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Vitamin D3: an ever green molecule

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1. ABSTRACT

Vitamin D3 is a key regulator of vertebrates homeostasis. It is synthesized from the precursor 7-dehydrocholesterol upon UVB exposure in the skin and then hydrolyzed in the liver in position 25, to be finally converted into its active form, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$ or calcitriol), in the kidneys. The biological activity of this molecule depends on its binding to the nuclear receptor VDR, which binds VDRE once complexed with RXR-alpha. Despite being present in different types of food, the best way to assume it at physiological levels remains the exposure to UVB radiation at certain hours of the day and at particular angles of the Earth's crust. There is plenty of evidence that altered levels of vitamin D3 are associated with pathological conditions, such as osteoporosis, cancer, immunological and infectious diseases. In this review, we discuss vitamin D3 metabolism, its role in several diseases and the link between vitamin D3 and immune cells.

2. INTRODUCTION

Vitamin D3 is a phylogenetically old compound, whose functions can be already found in the first terrestrial organisms. Early in evolution, organisms captured solar energy in the form of carbohydrates through the process of photosynthesis. Cellular mechanisms became increasingly linked to calcium for signal transduction and metabolic functions. Terrestrial animals developed an exoskeleton and an endoskeleton that subsequently allowed them to grow and developed a smaller footprint. Vertebrates left the waters and headed toward land. They needed a mechanism that would allow them to use calcium in biochemical processes. The solution was found using solar energy to increase the intestinal absorption of calcium through vitamin D3 (1). Some ancestral algae, such as diatoms (Emiliania uxlei) and phytoplankton, first used calcium for the formation of their support structures, producing ergosterol (provitamin D2). Ergosterol has a great capacity to absorb ultraviolet (UV) radiation, so the first organisms

used it to protect themselves from the UV damage to DNA, RNA and proteins (1). Nowadays, we know that vitamin D3 is a key molecule for human beings.

3. VITAMIN D3 AND UV RADIATION

Part of vitamin D3 is synthesized in the skin during exposure to UV rays, especially to UV having a length of less than 320 nm. UV light is divided into UVC, UVB and UVA. UVC is the most energetic and shortest of the UV bands and causes skin burns and DNA damage. UVA, known as the "tanning ray", is primarily responsible for darkening skin pigmentation. Most tanning beds have a high-performance UVA, with a small percentage of UVB. UVA is less energetic than UVB, so exposure to UVA will not result in a burn. UVA penetrates more deeply into the skin as compared to UVB, due to its wavelength (2).

Vitamin D3 is produced under exposure to UVB radiation. It is sometimes called the "burning ray" because it is the primary cause of sunburn (erythema). Although UVB causes sunburn, it also induces special skin cells called melanocytes to produce melanin, which is protective against UV rays. The effects of UV rays on skin colour are due to different mechanisms. The main role of melanic pigmentation is to protect from ultraviolet radiation. After a few minutes of sun exposure, an ephemeral skin tanning is the result of photo-oxidation of existing melanin. In two to three days, UVB rays induce an increase in the number of melanosomes in melanocytes, in the rate of melanin synthesis and transfer of melanin to keratinocytes. Upon UVB stimulation, keratinocytes stimulate melanogenesis, proliferation of melanocytes and formation of tyrosinase (3). UVB rays are not always available throughout the day: UVB are present only during midday hours at higher latitudes and only with considerable intensity in temperate latitudes and the tropics. Sun exposure before 10:00 am and after 2:00 pm has no effect on the production of vitamin D3.This means that sunning must occur between the hours we were told to avoid. Only sunning from 10:00 to 02:00 during the summer (or winter months in southern latitudes) for 20 to 120 minutes, depending on skin type and colour, is indeed effective for vitamin D formation, before burning occurs (4). After UVB stimulation, it takes about 24 hours for vitamin D to reach the maximum levels in the blood. Immediately after sun exposure, 30-60 minutes are required before vitamin D3 enters the bloodstream (5). Exposing hands, face and arms to the sun for 10-20 minutes, three times a week, leads to the production of only 200-400 IU (International Unit) vitamin D each time, an average of 100-200 IU per day, during the summer months. In order to achieve optimal levels of vitamin D, 85% of the body surface needs to be exposed to the midday sun (about 100-200 IU vitamin D is produced for every 5% of body surface exposed). Light-skinned people need 10-20 minutes of exposure, while dark-skinned people need 90-120 minutes (6). Latitude and altitude determine the intensity of UV light, in fact UVB is stronger at high altitude. Latitudes above 30 degrees (north and south) have insufficient sunlight for 2 to 6 months of the year, even at midday (7). Latitudes above 40 degrees have sufficient sunlight to reach optimal levels of vitamin D for 6 to 8 months of the year. In most northern or southern latitudes (45 degrees and above), even the summer sun is too weak to provide optimal levels of vitamin D (8, 9).

4. VITAMIN D SYNTHESIS

There are various forms of vitamin D. The two major forms are vitamin D2, commonly known as ergocalciferol, and vitamin D3, also known as cholecalciferol (or calcitriol) (10).

Vitamin D2 is synthesized by plants and fungi, but not by vertebrates, and it probably plays a protective role against ultraviolet radiation.

