Vitamin D and Crohn's disease in the adult patient: a review

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Abstract

Crohn's disease (CD) is characterized as a chronic immune-mediated inflammatory disorder of the gastrointestinal tract. Current consensus surrounding the cause of the disease suggests a complex interplay between genetic susceptibility, the intestinal microbiome and environmental factors, leading to the aberrant Th1 and Th17 cell driven autoimmune response.

Vitamin D deficiency is common in CD patients, and longstanding deficiency has been associated with reduced bone mineral density (BMD). Accumulating evidence now suggests that in addition to maintaining skeletal integrity, vitamin D also plays an integral role in regulating the general immune response, a function employed via its genomic actions on the vitamin D receptor (VDR). The VDR is expressed in all immune cells and both directly and indirectly targeted by the bioactive form of vitamin D, 1,25-Dihydroxyvitamin D ($1,25[OH]_2D$). Impaired regulation or deficiency of the vitamin has been linked to the promotion of self-reactive T cell development, loss of immune tolerance to self-structures and experimental colitis in animal models, whilst the subsequent administration of the vitamin in these models resulted in the improvement of immune-mediated symptoms. In addition, low vitamin D has been associated with disease activity in CD patients, and supplementation appears to be beneficial in improving clinical scores and reducing inflammation. Therefore, the primary aims of this paper were to review the molecular evidence supporting the immunoregulatory roles of vitamin D and its supplementation in the CD patient, based upon existing literature. The physiological processes, accepted serum concentration values and its well-recognized role in bone health were also summarized.

Introduction

Crohn's disease (CD), a subtype of inflammatory bowel disease (IBD), (Ulcerative colitis (UC), being the second subtype), is characterized as a chronic immune-mediated inflammatory disorder involving the host self-tissue damage of the gastrointestinal tract.[1–4] Although the exact pathogenesis of CD is unknown, it is believed to result from a dysregulated Th1 and Th17 cell driven immune response triggered by the host intestinal microbiome, and a range of poorly defined environmental factors, in genetically susceptible individuals.[3]

Vitamin D is derived from skin exposure to ultraviolet (UV) sunlight, and is well-described for its function in the maintenance of calcium and phosphorus homeostasis and skeletal integrity.[3–6] Deficiency in the vitamin is commonly found in the CD patient due to the inherent nature of the disease, and longstanding

deficiency is associated with reduced bone mineral density (BMD) and osteomalacia in the adult CD patient.[7–11]

In addition to bone health, accumulating evidence has revealed that vitamin D also plays an integral role in regulating the immune system, an action employed via its genomic effects on the vitamin D receptor (VDR). The VDR is expressed in all immune cells, and is directly and indirectly targeted by the bioactive form of vitamin D, 1,25-dihydroxyvitamin D (1,25[OH] $_2$D).[12–16] Both *in vivo* and *in vitro*, the active form of vitamin D has been shown to regulate the development and function of T-cells, anti-microbial peptides and dendritic cells (DCs), together with modulating the up-regulated Th1 cell response associated with immune-mediated disease pathogenesis.[12–17] In murine models, vitamin D deficiency or the impairment of its regulatory mechanisms, have been linked to the promotion of self-reactive T-cell and experimental IBD development. In these same models, the administration of $1,25(OH)_2D_3$ resulted in an improvement of these immune-mediated symptoms.[12–17] The association between vitamin D and its modulatory effect on disease activity in the CD patient has also recently been demonstrated in cross-sectional studies and small clinical trials, notwithstanding some discrepancies in the data.[18, 19] With this background, it is not surprising that there has been a recent upsurge in the literature, providing a variety of preliminary data relating to the role of vitamin D in CD. Therefore this review will focus on highlighting the immunoregulatory role of vitamin D and the evidence supporting its supplementation in the context of the CD patient. In addition the review will delineate the human synthesis, storage, and excretion of vitamin D, and the generally accepted serum concentration reference ranges as supported by the available evidence to date.

Methods

An extensive online search of the literature using the PUBMED (http://www.ncbi.nlm.nih.gov/pubmed) and EMBASE (http://www.embase.com/) databases was performed, which included the years 1966 through June 2013. Studies were restricted to the English language and found using the search terms “vitamin D”, “25(OH)D”, “25(OH)2D3”, “25(OH)2D”, “vitamin D3”, “vitamin D2”, “Crohn’s disease”, “Inflammatory bowel disease”, “disease activity”, “autoimmune disease”, “autoimmunity”, “immunomodulation”, “supplementation”, “deficiency”, “animal models”, “measurement”, “cholecalciferol”, “ergocalciferol” in the full text option. The titles and abstracts of all studies were scanned to exclude any studies which were clearly not relevant to the topic. In addition, all references cited in original studies and in all reviews were identified. The aim was to review the immunoregulatory role of vitamin D in the context of the CD patient.

