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REVIEW

Vitamin D metabolism, functions and needs: from science to health claims

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Abstract
Background Vitamin D is a nutrient long considered as essential for skeletal health but is now attracting interest from medical and nutritional communities as knowledge emerges of its biological function and its association with decreased risk of many chronic diseases.
Results A question emerges: How much more vitamin D do we need for these new functions of vitamin D? This review discusses vitamin D physiology and hypovitaminosis D and presents two vitamin D dietary policies: that according to regulatory authorities and that of nutrition scientists. Scientific evidence suggests that 25(OH)D serum levels should be over 75 nmol/L; otherwise, there is no beneficial effect of vitamin D on long-latency diseases. Current regulatory authority recommendations are insufficient to reach this level of adequacy. Observational and some prospective data show that vitamin D has a role in the prevention of cancer as well as immunity, diabetes and cardiovascular and muscle disorders, which supports the actions of 1α,25(OH)2D at cellular and molecular levels. The recent assessments done by the European Food Safety Authority should lead to new health claims.
Conclusions Vitamin D, through food fortification and supplementation, is a promising new health strategy and thus provides opportunities for food industry and nutrition researchers to work together towards determining how to achieve this potential health benefit.

Keywords Vitamin D · 25(OH)D · Supplementation · DRI · Health claim

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Introduction

In the early twentieth century, the curative properties of vitamin D on rickets were identified and its role then became confined to the prevention of disorders associated with bone health [1]. Today, the vitamin D receptor (VDR) and the activation enzyme, CYP27B1, have been identified in numerous cell types not involved in calcium and phosphorus homeostasis [1], suggesting involvement in other body functions, and studies report many cellular process affected by the active form of vitamin D, 1-alpha-25 dihydroxyvitamin D($1\alpha,25(OH)_2D$) [2, 3]. Accumulating data show that vitamin D status is positively correlated with health conditions such as cancer, immunity disorders, diabetes, muscle disorders and cardiovascular disease [2,

3]. The resulting concern is that suboptimal status may increase the risk of these diseases. This leads to questions such as: (1) What is considered as hypovitaminosis? (2) What is adequate vitamin D status? (3) What intake of vitamin D do we need?

It is the metabolite 25-hydroxyvitamin D [25(OH)D] that is the vitamin D status indicator. The serum level is determined by skin synthesis through sun exposure and/or dietary intake. Sun synthesis of vitamin D in recent years has been limited by changes in exposure to sun, such as clothing, being inside, and concern about skin cancer [2, 3]. Thus, dietary contributions are now emphasized. However, questions about vitamin D safety and adequacy have become subjects of great discussion between nutrition communities and regulatory authorities. This review aims to summarize the general understanding of vitamin D metabolism and the emerging knowledge of its physiology. We then discuss the definition of hypovitaminosis D, what constitutes adequate vitamin D status, and finally the debate between regulatory authorities and the scientific community regarding recommendations concerning vitamin D intake and safety. This should be informative when considering allowable health claims in Western countries.

Vitamin D metabolism

Synthesis and activation

Synthesis of endogenous vitamin D begins in the skin (see Fig. 1). The epidermis and dermis both contain 7-dehydrocholesterol (DHC). When UVB radiation (280–315 nm) passes through these skin layers, 7 dehydrocholesterol absorbs UVB photons inducing their conversion to previtamin D3. This photoisomerization is followed by previtamin D3 thermal-dependent isomerization, leading to formation of the vitamin D3 molecule, also known as cholecalciferol. During prolonged sun exposure, previtamin D3 is photoisomerized to lumisterol and tachysterol, both of which are biologically inactive. Because of this, vitamin D3 synthesis plateaus at about 10–15 % of the original 7 DHC content [4]. Once formed, vitamin D3 is preferentially bound to the vitamin D-binding protein (DBP), allowing its translocation into the general circulation [4].

Skin synthesis is limited by various determinants, including pigmentation, age, zenith angle of the sun, poor air quality and % of the skin surface area available for exposure. A recent study of sun-protective behaviour in the USA showed that wearing long sleeves or staying in the shade reduced vitamin D status [5]. Surprisingly sunscreen use, even those with a high sun protection factor (SPF), did not significantly affect vitamin D status; however, self-reported use does not necessarily imply total skin coverage. There have been numerous studies looking at skin pigmentation as a predominant factor for reducing vitamin D synthesis [6].

In addition to cutaneous synthesis, vitamin D can be obtained from the diet in the form of vitamin D3 (cholecalciferol) or occasionally as vitamin D2 (ergocalciferol). Whereas vitamin D3 is obtained from animal sources, vitamin D2 is present in fungi and mushrooms irradiated with UVB. In this article, vitamin D will denote the name of both types of molecules unless it is important to distinguish between these two forms. Vitamin D2 is deemed by some to be an "unnatural" form of vitamin D [7]. In terms of efficacy, a recent systematic review and meta-analysis showed that a bolus dose of vitamin D3 raises 25(OH)D more than a similar amount of vitamin D2, although this difference was not seen with daily supplementation of more modest doses [8]. Vitamin D2 is considered as an active substance and is not excluded as a source of dietary vitamin D by the Endocrine Society [9].

Before entering the circulation, ingested vitamin D is absorbed and then transported in chylomicrons. Once in the circulation, it binds DBP until it is released into the liver where it undergoes hydroxylation of the carbon molecule in the 25 position by one of four hepatic cytochrome P-450 enzymes. Three of them are microsomal forms, CYP2R1, CYP2J2 and CYP3A4, with CYP2R1 being the most physiologically important as this is the only 25-hydroxylase that causes rickets when it is non-functional. The fourth enzyme, CYP27A1, is mitochondrial [10, 11]. This metabolite, 25(OH)D (calcidiol), is the major circulating form of vitamin D and the last metabolite prior to conversion to the active form. Serum levels of 25(OH)D reflect both dietary and skin contributions as well as body stores. The serum concentration of 25(OH)D constitutes the main validated biomarker of vitamin D status [9, 10, 12].