Vitamin D3 is synthesized from 7-dehydrocholesterol in relatively large quantities in the skin of most vertebrate animals, including humans (11). Once produced by the skin (or ingested as food), it is hydrolyzed in the liver in position 25, by the mitochondrial enzyme 25-hydroxylase, forming 25-hydroxycholecalciferol (25OHD or calcidiol) (12, 13). Calcidiol is then transported through the bloodstream to the proximal tubule of the kidney, where 1-alpha-hydroxylase is responsible for calcitriol (1,25-dihydroxycholecalciferol or $1,25(OH)_2D$) synthesis (Figure 1). 1-alpha-hydroxylase levels are increased by parathyroid hormone (PTH), secreted by parathyroid gland. Thereafter, the so-formed calcitriol is released into the blood stream. Its ability to bind to a transporter protein, vitamin d binding protein (VDBP), enables it to reach other target districts (14). The biological action of vitamin D3 is due to its binding to vitamin D3 receptor (VDR) and retinoid X receptor alpha (RXR-alpha) in the nucleus of many cells of the body, including brain, gonads, skin, prostate and breast (14).

The complex VDR-RXR-vitamin D3 targets the DNA sequence VDRE (VDR responsive element). Furthermore, the VDR nuclear receptor is involved in many processes, such as proliferation and differentiation (16). Its presence was seen in many cells of the immune system, including monocytes, macrophages and activated T and B cells (16).

5. VITAMIN D3 PATHWAYS

The action of vitamin D3 is carried out through its receptor VDR in many different cells. VDR is a nuclear receptor and ligand-activated transcription factor (17, 18), composed of a highly conserved DNA binding domain and an alpha-helical ligand binding domain (19). The ligand-bound VDR activates transcription by heterodimerization with retinoid X receptor (RXR), which is essential for high-affinity DNA binding to cognate vitamin D response elements (VDREs) located in the regulatory regions of $1,25(OH)_2D$ target genes (17, 18, 20, 21, 22). VDRE motifs can also function as response elements for the VDR and related RXRs (23), thus partially integrating $1,25(OH)_2D$ and retinoid signalling. DNA-bound VDR/RXR heterodimers recruit numerous co-regulatory proteins, which control histone modifications, chromatin remodelling, RNA polymerase II binding and

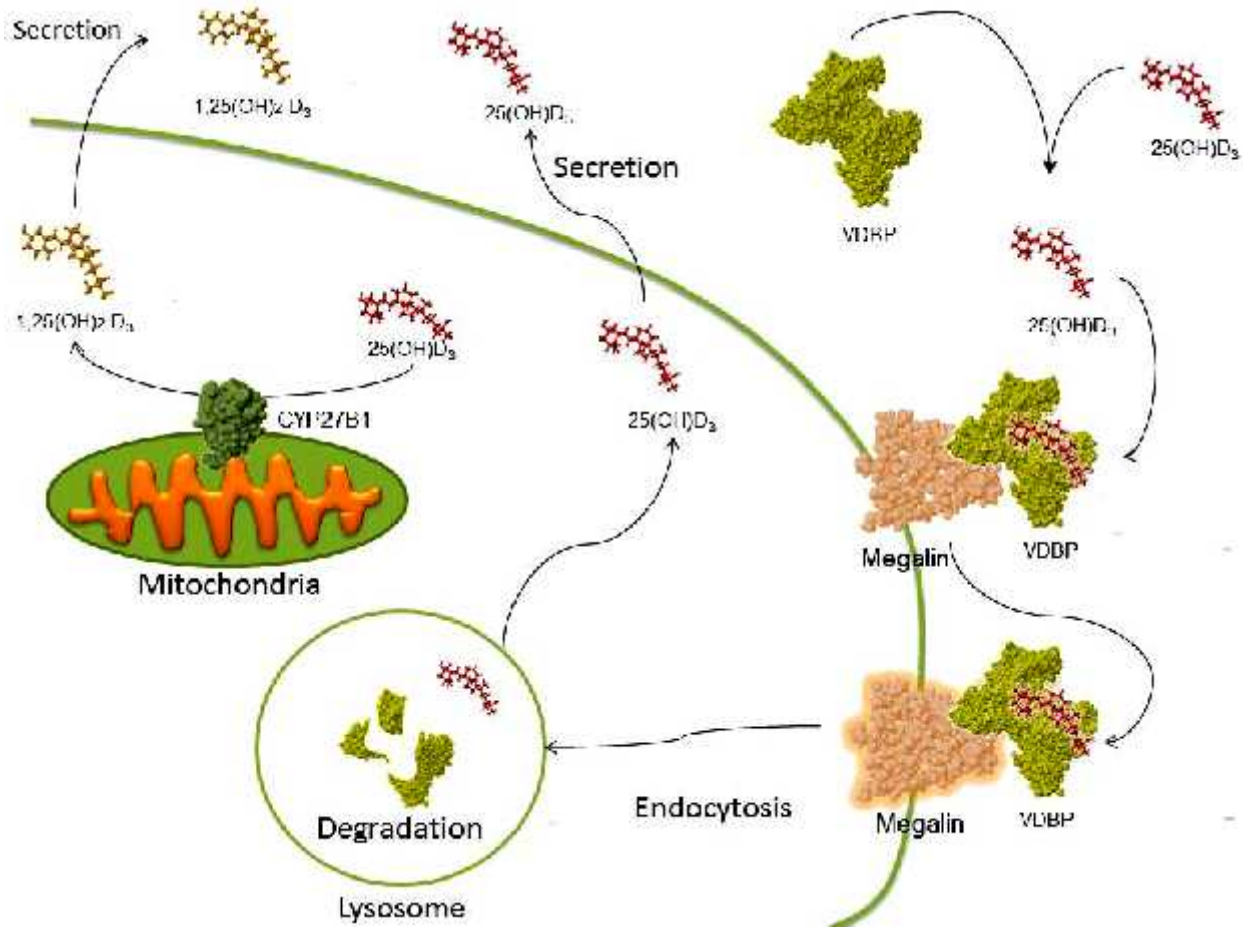

Figure 1. Megalin role in renal uptake and activation of 25OHD3. Filtered 25OHD3–VDBP complexes are endocytosed by the proximal tubular epithelium and delivered to lysosomes, where VDBP is degraded and 25OHD3 is released to the cytosol; 25OHD3 is either secreted or hydroxylated in the mitochondria to $1,25(OH)_2D3$ before release into the interstitial fluid and complex formation with VDBP.