Vitamin D overview

Synthesis

Vitamin D is a "steroid-like" hormone, that exists in two forms; cholecalciferol (25[OH]D_3) also known as 'vitamin D3' (VD3), which is the naturally occurring form in humans, and ergocalciferol (25[OH]D_2) also known as 'vitamin D2' (VD2).[20] Food and supplements contain both forms of vitamin D (D2 and D3) however VD3 is rare in foods, and originates only from 7-dehydrocholesterol (7-DHC) in animal sources such as oily fish and egg yolk, while VD2 is mostly acquired from yeast and plant sterol sources (Table 1).[14, 20, 21] The primary source of pre-vitamin D3 is produced from UV irradiation of 7-DHC present in the skin, which rapidly undergoes isomerization to form vitamin D3, a process stimulated by body heat.[5, 14] The human 'skin derived' VD3 is the easiest and most cost-effective means of obtaining vitamin D, and adequate levels can be acquired from 5 to 30 minutes (depending on season) of sun exposure to the skin on the face, arms, back or legs twice every week, even on hazy or cloudy days.[22] However, exposure may need to be increased by 5 to 16 times this amount in individuals with darker pigmented skin due to the higher melanin content reducing UV permeation to the skin (Table 1).[20]

The dietary form of vitamin D is fat soluble in nature, which once digested, is required to undergo two sequential hydroxylations in the body to become biologically active (Figure1). Upon digestion, vitamin D is delivered via plasma to the liver by either chylomicron remnants, vitamin-D binding protein (DBP) or transcalciferon.[23] Vitamin D is then metabolized in the liver by the cytochrome P450 enzyme '25-hydroxylase' into '25-hydroxy vitamin D' (25[OH]D), the major circulating and storage form of vitamin D in the body.[24] There are two ways in which 25(OH)D can be converted into its biologically (hormonally) active form calcitriol or '1,25-hydroxy vitamin D' (1,25 $[OH]_2$D), one being in the proximal tubule of the kidneys.[25, 26] Renal conversion of the metabolite functions to regulate calcium metabolism, and is under tight control by the parathyroid hormone (PTH) and fibroblast growth factor (FGF23) in response to serum levels of calcium and phosphate, through the interaction between 1,25$(OH)_2$D with the VDR in the small intestine and on osteoblasts, and via the regulation of renal 25-hydroxyvitamin D-1-α-hydroxylase (1α-OHase) (CYP27B1), the latter acting as a rate limiting step in the pathway (Figure 1).[20, 27] The second conversion mechanism can take place within immune cells, such as T lymphocytes, B lymphocytes, macrophages and DCs. These cells possess 1α-OHase and the VDR, enabling the localized activation of circulating 25(OH)D into 1,25$(OH)_2$D or 1,25$(OH)_2D_3$, and the ability to respond in an autocrine or paracrine fashion to the metabolites.[28] Unlike renal 1α-OHase, cellular 1α-OHase is not up-regulated by PTH, and thus immune cell production of 1,25$(OH)_2$D is dependent on serum 25(OH)D concentrations.[29]

Storage, circulation and excretion

In the human body, 25(OH)D is the major storage and circulating form of vitamin D. Highest concentrations are found in the plasma, and usually measured in the serum,

although adipose and muscle tissue are believed to store the largest pool of 25(OH)D.[30, 31] The metabolites in the vitamin D pathway are circulated by DBP carrying 85-90%, and albumin, which carries the remaining 10-15%.[31] *In vivo*, the circulating half-life of 25(OH)D is approximately 2-3 weeks,[32] however can last up to three months when considering adipose tissue store release.[15, 32] For excretion, 25(OH)D, 1,25$(OH)_2$D and 1,25$(OH)_2D_3$ are converted into the inactive metabolites 24,25$(OH)_2$D and 25(OH)D-26,23-lactone, by the enzyme 24-hydroxylase, allowing final elimination via urine and bile (Figure 1).[33, 34]

Vitamin D Binding Protein (DBP)