For the endocrine functions of vitamin D, the proximal tubule of the kidney is the main site for CYP27B1 (1α-hydroxylase) activity [11]. This enzyme is responsible for conversion of 25(OH)D to the active metabolite $1\alpha,25(OH)_2D$ (calcitriol). Once made in the kidney, this active metabolite enters the general circulation, allowing it to act in distant organs and cells in a hormone-like manner. The two primary functions of circulating $1\alpha,25(OH)_2D$ are (1) to increase the efficiency of intestinal calcium and phosphorus absorption and (2) to induce preosteoclasts to become mature osteoclasts [13]. Other known roles include down-regulation of renin production in the kidney and stimulation of insulin secretion in the beta islet cells of the pancreas [13].

Extrarenal conversion of 25(OH)D to $1\alpha,25(OH)_2D$ can occur in numerous organs or tissues such as the muscles,

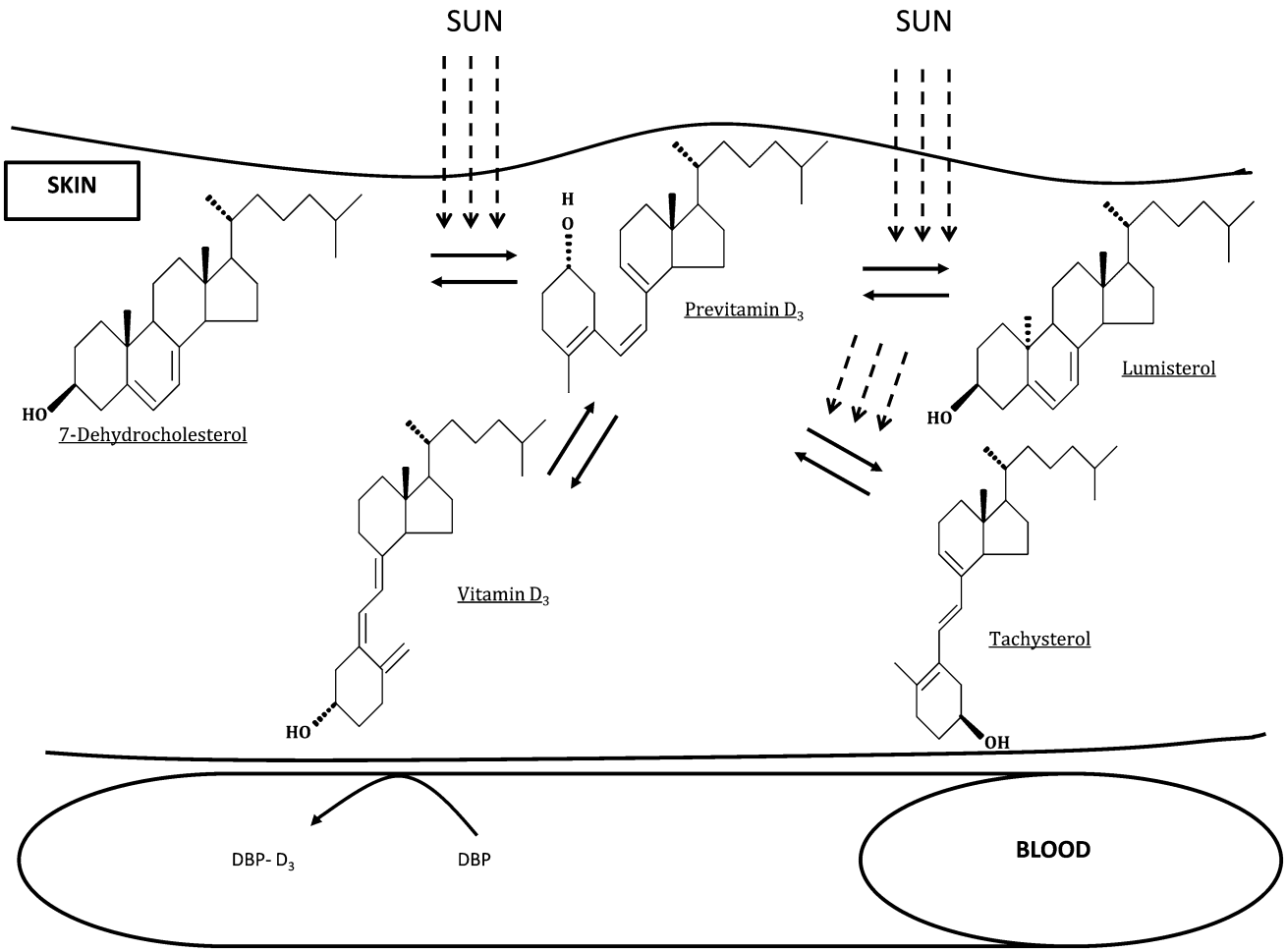

Fig. 1 Schematic of vitamin D3 synthesis in skin, adapted from [4]

colon, prostate, immune system or pancreas, all of which express CYP27B1. These "ectopic" sites can thus supply local needs of active vitamin D in a paracrine/autocrine manner. The most well-known example of this is its production in the macrophage cells of the antimicrobial peptide cathelicidin, a peptide capable of promoting innate immunity and inducing the destruction of infectious agents such as *M. tuberculosis* [13].