transcriptional initiation (24, 25, 26, 27, 28). The ligand-bound VDR can also repress transcription. For example, the presence of $1,25(OH)_2D$ VDR/RXR heterodimers can displace DNA-bound nuclear factors of activated T cells, thus repressing cytokine gene expression (29, 30). While numerous VDREs have been identified in relatively promoter-proximal locations, a recent work has provided evidence that the DNA-bound VDR can function at distances as great as 75 kb to regulate adjacent target gene transcription (31).

1-alpha-hydroxylase (or CYP27B1) is important for vitamin D3 immunological actions. Activated macrophages and dendritic cells (DCs) express CYP27B1 (16, 32, 33, 34, 35), which, unlike the renal enzyme, is not regulated by Ca2+ homeostatic signals but is primarily regulated by immune inputs, mainly gamma interferon and agonists of the Toll-like receptor (TLR) pattern recognition receptors. Microarrays studies have shown that human macrophages, stimulated by bacterial lipopeptides, signal through TLR1-TLR2 and induce the expression of both CYP27B1 and VDR (36). Further studies showed a correlation between lipopolysaccharide (LPS) stimulation and expression of CYP27B1 via TLR4-CD14 (37, 38). Furthermore, CYP24-SV transcripts (a splice variant of the enzyme that starts the catabolism of $1,25(OH)_2D$) is also capable of hydrolyzing 25OHD at 1-alpha-position, forming $1,25(OH)_2D$ (39). This observation suggests that, in macrophages, robust $1,25(OH)_2D$ signalling is maintained over an extended period of time, which would be advantageous for combating intracellular pathogens (Figure 2).

6. VITAMIN D3 AND FOOD

Very few foods in nature contain vitamin D: fish flesh (such as salmon, tuna, and mackerel flesh) and fish liver oils are among the best sources (40, 41)). Small amounts of vitamin D are found in beef liver, cheese and egg yolks. Some mushrooms provide vitamin D2 (ergocalciferol) in variable amounts (42-43). Mushrooms with enhanced levels of vitamin D2 from being exposed to ultraviolet light under controlled conditions are also available. Several food sources of vitamin D are listed in Figure 3.

