active form.

In children, vitamin D deficiency causes rickets, a disease characterized by inappropriate bone mineralization, resulting in softening of the bone followed by deformation of the skeleton. Causes of rickets include prolonged breastfeeding without age-recommended vitamin D supplements, especially if breastfeeding mothers have vitamin D deficiency, intensive use of sunscreens, and lack of outdoor activities. Rickets is also more common among immigrants from Asia, Africa and the Middle East, possibly due to genetic differences in vitamin D metabolism and a different cultural behavior resulting in a low sun exposure [41].

There are also found mentioned in literature other effects of vitamin D on health of children and adolescents, including prevention of immune diseases (asthma, diabetes type 1), infectious diseases (respiratory infections, flu) and cardiovascular disease [42]. In our country there are still cases of rickets, although since 2002 there is an on-going program for the prophylaxis of rickets with vitamin D.

4. GLOBAL VITAMIN D STATUS

A global report has shown a prevalence of vitamin D deficiency in almost all regions investigated. There are still insufficient data from some parts of Asia and most of Africa. The degree of deficiency varies from region to region. While levels of 25 (OH) vitamin D below 30 ng/ml are present in most populations, levels below 10 ng/ml, which represent a pronounced deficiency, are most common in risk populations, especially in the elderly. In regions such as southern Asia and the Middle East, levels of less than 10 ng/ml are common for the entire population, from newborn to the elderly.

Vitamin D insufficiency risk factors include older age, female gender, latitude, cold season, dark skin, factors that influence sun exposure such as clothing or cultural practices, specific diet etc. In addition to these factors, a significant influence on the level of vitamin D in a given population has national food enrichment policies with this nutrient.

Skin color and cultural practices seem to mask the effects of other factors, such as latitude, evidenced by elevated levels of vitamin D measured in the north compared to southern Europe and the severity of the deficiency of South Asia and Middle East.

Seasonal variation is also observed in most populations, regardless of latitude, except for areas with a very warm climate where individuals are accustomed to covering their bodies in the summer.

Age seems to have the same effect regardless of the area, but the influence of supplementation and diet on the vitamin D level is as variable as possible [43].

Vitamin D status in the European population. Vitamin D status in Europe varies greatly from one country to others [44]. Levels of 25(OH) vitamin D of less than 10 ng/ml were found in percentages from 2% to 30% of the adult population, a situation which may increase for institutionalized people to 75% [45].

In a study from France have been showed that the adult population aged between 35 and 65 recorded an average of 25(OH) vitamin D of 17.2 ng/ml in the north and 37.5 ng/ml in the south-west [46]. In this study, the correlation of the 25(OH) vitamin D level in serum with latitude was the one expected.

In the Netherlands, LSA (Longitudinal Aging Study Amsterdam) determined an average level of 25(OH) vitamin D lower than 10 ng/ml in the serum of 8% of male individuals and 14% of the female population serum [47].

Similar data was obtained in Switzerland following the MONICA project (Swiss Monitoring of Trends and Determinants in Cardiovascular Disease) [48]. Also in Switzerland, following a study in elderly care centers, it was observed that 90% of female subjects had levels of 25(OH) D of less than 20 ng/ml, a much higher percentage compared to 57% of non-institutionalized women [49].

In Italy, an average level of 25 (OH) D in the postmenopausal women's serum was 18 ng/ml, 30% of them having serum concentrations below 10 ng/ml [50].

In Greece, the mean serum concentrations of 25(OH) D in the breast feeding population are approximately 10 ng/ml, while in their mother's serum is around 12 ng/ml. In the case

of adolescents of the same country, a median of less than 10 ng/ml was recorded for 47% of them in winter [51].

Immigrants from Asian countries have an increased risk of vitamin D deficiency [52]. The serum level of 25(OH) D was less than 10 ng/ml in 40% of Netherlands non-western immigrants [53]. Pregnant women from the same non-western regions have a higher risk, with studies showing 25(OH) D levels of less than 10 ng/ml for more than 80% of mothers coming from Turkey and Morocco. It has been found worryingly low levels below the detection limit for 22% of pregnant women from Turkey [54].

In northern Europe the determining factor for higher values of 25 (OH) D is considered the consumption in large amounts of oily fish and cod liver oil, equivalent to up to 400 IU or 10 µg of vitamin D per day [55].

In a Netherlands study, factors that have been shown to influence vitamin D status were the time spent outdoor, body mass index, oily fish and margarine consumption (enriched with 3 IU/g), and administration of vitamin D supplements [56].

The population in northern Europe may exhibit sunlight-seeking behavior which, in combination with the fact that most of them have light-colored skin, increases vitamin D levels, while southern Europeans tend to avoid the sun and have darker skin, resulting in a lower vitamin D synthesis level.

5. CONCLUSIONS

It can be concluded that deficiency of vitamin D [serum concentrations of 25(OH) D lower than 10 ng/ml] is more commonly found in southern than in northern Europe. The risk groups are the elderly (especially those institutionalized), teenagers and non-western immigrants. Depending on the threshold below which the level of vitamin D is considered to be insufficient, 20 ng/ml or 30 ng/ml, the percentage of population with levels below these limits is high or very high in most European countries.

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