Regulation

As previously described, UVB radiation (UVB) induces conversion of 7-dehydrocholesterol to previtamin D3, but this exposure also leads to an increase in epidermal melanin content. Involving both a transcriptional and post-transcriptional response, this pigment increases in response to an increase in the number of melanocytes [14]. Vitamin D3 production is down-regulated by the tanning response because melanin competes with 7-dehydrocholesterol to absorb UVB photons, thus decreasing the efficiency of conversion to previtamin D3 [4]. Another determinant affecting the skin synthesis efficiency is the photoisomerization of previtamin D3 to two biologically inert products, lumisterol or tachysterol, thus limiting thermal isomerization to vitamin D3 [4].

Previously stimulated by a low blood calcium level, the parathyroid hormone (PTH) induces an increase in 1α-hydroxylase activity by increasing CYP27B1 expression in the kidney. Conversely, $1,25(OH)_2D$ is a negative feedback signal for both release of PTH into the circulation and CYP27B1 expression. Regulation of CYP27B1 expression by PTH and $1,25(OH)_2D$ seems to be confined to the kidney since several extrarenal sites have demonstrated other control systems [11, 15]. $1,25(OH)_2D$ also stimulates its own inactivation by increasing CYP24A1 expression. This enzyme is involved in hydroxylation of $1,25(OH)_2D$ and 25(OH)D on carbon 24 and, to a lesser extent, carbon 23. This mechanism generates metabolites that are more polar and less biologically active and that are subsequently metabolized and excreted in the bile [13].

Physiological actions

Mechanism of action

The 1α,25$(OH)_2$D metabolite acts through the VDR, which belongs to the nuclear receptor superfamily. When activated by 1α,25$(OH)_2$D, this receptor dimerizes with the retinoid X receptor RXR and then the 1α,25$(OH)_2$D-VDR-RXR complex binds up or down to vitamin D-responsive elements (VDREs) regulating the transcription of various genes in the target cells [12]. Indeed, Ramagopalan et al. [16] identified more than 2,700 human genome sites involved in VDR binding, and 1α,25$(OH)_2$D could affect the expression of as many as 229 genes. At least 37 cell types express VDR [17, 18], and this highlights the "pleiotropic" nature of VDR, which is involved in a broad range of health conditions and physiological functions.

However, 1α,25$(OH)_2$D is also involved in reactions that occur faster than those supported by a genomic response. These rapid responses might be explained by the presence of the cellular plasma membrane–binding VDRs sometimes described as associated with caveolae domains [19–21]. Membrane-bound VDR, through second messengers, could, for example, instigate rapid intestinal absorption of calcium (transcaltachia), the secretion of insulin by β cells in the pancreas, Ca^{2+} influx in muscle cells and the rapid migration of epithelial cells. The membrane-mediated rapid signalling process remains poorly understood and several models are still under debate [21]. An overview of vitamin D metabolism and its physiological action is presented in Fig. 2.

Effects on the skeletal system

1α,25$(OH)_2$D enhances intestinal calcium absorption via its nuclear VDR that up-regulates the expression of the epithelial calcium channel (TRPV6) and a calcium-binding protein (calbindin 9K) [13]. 1α,25$(OH)_2$D is also involved in the rapid intestinal absorption of calcium (transcaltachia) [22], as previously described, and enhances the efficiency of intestinal phosphate absorption. Indeed Fleet et al. [23] showed diminished intestinal phosphate absorption in animals with vitamin D deficiency and restored absorption efficacy on injection of vitamin D. Although the underlying mechanisms are not clear, a sodium–phosphate cotransporter (NaPi-IIb) may be involved. Vitamin D activity can also modulate renal phosphate reabsorption as 1α,25$(OH)_2$D stimulates synthesis of fibroblast growth factor 23 (FGF 23), a protein that acts to increase renal phosphate excretion. Indeed, FGF 23 decreases expression of the renal sodium–phosphate cotransporters NaPi-IIa and NaPi-IIc in the proximal tubule [24, 25].

1α,25$(OH)_2$D indirectly stimulates osteoclastogenesis by promoting the maturation of preosteoclasts to multinucleated osteoclasts. 1α,25$(OH)_2$D initially induces expression of membrane-bound RANKL by interacting with its VDR in osteoblasts. This osteoblast membrane factor then binds to its cognate receptor RANK localized on preosteoclast membranes. In preosteoclasts, the RANKL–RANK interaction triggers strong activation of the $\kappa\beta$ nuclear factor responsible for the maturation signal [13, 26]. Once mature, osteoclasts have potent bone resorption activity, resulting in a release of calcium and phosphate into the general circulation. By increasing calcium resorption, osteoclast activity may also support bone neo-mineralization [27].

Effect on non-skeletal systems

Discovery of the further effects of 1α,25$(OH)_2$D activity has gone hand in hand with a better understanding of how 1α,25$(OH)_2$D functions in cells, that is, whether by nuclear VDR or not, via 1α hydroxylase induction and/or by 24 hydroxylase induction. With these new targets for vitamin D has come the realization of the full health impacts of this vitamin. The following is an account of some of the diseases believed to be influenced by vitamin D status:

Cancer

Experimental evidence supports a reduction of risk of many cancers through the action of 1α,25$(OH)_2$D in suppressing the proliferation and stimulating differentiation of cancer cells. The cyclin/cyclin-dependent kinase (CDK) complex acts to ensure the phosphorylation of target proteins involved in cell cycle progression. Cyclin/CDK complexes are regulated by numerous proteins such as CDK inhibitors (CKIs). When associated with CDKs, CKIs prevent CDKs/cyclin complexes from forming. In the normal cell cycle, 1,25$(OH)_2$D induces growth arrest by regulating the transcription of cyclins and CKIs by enhancing the expression of three CKIs, p21, p27 and p53 [28–30], that may be involved in G1 cell arrest by silencing the phase S-dependent cyclin/CDK complex [30, 31]. This hypothesis is supported by the study by Hager et al. [32] showing that 1,25$(OH)_2D_3$ triggers p21 and p27 expression and concomitant hypophosphorylation of the retinoblastoma protein, leading to G0/G1 cycle cell arrest. Another suggested mechanism is that 1α,25$(OH)_2$D may exert its antiproliferative properties through its effect on the anti-Wnt-β-catenin pathway. Under normal conditions, the Wnt-β-catenin-TCF4 pathway induces expression of the genes involved in cycle cell control (C-MYC, PPARδ, etc.) [30, 33]. However, Palmer et al. [33] have shown that in a model of colon cancer, with vitamin D signalling, nuclear

β-catenin is translocated to the plasma membrane, thus depriving the nucleus of the β-catenin-TCF4 transcription signal. This is consistent with the antiproliferative properties of $1\alpha,25(OH)_2D$ since C-myc promotes cell growth.

Other antitumour effects include apoptosis. Studies report that vitamin D activity regulates pro- and antiapoptotic factors in support of the apoptotic process [30]. $1,25(OH)_2D$ decreases expression of the antiapoptotic proteins BCL_2 and $BCL\text{-}X_L$ and activates expression of BAX, BAK and BAD known to be pro-apoptotic proteins. $1,25(OH)_2D$ may also accelerate telomere shortening by inhibiting telomerase activity [30]. Telomeres are structures that cap the ends of linear chromosomes to preserve chromosomal integrity. In normal somatic cells, which do not express telomerase (except stem cells), telomeres shorten with every cell division; a short telomere is normally a signal for cell senescence and death. Telomerase maintains telomere length and is essential for cell immortalization [34–36]. Further, $1\alpha,25(OH)_2D$ may act as a potent antiangiogenic/antimetastatic factor. Indeed Mantell et al. [37] reported that $1\alpha,25(OH)_2D3$ can counteract both in vivo and in vitro VEGF-induced endothelial cell proliferation. $1\alpha,25(OH)_2D$ preserves the normal cell phenotype by diminishing the tumour-invasive potential. Indeed, $1\alpha,25(OH)_2D3$ can up-regulate expression of several proteins (E-cadherin, occludin, zonulaoccludens 1 and 2) involved in cell adhesion and intercellular junctions [33]. These proteins help maintain cell phenotypes and tissue structure. For example, E-cadherin is a cell–cell adhesion molecule that could be silenced in carcinoma cells undergoing epithelial–mesenchymal transition (EMT) [38]. Malignant EMT is thought to confer greater invasive potential [38].

Immunity

Vitamin D is important for stimulation of innate immunity. Studies report that $1\alpha,25(OH)_2D$ enhances the antimicrobial properties of monocytes and macrophages. $1\alpha,25(OH)_2D$ enhances both chemotaxis and the phagocytic capabilities of macrophages [39]. Furthermore, it also activates both the cathelicidin gene (CAMP) and defensin $\beta 2$ expression [40], both of which are antibacterial peptides capable of destroying the microbe cell membrane [15].