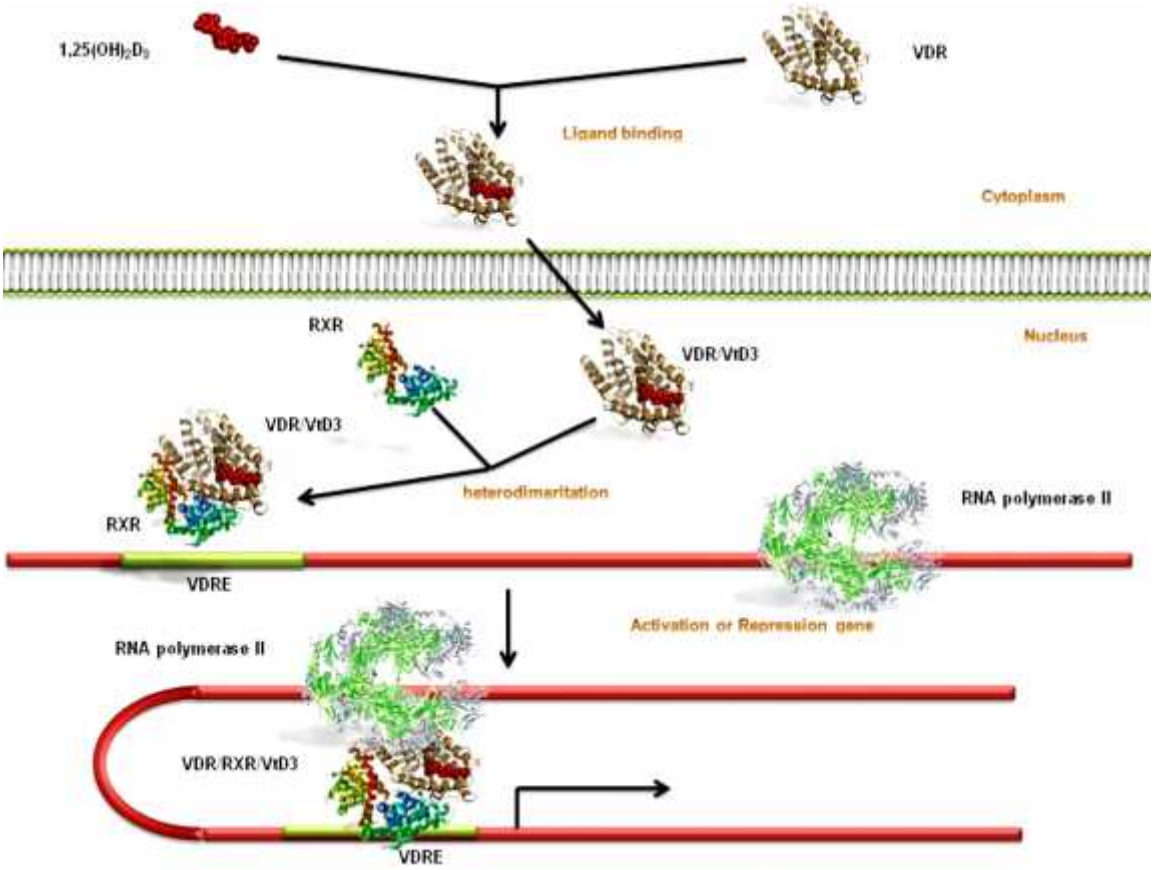

Figure 2. Vitamin D3 pathways. The biological action of vitamin D3 is performed by binding its receptor, called VDR (vitamin D3 receptor) and RXR-alpha (retinoid X receptor alpha) in the nucleus of several cell types. The VDR-RXR-vitamin D3 complex targets the DNA sequence VDRE (VDR responsive element) in many targets genes, modulating their upregulation or downregulation.

The adequate dietary intake of vitamin D has been established by the Food and Nutrition Board at the Institute of Medicine of The National Academies (formerly National Academy of Sciences). The intake levels are based on age (Figure 4).

7. VITAMIN D3 DEFICIENCY

The association between low levels of vitamin D3 and susceptibility to infections was revealed in childhood rickets, where a correlation between calcium metabolism dysfunction and lung infections has been found (44). Of note, before the introduction of antimicrobial drugs in 1950, vitamin D3, which is present in the cod liver oil, was used in the treatment of tuberculosis (TB) (45). More recently, epidemiologic studies demonstrated a strong association between seasonal variations in vitamin D levels and the incidence of several infectious diseases, including septic shock (46), respiratory infections (47) and influenza (47, 48).

25OHD levels below 20 ng/mL (50 nmol/liter) define vitamin D3 deficiency (49, 50). 25OHD levels inversely correlated with those of PTH, which induces CYP27B1 to convert 25OHD to $1,25(OH)_2D$, until 25OHD concentration reaches 30 to 40 ng/mL: at this point, PTH levels decrease (49, 50, 51). Higher 25OHD levels (21) correlated with increased calcium absorption in the gut up to 65% (52). On the basis of these data, a level of 25OHD ranging between 21 and 29 ng/ml may be indicative of a relative vitamin D insufficiency, while a level of 30 ng/mL or higher may be considered as an indicator of sufficient vitamin D (53). Levels of 25OHD greater than 150 ng/ml cause vitamin D3 intoxication.

In older people it is very common to find a lack of vitamin D3. Individuals who live at the equator usually have levels of 25OHD higher than 30 ng/ml (or 30% above normal levels) (54, 55). In Europe, where very few foods are fortified with vitamin D, children and adults could be at higher risk of vitamin D3 insufficiency (49, 56, 57, 58, 59). In countries where, either for environmental and religious reasons, the body is fully covered, and, as such, not exposed to the sun, vitamin D3 plasma levels are lower, at around 20 ng /ml (60, 61).

The interaction between $1,25(OH)_2D$ and VDR increases the efficiency of intestinal calcium absorption up to 30-40% and phosphorus absorption up to approximately 80% (37, 62). When 25OHD levels are less than 30 ng/ml,

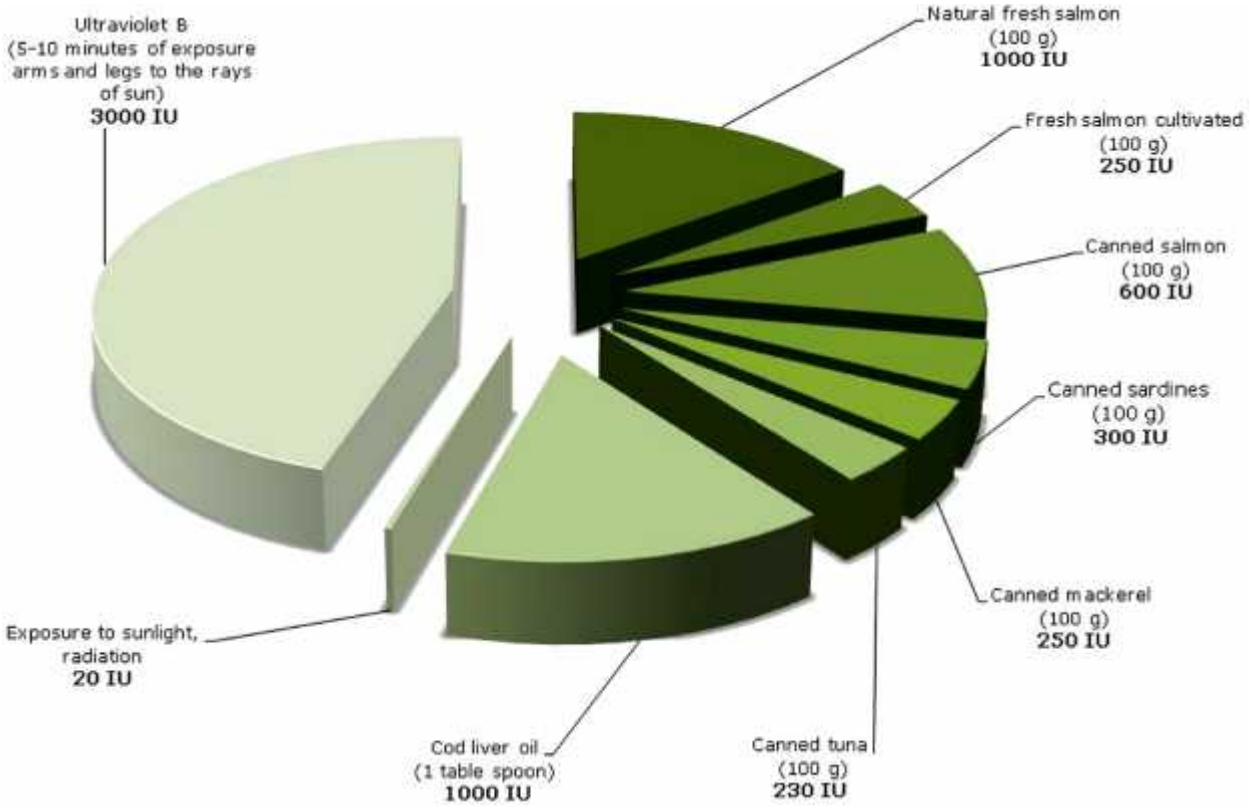

Figure 3. Foods high in vitamin D. Very few foods naturally contain vitamin D. The optimal way to intake vitamin D3 is exposure to UVB radiation. 90% of available vitamin D3 is absorbed indeed through UVB radiation and the remaining 10% comes up from dietary sources.

there is a significant reduction in intestinal calcium absorption, with an increase of PTH. PTH stimulates the kidneys to produce $1,25(OH)_2D$, thus leading to an increase in calcium serum levels. PTH also activates osteoblasts, stimulating the transformation of preosteoclasts to mature osteoclasts. Osteoclasts dissolve the mineralized matrix of bone collagen, resulting in osteopenia and osteoporosis and therefore increasing the risk of fractures (63, 64). Whereas osteoporosis is not associated with bone pain, osteomalacia has been associated with isolated or generalized bone pain (65, 66). The cause is thought to be hydration of the demineralised gelatin matrix beneath the periosteum; the hydrated matrix pushes outward on the periosteum, causing throbbing and aching pain (49). A proper intake of vitamin D3, either through sun exposure or through nutritional supplements, appears essential for well-being.

8. VITAMIN D3 AND DISEASE

Several diseases have been related to low vitamin D3 levels (< 30ng/ml), like cardiovascular disease, osteoporosis, fractures, bacterial infections, cancer and autoimmune diseases (67). Immune system dysregulation is thought to be involved in the development of many pathologic processes. Considering vitamin D3 key-role in immune modulation, it is not surprising to find a large number of studies focusing on the relationship between vitamin D3 and morbidities.

8.1. Osteoporosis

Although osteoporosis is a multifactorial disease, vitamin D deficiency can be an important contributing factor. Without sufficient vitamin D levels, calcium absorption cannot be maximized and the resulting elevation in PTH secretion by the parathyroid glands results in increased bone reabsorption, which may lead to osteoporotic fracture. The results of most clinical trials suggest that vitamin D supplementation can slow bone density loss or decrease the risk of osteoporotic fractures in men and women who are unlikely to assume enough vitamin D (68). Approximately 33% of women aged 60-70 years and 66% of those aged 80 years or older have osteoporosis (68, 69). Since bone loss occurs without symptoms, osteoporosis is often considered a 'silent disease'. Continuous bone reabsorption alters and weakens the bone structure, so that relatively minor injuries or falls can cause fractures or vertebral collapse. The resulting fracture may lead to loss of mobility and independence, with 25% of individuals requiring long term care (70). Currently, there are a number of pharmacologic treatments for osteoporosis which provide improvements in bone mass and reduction in fracture risk, mainly based on calcium and vitamin D supplementation. Menopause often leads to increased bone loss, with the most rapid rates of bone loss occurring during the first five years after menopause (71). A drop in estrogen production after menopause results in increased bone reabsorption and decreased calcium