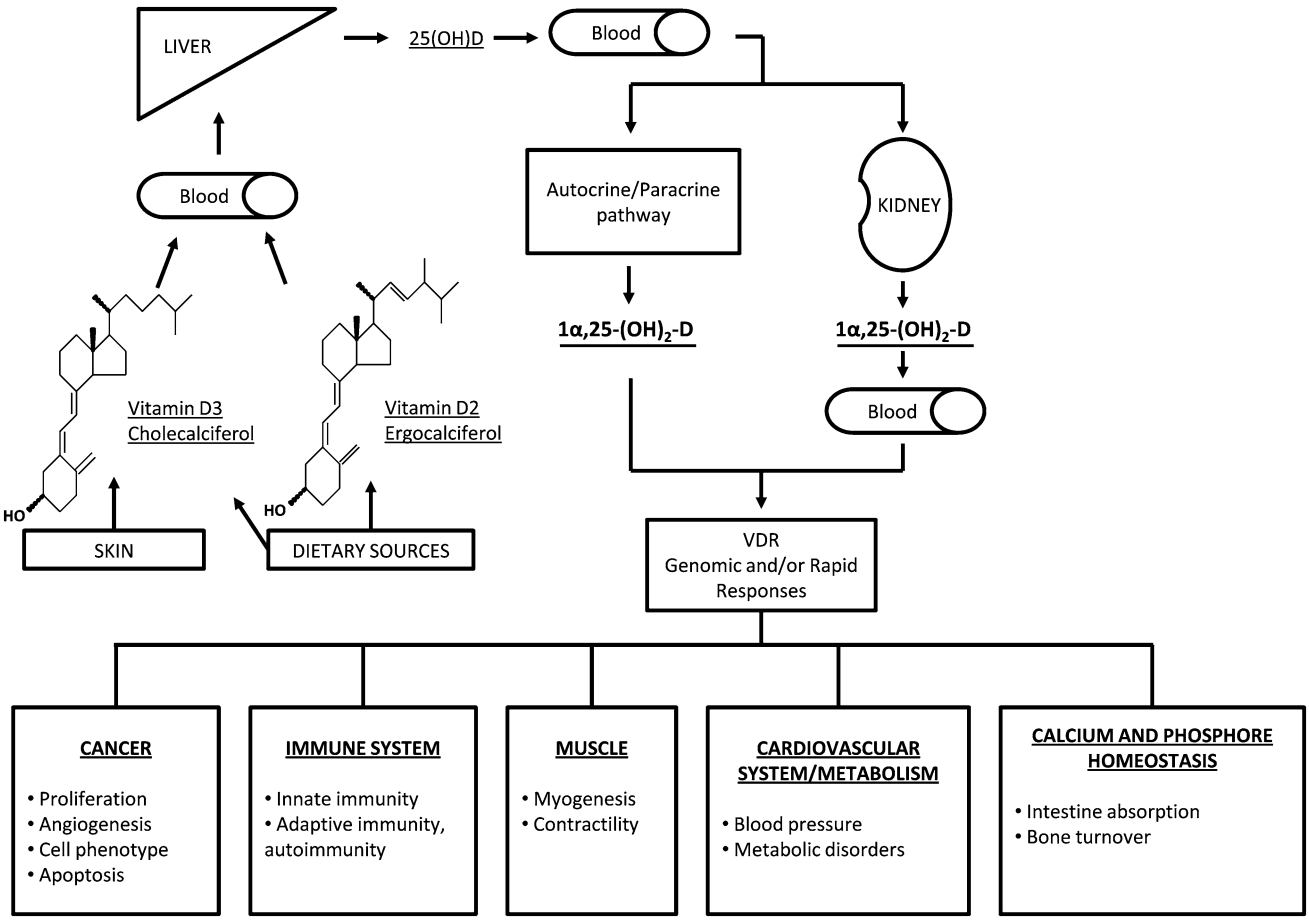

Fig. 2 Schematic of vitamin D metabolism and physiological actions

Interestingly, TLR1/2 activation of macrophages triggers VDR and 1-α hydroxylase expression, potentiating antimicrobial 1α,25(OH)$_2$D effects [41].

1α,25(OH)$_2$D can mitigate adaptive immunity [42]. VDR agonists have potent immunomodulatory effects on dendritic cells (DC). Indeed VDR activation gives dendritic cells tolerogenic properties. These immature dendritic cells are characterized by decreased levels of marker expression such as MHC class II, costimulatory molecules (CD 40, CD 80 and CD 86) and, respectively, increased and decreased synthesis of IL-10 and IL-12 [15, 39, 42]. Consequently, tolerogenic DC leads to a decrease in Th1 and Th17 cell response and an increase in T regulatory cells [15, 39, 42]. On T cells, the 1α,25(OH)$_2$D balances the T-cell profile in the same manner by decreasing Th1 cytokines (IL-2 and IFN-γ) and Th17 cytokine (IL-17, IL-21) production [39]. Furthermore, 1α,25(OH)$_2$D decreases differentiation, proliferation and immunoglobulin production of B cells and also triggers their apoptosis [15, 39, 42]. The modulation and mitigation of the adaptive immunity responses by vitamin D are also likely to be beneficial in autoimmune afflictions [15, 42].

Muscle function

A link between poor vitamin D status and muscle disorders is evident when osteomalacia and rickets are considered. VDRs are present in both the nucleus and plasma membrane of mammalian skeletal muscle cells [43]. Cell surface VDRs may be present in muscle cells as suggested by the fact that 1α,25(OH)$_2$D can induce fast effects, such as calcium uptake (1–15 min), that cannot be explained by the genomic pathway [44]. Several cellular and molecular level indications of atrophy and contractility disorders occur in hypovitaminosis D. 1α,25(OH)$_2$D modulates muscle cell calcium exchange and intracellular calcium. Calcium homeostasis regulation is an essential element for muscle contraction and relaxation. Stimulation by 1α,25(OH)$_2$D results in enhanced calcium intake and release of intracellular calcium stored in muscle cells. This cytosolic calcium influx has been identified as being mediated via voltage dependent calcium channels (VDCC) and the store-operated Ca^{2+} channel (SOC) [21, 45]. From cultured chick skeletal muscle cell studies, Ca^{2+} channels (CRAC) may also contribute to 1α,25(OH)$_2$D-induced calcium influx [46]. Various intracellular pathways have been identified to mediate 1α,25(OH)$_2$D Ca^{2+} channel activation. These effects involve G-protein stimulation that in turn activates phospholipase C and adenylyl cyclase pathways that ultimately activate PKC and PKA [21, 44, 47, 48].

1α,25(OH)$_2$D modulates muscle cell proliferation, differentiation and consequently myogenesis. Mitogen-activated protein kinase (MAPK) signalling pathways relay extracellular signals to activate intracellular targets, resulting in modulation of gene expression, proliferation, differentiation or apoptosis. As reviewed by Boland [21], there is compelling evidence that 1α,25(OH)$_2$D3 activates at least three MAPKs in muscle cells. Boland suggested that 1α,25(OH)$_2$D3 stimulates the MAP ERK-1/2 cascade through several possible mechanisms: tyrosine kinase, calcium and protein kinase C activation. Two other MAPK subfamilies, P38 MAPK and JNK-1/2, have been shown to be promoted in the presence of 1α,25(OH)$_2$D3. Moreover, vitamin D is thought to exert its beneficial effects on muscle physiology by balancing hyperparathyroidism. Indeed, an excess of PTH has been associated with various muscle, tissue and functional abnormalities [44].