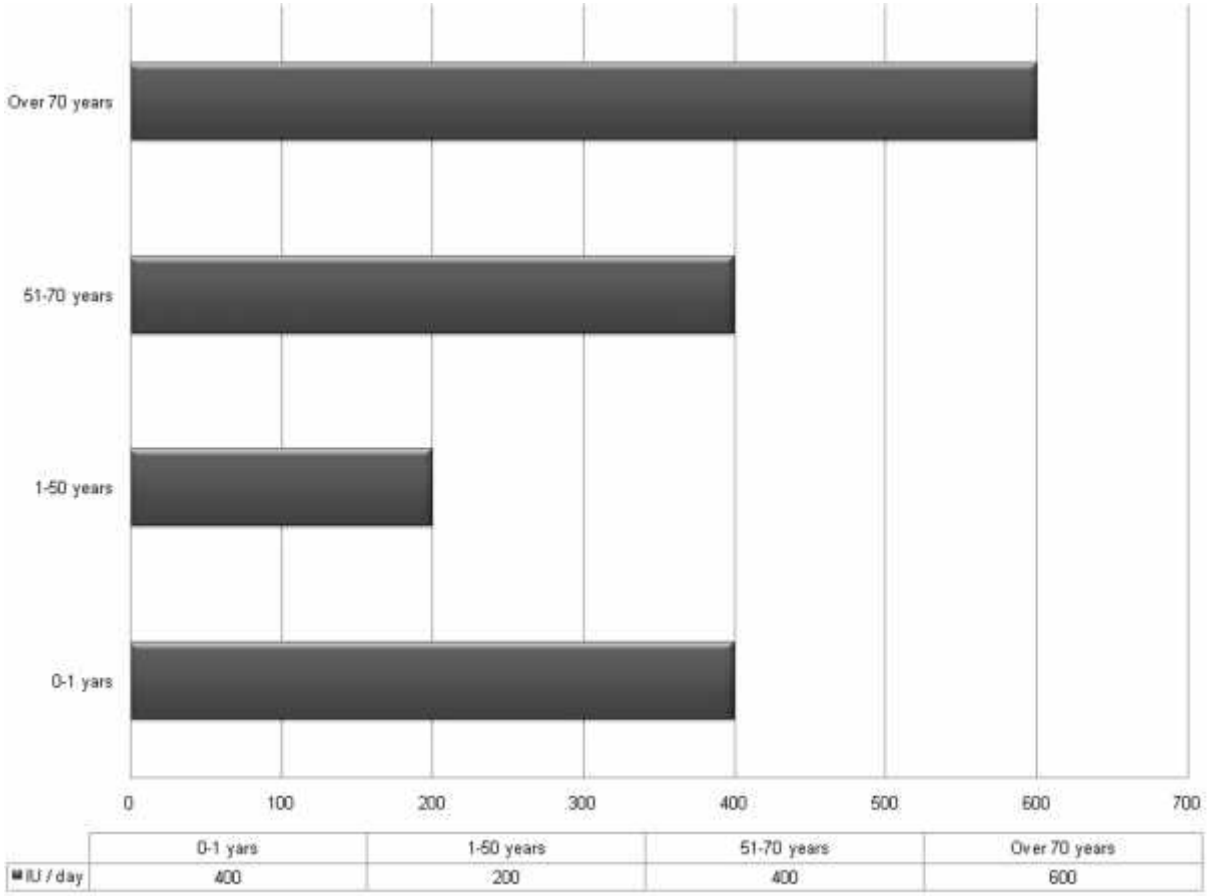

Figure 4. Recommended vitamin D Intake. The optimal vitamin D intake is linked to the age. In the first year, children needs 400 IU per day, people aged 1-50 years need 200 IU per day, people aged 51-70 years need 400 IU per day, people over the age of 70 need up to 600 IU per day.

absorption (72). A decrease in bone mass of 3%–5% per year is often seen during the years immediately following menopause; a less severe decrease in bone mass usually occurs after 65 (73). Post-menopausal hormone therapy can decrease the incidence of osteoporosis and reduce fracture risk. However, since many women have to discontinue or avoid hormone therapy after menopause, it is imperative for health care providers to actively identify those women who are at risk for bone thinning and fractures. In fact, counselling regarding weight bearing, exercise and calcium/vitamin D intake is particularly important during the perimenopause.

8.2. Cardiovascular disease

Vitamin D deficiency has been associated with congestive heart failure (74) and with high blood levels of several inflammatory factors, including C-reactive protein (CRP) and interleukin-10 (75, 78). Of note, living at higher latitudes increases the risk of hypertension and cardiovascular disease (75, 76). In a study of patients with hypertension who were exposed to UVB radiation three times a week for 3 months, 25OHD levels increased by approximately 180% and blood pressure became normal (both systolic and diastolic blood pressure reduced by 6 mmHg) (77).

The two most important arterial complications leading to cardiovascular events are intima and media calcification. Arterial intima calcification is associated with atherosclerosis and leads to plaque formation and rupture with subsequent blood vessel occlusion. Arterial media calcification is associated with proliferation of vascular smooth muscle cells and leads to calcification and stiffening of the vessel wall (79). Vitamin D can inhibit various aspects of inflammation related with intimal and medial calcification. We will explain how further on.

8.3. Cancer

People living at higher latitudes are at increased risk for Hodgkin's lymphoma as well as colon, pancreatic, prostate, ovarian, breast cancer and are more likely to die from cancer, as compared with people living at lower latitudes (80, 81). The sunlight hypothesis (assuming that sunlight is a surrogate for vitamin D circulating levels) has been proposed to explain the higher risk for several types of cancer (82, 83) including colorectal cancer (CRC) (84), prostate cancer (PCa) (85, 86) and breast cancer (BCa) (87). The evidence is stronger for CRC: circulating 25OHD levels and vitamin D intake are indeed inversely associated with CRC incidence and recurrence (88, 89). In addition, higher pre-diagnosis plasma 25OHD levels were associated

with a significant improvement in overall survival in CRC patients (90).

Calcitriol exerts antiproliferative and prodifferentiating effects; *in vivo* studies in animal models of cancer demonstrated that calcitriol retards tumor growth (91, 92, 93). Calcitriol inhibits the proliferation of many malignant cells by inducing cell cycle arrest and the accumulation of cells in the G0/G1 phase of the cell cycle (92, 94). In many neoplastic cells calcitriol also induces differentiation, resulting in the generation of cells expressing a more mature and less malignant phenotype. These mechanisms are specific to each cell type and include the regulation of signaling pathways involving beta-catenin, Jun-N-terminal kinase (JNK), phosphatidyl-inositol 3-kinase, nuclear factor-kappaB (NF-kappaB) as well as the regulation of the activity of several transcription factors, such as the activator protein-1 (AP-1) complex and CCAAT/enhancer-binding protein (C/EBP) (91,95).

Calcitriol induces apoptosis in several cancer cells, although this effect is not uniformly seen in all malignant cells. These effects are related to the inhibition of antiapoptotic proteins, such as Bcl2 (96, 97) and the enhanced expression of proapoptotic proteins, such as Bax and Bad (98). Calcitriol reduces the invasive and metastatic potential of many malignant cells, by blocking angiogenesis and regulating the expression of key molecules involved in invasion and metastasis (99). Calcitriol directly modulates basal and cytokine-induced NF-kappaB activity in many cells, including human lymphocytes (100), fibroblasts (101) and peripheral blood monocytes (102). The addition of a VDR antagonist to colon cancer cells up-regulates NF-kappaB activity by decreasing the levels of IkB, thus suggesting that VDR ligands are able to suppress NF-kappaB activation (103).

8.4. Multiple sclerosis

Some studies have shown that the number of patients with MS increases with distance from the equator (104, 105). Patients with MS have relatively low serum 25OHD; furthermore, disease activity may increase when UVB exposure is limited (106, 107). In mice with autoimmune encephalitis (EAE), Cantorna *et al* observed that vitamin D pre-induction treatment prevented disease development, while post-induction treatment ameliorated disease activity (108). Vitamin D has *in vitro* anti-inflammatory actions, including enhanced Th2 and decreased Th1 cytokine production and enhanced macrophage phagocytosis (109). Several studies have shown that MS patients positively respond to the somministration of vitamin D3 or to sun exposure (105, 107, 110). During sun exposure, 25OHD levels in these patients augmented up to 220 nmol/liter, without any problem (111). Furthermore, patients with MS tolerated a pilot dose-escalation trial up to 40,000 IU/day (112). In a recent study, Burton *et al* have shown in a cohort of forty-nine patients (25 under treatment and 24 controls) treated with high doses of 25OHD (10,000 IU/day), that 25OHD treatment was safe and able to play immunomodulatory effects (113).

9. VITAMIN D3 AND INFECTION

9.1. Vitamin D3 and bacterial infections

The anti-inflammatory role of vitamin D has been documented in various diseases, such as multiple sclerosis, diabetes mellitus, psoriasis, and prostate cancer (114). In the 1980s, Rook *et al* (115) and Crowle *et al* (116) demonstrated that vitamin D enhanced bactericidal activity of human macrophages against Mycobacterium tuberculosis, the causative agent of tuberculosis. This discovery led to a new era of interest regarding the role of vitamin D in determining the pathogenesis and the immune response to bacterial pathogens. Liu *et al* demonstrated that stimulation of macrophage TLR2/1 complex by Mycobacterium tuberculosis-derived antigens upregulated the expression of both VDR and CYP27B1 (117). Moreover, intracellular $1,25(OH)_2D$ led to the induction of the antimicrobial peptide cathelicidin and to the intracellular killing of Mycobacterium tuberculosis (117). When vitamin D is deficient, infected macrophages are unable to produce sufficient $1,25(OH)_2D$ to upregulate production of cathelicidin.

Vitamin D is also known to regulate the expression of β-defensin (118), another antimicrobial peptide with multiple effector functions within the immune system. Endoscopic studies in humans have demonstrated that β-defensin is secreted in the gastric mucosa after infection by Helicobacter pylori (119) and may therefore constitute a major aspect of immune defence against this bacterial pathogen at the mucosal surface. Additional studies also suggested that vitamin D may be responsible for upregulation of the oxidative burst in activated macrophages (120), thus further augmenting bacterial killing. Studies on VDR polymorphisms in humans support the hypothesis that variability in vitamin D status and host genes encoding vitamin D-responsive elements affect the immune response to bacterial pathogens other than Mycobacterium tuberculosis (121, 122). Therefore, much of what we have learnt from the interaction between host vitamin D status and Mycobacterium tuberculosis infection can enhance our understanding about the immunomodulatory properties of vitamin D in other bacterial diseases, although more studies are needed to extend this observation to other clinical settings.

9.2. Vitamin D3 and viral infections

Vitamin D modulates cytokine profile in animal models of autoimmune disease, limiting excessive production of proinflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin-12 (123). The host vitamin D status may contribute to immunoregulatory functions in the setting of viral respiratory infections, modulating the cytokine responses to various microbial species (124).

Recent data on human immunodeficiency virus (HIV) infection and vitamin D3 have demonstrated increased prevalence of vitamin D deficiency in HIV-infected patients in comparison with uninfected hosts (74, 125). Laboratory models of HIV infection have shown that pre-treatment of human monocytes and macrophages with

$1,25(OH)_2D$ prevents HIV infection in certain cell lines (126), while increasing HIV replication in others (127). Another recent study demonstrated that cathelicidin, an antimicrobial peptide, whose expression is partly regulated by vitamin D, may directly inhibit HIV replication (128). Patients with AIDS with abnormally low $1,25(OH)_2D$ levels (< 20ng/ml) had shorter survival than controls (p value less than 0.01). These results indicate that $1,25(OH)_2D$ serum levels correlate with the degree of immunodeficiency in HIV infection. Moreover, low $1,25(OH)_2D$ is associated with increased incidence of AIDS-events and reduced survival time (129).

10. VITAMIN D3 AND IMMUNE CELLS

VDR is present in most cells of the immune system, including T lymphocytes, neutrophils and antigen-presenting cells, such as macrophages and DCs (130, 131). $1,25(OH)_2D$ inhibits DCs maturation and directly acts on T lymphocytes to inhibit T-cell proliferation (132). $1,25(OH)_2D$ signalling represses the transcription of genes encoding key T helper 1 (Th1) cytokines, such as gamma interferon and interleukin-2 (29, 132). The net effect of $1,25(OH)_2D$ is to polarize T-helper responses toward a more regulatory Th2 phenotype, which is considered a key component of its ability to suppress Th1-driven autoimmune responses (132) .