Cardiovascular

Observational and controlled studies show that adequate vitamin D status is beneficial for good cardiovascular health. Vitamin D may be beneficial for preventing cardiovascular disease. As excessive levels of PTH are associated with increased blood pressure [49], 1α,25(OH)$_2$D can indirectly modulate blood pressure by decreasing PTH levels. Vitamin D interferes with the renin–angiotensin system (RAS) that regulates blood pressure. Li et al. [50, 51] showed that 1α,25(OH)$_2$D and its analogues can reduce renin synthesis. Renin is a protease responsible for conversion of angiotensinogen to angiotensin I, which in turn is a precursor of angiotensin II in the RAS. Consequently, 1α,25(OH)$_2$D may also reduce hypertension by slowing down RAS.

The heart may be a significant physiological target for 1α,25(OH)$_2$D3. In rat cardiomyocytes, Simpson et al. [52, 53] showed that 1α,25(OH)$_2$D3 can rapidly (within 15 min) modulate sarcomere contraction. This effect is likely to be mediated through membrane-bound VDR. They also demonstrated that VDR interacts with caveolin-3 in t-tubules and this interaction was shown to be affected by 1α,25(OH)$_2$D3 treatment. In the caveolae, the caveolin proteins interact with and modulate various signalling pathways. The authors thus hypothesized that 1α,25(OH)$_2$D3 affects VDR/caveolin 3 interaction that in turn activates signalization pathways to finally modulate cardiomyocyte contraction.

Diabetes

The relationship between vitamin D status and type 2 diabetes seen in observational studies [54] can be supported experimentally. For example, Maestro et al. [55] reported that 1,25(OH)$_2$D can trigger transcription of the human insulin receptor gene in U-937 human promonocytic cells. The activities where vitamin D (as 1α,25(OH)$_2$D3) may be

acting include pancreatic beta cell function, insulin sensitivity in peripheral target cells and, indirectly, systemic inflammation [56]. This activity could be associated with better insulin sensitivity, which would indirectly reduce cardiovascular risk as uncontrolled glycaemia is a major risk factor for cardiovascular disease [57].

Physiopathology

People with severe hypovitaminosis D experience rickets (especially infants and children) or osteomalacia (especially adults). While hypovitaminosis D usually results from malnutrition or lack of sun exposure, there are inherited disorders that disrupt vitamin D metabolism [58], as shown in Table 1.

Osteomalacia is a disease characterized by impaired mineralization of osteoid resulting in an accumulation of immature or non-mineralized bone [59]. This osteoid is mechanically weaker than mineralized bone and may bend and even break under load. Osteomalacia is also characterized by muscle atrophy, bone pain and fatigue [10]. At a lesser degree of hypovitaminosis, vitamin D insufficiency increases the risk of osteopenia that can result in osteoporosis. Osteoporosis is due to an unfavourable balance in bone remodelling/resorption whereby a loss of bone mineral density results in an increased risk of fractures and falls. This disease is common in older adults in whom calcium and vitamin D absorption can decrease with ageing. Rickets has the same aetiology as osteomalacia, but "rickets" is attributed to the disease affecting infants and children. If not treated in time, this disease can have serious and irreversible repercussions on growth and skeleton morphology, as well as respiratory problems [10].

Vitamin D-dependent rickets type I (VDDR-1) is an inherited disorder, transmitted as an autosomal recessive disorder caused by mutation of the 1α-hydroxylase gene [58]. Therefore, it results in reduced 1α-hydroxylase activity and less active metabolite production. Characteristic biochemical abnormalities are hypocalcaemia, secondary hyperparathyroidism and normal serum 25(OH)D3 levels whereas the $1,25(OH)_2D3$ serum level is clearly low. Treatment therefore consists of $1,25(OH)_2D$ supplementation to meet needs. Vitamin D-dependent rickets type II (VDDR-2), sometimes termed "vitamin D-resistant rickets", is also a genetic autosomal recessive disorder characterized by insensitivity of the target organs/cells to $1,25(OH)_2D$. It is caused by mutations on the gene coding for VDR [60, 61]. VDDR-2 is easily distinguished from VDDR-1 by elevated serum levels of $1\alpha,25(OH)_2D$ in VDDR-2. Treatment involves high doses of $1\alpha,25(OH)_2D$ and/or calcium.

What is the right target level to ensure optimal status?

Beyond its well-known skeletal functions, vitamin D is necessary for the normal functioning of other human body systems including those related to immunity, the cardiovascular system and cancer. This then leads to the question of how much vitamin D is needed to maximize $1\alpha,25(OH)_2D$ actions. There is a consensus that 25(OH)D is the best biomarker of vitamin D status, but there is still controversy about the serum concentration associated with an optimal status. The literature distinguishes two forms of hypovitaminosis D: that deemed "deficiency" and that called "insufficiency". Deficiency corresponds to a level of 25(OH)D below ~25 nmol/L [62], a level just sufficient to prevent rickets or osteomalacia. The Institute of Medicine (IOM) defines the desired serum 25(OH)D level as 50 nmol/L; however, as highlighted by Holick et al. [63] on behalf of the Endocrine Society, that recommendation is

Table 1 Rickets or osteomalacia resulting from inherited conditions

Pathologies	Aetiologies	Treatments
VDDR-1	Non-active 1α-hydroxylase gene leading to decrease in 1,25 (OH)2 production	1,25 $(OH)_2$ D supplementation
VDDR-2	Mutations incapacitating VDR	Depending on the severity of vitamin D resistance, some patients respond to very high doses of vitamin D (up to 50,000 IU daily of vitamin D2), or 1α,25(OH)2D (up to 12.5 μg/day) and/or calcium (up to 14 g/day by intravenous infusion and/or oral doses up to 6 g)
VDDR-3	Abnormal expression of hormone response element–binding protein (HRBP) limiting VDR binding on VDRE	High dose of 1α,25(OH)2D (up to 12 μg/day) and oral dose of calcium (up to 1 g)
CYP2R1 protein deficiency	Incapacitating mutation of CYP2R1 gene reduction in 25 hydroxylation activity	Vitamin D supplementation

Reference sources: [10, 58, 76–80]

exclusively based on data relative to bone health and may even be underestimated for calcium metabolism and bone health. Instead, they propose that the lower threshold for health is 75 nmol/L based on three rationales. First, at 80 nmol/L of 25(OH)D, vitamin D is no longer a limiting factor for calcium absorption [13, 64, 65]; second, at this level, PTH levels are minimized [13]; and lastly, Priemel et al. [66] in their study of German adults who had died accidentally found that only above 75 nmol/L 25(OH)D was the pathological proportion of unmineralized osteoid present.

In between "deficient" and "normal", there is a gap termed "insufficiency", linked to several outcomes that are associated with long latency diseases or dysfunctions [10, 67]. Others argue that the optimal status for many pathologies such as lower-extremity function, dental health, falls and fracture risk is met only at levels above 90–100 nmol/L of 25(OH)D [68]. This suggests that the optimal 25(OH)D serum level should be defined in terms of emerging autocrine functions and associated diseases.

Recommendations

Government positions

The US and Canadian governments requested the Institute of Medicine (IOM) to update its 1997 report on Dietary Reference Intakes (DRIs) of calcium and vitamin D. In late November 2010, the IOM published new DRIs for vitamin D. The new Recommended Dietary Allowances (RDAs) range between 600 and 800 IU/day; these are "values sufficient to meet the needs of virtually all healthy persons" according to the IOM report, keeping in mind that 50 nmol/L is the level the IOM found to be adequate "for good bone health for practically all individuals" [69].

Most European countries have their own recommendations for vitamin D intake. Table 2 gives the recommendations for France and the UK compared to IOM levels. The European Food Safety Authority (EFSA) does not suggest anything concerning vitamin D intake, but in 2006, it did released a report on tolerable upper intake levels for vitamins and minerals [70].

Other recommendations

There is a debate concerning what daily intake should be advised. Some researchers [68, 71] argue that 25(OH)D levels should be above 75–80 nmol/L to supply the needs of non-musculo-skeletal functions such as immunity and cancer prevention; the recommended intake of 600 IU/day for adults is not therefore clearly insufficient to reach this level in most people [72]. The US Endocrine Society recommends an intake of at least 1,000 IU to raise the blood level of 25(OH)D consistently above 75 nmol/L [73].

Safety will always be a concern; in 2010, however, higher upper intake level recommendations (UL) were made (see Table 3). Many researchers [68, 74, 75] argue that daily vitamin D intake well above these new ULs is safe. Hathcock et al. [68] suggest a UL at 250 μg/day (10,000 IU/day) based on trials that show a no-observed-adverse-effect-level (NOAEL) up to 250 μg/day. The lowest-observed-adverse-effect-level (LOAEL) was up to 1,925 μg/day.

Health claims

A health claim refers to any commercial advertising message that highlights or suggests a relationship between a nutrient, a food or a food constituent and health. Many countries permit health claims related to vitamin D (Table 4), but the regulatory authorities' responsibility is to ensure that permitted claims are scientifically substantiated, not misleading and clearly worded to avoid misinterpretation by consumers.

The FDA, Health Canada and EFSA have different approaches to assessing health claims. Here, we focus on the recently developed approach of the EFSA, for whom there are three kinds of health claim. The first are claims about "general function" assessed under Article 13.1 of the EU Regulation. The EFSA will establish a list of approved claims (to be published in 2012) on the basis of claims submitted by EU Member States. These application and authorization procedures are now effectively closed, and health claims in future applications must be made under Articles 13.5 and 14 (health claims related to vitamin D and pursuant to Article 13.1 are noted "A]" in Table 4).

The second claims are about "new functions" and are based on newly developed scientific evidence and refer to Article 13.5 (health claims related to vitamin D and pursuant to Article 13.5 are noted "B]" in Table 4). The third type of claim relating to "reduction in disease risk and child development or health" is assessed under Article 14 (health claims related to vitamin D and pursuant to Article 14. are noted "C]" in Table 4).

The EFSA publishes scientific opinions relative to each of these claims. On the basis of the EFSA's opinions, the list of authorized health claims will be adopted progressively by the European Commission.

Summary

The active metabolite of vitamin D, $1\alpha,25(OH)_2D$ can induce biological responses either through binding to

Table 2 Vitamin D recommendations for different countries

Organization and date	Age group/ pregnancy	Recommendation (daily)	Comment
Institute of Medicine IOM (2010)	1–50 year	600 IU (15 μg)	
	Pregnancy	600 IU (15 μg)	
	51–70 year	600 IU (15 μg)	
	71+	800 IU (20 μg)	People aged 71 and older may need as much as 800 IUs per day because of potential changes in people's bodies as they age
The Endocrine Society	0–1 year	400 IU (10 μg)	We suggest that obese children and adults and children and adults on anticonvulsant medications, glucocorticoids, antifungals such as ketoconazole, and medications for AIDS be given at least two to three times more vitamin D for their age group than recommended to satisfy their body's vitamin D requirements
	1–70 year	600 IU (15 μg)	
	71+	800 IU (20 μg)	
Osteoporosis Canada (2010)	19–50 year	400–1,000 IU (10–25 μg)	
	51+ year	800–2,000 IU (20–50 μg)	
Canadian Cancer Society	19+ year	1,000 IU (25 μg)	
Anses (at the time of writing)	1–3 year	400 IU (10 μg)	The dietary recommended intake has been defined considering that endogenous production covers 50–70 % of daily requirements for this vitamin (translated from French)
	4–19 year	200 IU (5 μg)	
	Adults	200 IU (5 μg)	
	Pregnancy/ breastfeeding	400 IU (10 μg)	
	Elderly	400 IU (10 μg)	
Department of health UK (2009)	6 month to 5 year	7 μg (280 IU)	Unless they are drinking 500 mL (a pint) or more of infant formula a day at any time during this age range
	6–65 year	10 μg (400 IU)	People who are not exposed to much sun, e.g. people confined indoors for long periods and those who cover their skin for cultural reasons
	Pregnancy/ breastfeeding	10 μg (400 IU)	
	65+	10 μg (400 IU)	