Activated macrophages and DCs express CYP27B1 (32, 33, 34, 35), which, unlike the renal enzyme, is not regulated by Ca2+ homeostatic signals but is regulated primarily by immune inputs, mainly gamma interferon and agonists of the TLR pattern recognition receptors. Liu *et al* found in microarray studies that signalling through human macrophage TLR1/2 heterodimers, stimulated with bacterial lipopeptides, induced the expression of both CYP27B1 and VDR (36). Most importantly, they showed that in TLR2/1-stimulated human macrophages cultured in presence of human serum, downstream VDR-driven responses were strongly dependent on serum 25OHD concentrations. VDR-driven responses were strongly attenuated or absent in serum from vitamin D-deficient individuals, a defect that could be overcome by 25OHD supplementation. Moreover, consistent with previous findings (133, 134), 25OHD serum levels from African-Americans were found to be markedly lower than those of Caucasian Americans (135). This study also provided a clear demonstration of the dependence of immune responses on circulating 25OHD levels. Similarly, stimulation of the TLR4-CD14 receptor complex by LPS induced CYP27B1 expression (136), in line with other studies (137, 138).

Remarkably, while expression of CYP24, the mitochondrial enzyme that initiates $1,25(OH)_2D$ catabolism, is exquisitely sensitive to the presence of $1,25(OH)_2D$, the negative feedback loop appears to be defective in macrophages. Ren *et al* have recently shown that while expression of CYP24 transcripts is induced by $1,25(OH)_2D$ in macrophages, as in other cells, the corresponding enzymatic activity is virtually undetectable (39). In macrophages, $1,25(OH)_2D$ induces the expression of a splice variant form (CYP24-SV) that encodes a truncated enzyme, lacking of the critical amino-terminal mitochondrial targeting sequence (39). Although the substrate binding pocket of CYP24-SV is apparently functional, the enzyme, trapped in the cytosol, appears to be catalytically inactive. This suggests that, in macrophages, robust $1,25(OH)_2D$ signalling is maintained over an extended period of time, which would be advantageous for combating intracellular pathogens, such as Micobacterium tuberculosis. It also provides at least part of the molecular basis for the excessive production of $1,25(OH)_2D$ by macrophages in granulomatous diseases, such as sarcoidosis (139).

The expression of the co-receptor of TLR4, CD14, is strongly induced by $1,25(OH)_2D$ in human cells (140). Vitamin D signalling enhances the expression of TLR2 approximately twofold in human keratinocytes. Given that TLR2 and TLR4 recruitment enhances vitamin D signalling, by upregulating VDR and CYP27B1 expression, the effects of $1,25(OH)_2D$ on TLR2 and CD14 in keratinocytes represent a positive feedback loop. Treatment of human monocytes with $1,25(OH)_2D$ suppressed the expression of both TLR2 and TLR4 mRNA and protein in a time- and dose-dependent manner (141). The authors speculated that downregulation of pattern recognition receptors by $1,25(OH)_2D$ in antigen presenting cells (APCs) may contribute to the capacity of $1,25(OH)_2D$ to attenuate excessive Th1-driven inflammatory responses and potential downstream autoimmunity (79). In addition, while there are conflicting results concerning the role of vitamin D signalling in controlling HIV infection, it should be noted that human cathelicidin was found to inhibite the replication of a number of HIV isolates (142) and that the human and porcine homologues reduced the infectivity of lentiviral vectors (143), thus suggesting that vitamin D signalling may play an antiretroviral activity (Figure 4).

In a recent study Marina Rode von Essen *et al* (144) have shown that vitamin D controls T cell antigen receptor signalling and activates human T cells. Naive human T cells have very low expression of VDR and PLC-γ1. However, TCR signals through the alternative p38 pathway induced VDR expression. VDR binds $1,25(OH)_2D$, translocates to the nucleus and activates the gene encoding PLC-γ1, which results in the accumulation of PLC-γ1 protein in the cytoplasm of primed T cells, approximately 48 hours after the initial activation signals (144). Because of PLC-γ1 central role in classical TCR signalling and T cell activation (145,146), the differences in PLC-γ1 expression in naive and primed T cells might explain the process of functional avidity maturation observed in T cells.

11. CONCLUSIONS

Vitamin D3 has a critical in human life. Direct correlations were found between many diseases and vitamin D3 deficiency (47, 48) due to the lack of sun exposure, to geographical or cultural reasons (60, 61), or low daily dietary intake (49, 56, 57, 58, 59). It has been widely shown that people living farther from the equatorial

region are more susceptible to vitamin D3 deficiency and autoimmune diseases (104, 105, 106, 107). Moreover, the importance of vitamin D3 in viral and bacterial infections has also been shown (115, 116, 119, 124).

Introduction in the diet of foods rich in vitamin D3 may certainly play a crucial role in vitamin D3 levels for well-being in individuals with vitamin D3 deficiency .

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Abbreviations: $1,25(OH)_2D$: 1,25-dihydroxyvitamin D, 25OHD: 25-hydroxyvitamin D, APCs: antigen presenting cells, AP-1: activator protein-1, DCs: dendritic cells, EAE: autoimmune encephalitis, HIV: human immunodeficiency virus, IU: International Unit, JNK: Jun-N-terminal kinase, LPS: lipopolysaccharide, NF-kappaB: nuclear factor-kappaB, PTH: parathyroid hormone, RXR: retinoid X receptor, Th1: T helper 1, TLRs: toll-like receptors, TNF-alpha: tumor necrosis factor-alpha, UV: ultraviolet, VDR: vitamin D receptor, VDBP: vitamin D binding protein, VDRE: vitamin D responsive element,

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