Reference sources: IOM [69], The Endocrine Society [73], Osteoporosis Canada [71], Canadian Cancer Society [81], Anses [82], Department of Health UK [83]

membrane-bound VDR or through nuclear VDR. In turn, VDR activation leads to activation of several cell-signalling pathways involving genomic or other processes. Signalling pathways are ultimately involved in a wide range of identified biological effects even beyond calcium and bone health–related effects. Among physiological cell effects referenced in the bibliography, many provide clarification of the role of vitamin D in the prevention of bone, muscle and cardiovascular disorders and for cancer immunity.

The level of the circulating metabolite of vitamin D, 25(OH)D, has become associated with health benefits. It now appears that 75 nmol/L is the low end of the threshold for adequate vitamin D status. This value provides sufficient vitamin D activity for both musculoskeletal and non-musculo-skeletal criteria. As there is no unique biomarker for autocrine functions, optimal status still needs to be defined and may vary depending on the expected health outcome. Although the IOM has recently revised upwards both the recommended daily intake and the upper level, there has been inertia by other regulatory authorities in revising health claims related to intake or safety. For example, the French-recommended daily

Table 3 Upper levels for vitamin D

	Age group	Tolerable upper intake level (UL)	Comment
European Food and Safety Authority (EFSA)	0–10 year	25 μg/day (1,000 IU)	Depending on the amount of sunlight the risk of adverse effects at an intake at the upper level could increase. It should also be noted that higher doses of vitamin D might be needed, particular in the elderly, to achieve optimal serum levels of 25(OH)D for optimal mineralisation of the skeleton. The UL for adults does also apply to pregnant and lactating women
	11 year+	50 μg/day (2,000 IU)	
IOM	0–6 month	1,000 IU	
	6–12 month	1,500 IU	
	1–3 year	2,500 IU	
	4–8 year	3,000 IU	
	9 year+	4,000 IU	The UL also apply to pregnant and lactating women

Reference source: EFSA [70], IOM [69]

Table 4 Health claims related to vitamin D in the USA, Canada and the EU

	Health claims	
Food and Drug Administration FDA	Calcium, vitamin D and osteoporosis: Adequate calcium and vitamin D, as part of a well balanced diet, along with physical activity, may reduce the risk of osteoporosis	
Canadian Food Inspection Agency	A healthy diet with adequate calcium and vitamin D, and regular physical activity, help to achieve strong bones and may reduce the risk of osteoporosis	
EFSA	Helps to absorb calcium in the gastrointestinal tract and keeps a balance of calcium in the organism	A]
	Is necessary for the absorption and utilisation of calcium and phosphorus	
	Is necessary for calcium uptake in bones*	
	Is necessary for the normal absorption and utilization of calcium and phosphorus	
	Is needed/important for the structure of bones/healthy bones	A]
	Helps build and maintain strong/healthy bones	
	Is necessary for adequate bone density	
	Is needed for teeth mineralization	
	Is important for the structure of healthy teeth	
	Contributes to promote teeth mineralization	
	Is necessary for normal bone and tooth formation	
	Helps build and maintain strong muscles	A]
	Is needed for proper functioning of the muscles	
	Helps maintain muscle function in ageing	
	Contributes to normal cell division	
	Is important for the immune system/natural defences	A]
	Vitamin D calcium and reduction of bone loss; at least 1,200 mg of calcium from all sources or at least 1,200 mg of calcium and 800 IU (20 μg) of vitamin D from all sources should be consumed daily in order to obtain the claimed effect. The target population is women 50 years and older	C]

Reference sources: FDA [84], Canadian Food Inspection Agency [85], EFSA [86–90]; A] health claims related to vitamin D and pursuant to Article 13.1; B] health claims related to vitamin D and pursuant to Article 13.5; C] health claims related to vitamin D and pursuant to Article 14.; * Wording submitted to EFSA

intake is a concern: while some researchers argue that 1,000 IU/day should be a minimum, the level set in France (200 IU) remains three times lower than that released by the IOM (600 IU). However, we acknowledge that the latest EFSA opinion on generic health claims for vitamin D is positive, as the panel has favourably received several health claims related to non-musculo-skeletal functions.

Finally, vitamin D emerges as a promising nutrient for new health strategies, but further research is needed to clarify non-classical functions of vitamin D and their underlying mechanisms. Vitamin D, through food fortification and supplementation, represents a nutrient with great potential for the development of innovative products that should attract food industry research and development funding.

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Conflict of interest Ghislaine Gerber, Jean-Michel Maixent, Stéphane Sadrin, Sébastien L. Peltier and Sylvain Battault are members of the SYNADIET scientific committee, the national union for dietary supplements in France. Sébastien L. Pelier and Sylvain Battault are employees of the company Laboratoire Lescuyer. Ghislaine Gerber is the head of the company Laboratoire Holistica. Stéphane Sadrin is an employee of the company Laboratoire Codifra-Denel. Susan J. Whiting has no conflicting interests.

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