Vitamin D binding protein is the major circulatory protein for metabolites in the vitamin D pathway. The entry of the DBP-bound vitamin D metabolites (i.e., 25[OH]D and 1,25$[OH]_2$D) into the cell can occur either through endocytosis (in which case DBP is also taken up),[35] or diffusion, though only small amounts of DBP-bound vitamin D metabolites passively diffuse across cell membranes.[15, 36] Once the metabolite has entered the cell via endocytosis, DBP plays a role in regulating the intracellular actions of 25(OH)D and 1,25$(OH)_2$D, and thus their availability to the target organ.[37–39] In a small cohort of 49 adults, Powe et al[31] demonstrated that DBP modulates the relationship between vitamin D and bone density, as a positive correlation was found between BMD and free bioavailable 25(OH)D, however not with total 25(OH)D. The authors also found that DBP concentrations and BMD were negatively correlated (r= -0.296). Similarly, in the renal tubular cells, DBP also facilitates the cellular uptake of bound 25(OH)D enabling its presentation to renal 1α-OHase, further regulating the second hydroxylation reaction of 25(OH)D into its hormonally active form.[36, 39, 40]

There are three primary single nucleotide polymorphisms for the Gc gene that codes for DBP, resulting in six inheritable diplotypes, each with varied functionality regarding the binding affinity of DBP for 25(OH)D or its metabolites.[41–46] Whilst the significance between the functional variations of these Gc polymorphisms are not yet known, it has been suggested that they may result in different circulating and intracellular levels of vitamin D between individuals.[15, 47] Therefore, if these variations prove functional, future determinations of vitamin D status would require the measurement of the DBP genotype and circulating 25(OH) vitamin D3.[15] However to date, studies have failed to establish a consistent association between a DBP genotype and risk of specific diseases.[48–50]

The Vitamin D Receptor (VDR)

The biological effects of VD3 are regulated by the VDR, a nuclear receptor expressed in many cell types.[30, 51, 52] Upon entry into the cell or through intracellular conversion from 25(OH)D, the metabolites 1,25$(OH)_2$D or 1,25$(OH)_2D_3$ will bind to nuclear VDR forming a VDR-complex.[33] The VDR-complex promotes heterodimerization with the retinoid-X receptor (RXR), ultimately functioning as a ligand-activated transcription factor that will bind vitamin D response elements

(VDRE) in the promoter regions of target genes.[27] The complex has been shown to both promote and suppress the transcription of several genes when combined with transcription factors and co-regulatory proteins.[33]

Similarly to DBP, allelic variations in the VDR gene have subtle effects in protein production and function.[17, 53–56] One such recognized variant, X-linked hypophosphatemia (XLP), also referred to as 'vitamin D resistant rickets', is a sex linked dominant familial disorder resulting in excess phosphate excretion and impaired bone mineralization. Patients are resistant to vitamin D supplementation and treatment includes oral phosphate, calcitriol and surgery if necessary.[55] It is believed that other VDR variants may act as predisposing factors in immune-mediated disease development, [24, 57, 58] however, at this time inconsistent results surrounding VDR polymorphisms, a functional phenotype, and various disease states have been reported.[15, 56] Furthermore, it is likely other genes and complex interactions between these genes contribute to the extremely complex vitamin D regulatory system which may also affect susceptibility to immune-mediated disease, and is being actively researched.[13]

Determination of Vitamin D status: serum 25(OH)D

In the human body, serum 25(OH)D (a summation of both VD3 and VD2) is the recommended index to determine whether a patient has adequate, depleted or intoxicative amounts of vitamin D.[59–65] Despite 1,25$(OH)_2$D being the biologically active form, it is not recommended as a measurement index for vitamin D status as it only has a half-life of 4-6 hours, with circulating levels about one thousandth (measured in the picomolar range) that of 25(OH)D.[66] In addition, during vitamin D deficiency, intestinal calcium absorption is reduced, which transiently lowers ionized calcium triggering calcium sensors in the parathyroid gland to increase PTH production and secretion.[67] The PTH in turn acts to increase renal re-absorption of calcium, increase skeletal calcium mobilization, and increase the production of 1,25$(OH)_2$D in the kidneys,[60, 63, 64] resulting in 'normal' or elevated serum 1,25$(OH)_2$D concentrations, thus making measurement of this metabolite useless to determine vitamin D status.[66]

Although there is no absolute consensus for what a normal range for 25(OH)D should be, vitamin D deficiency or hypovitaminosis D is generally defined as a serum 25(OH)D below ≤20ng/mL (a range still sufficient to prevent secondary hypoparathyroidism),[68, 69] with serum 21-29 ng/mL regarded as vitamin D 'insufficient', and serum ≥30-36ng/mL being vitamin D 'sufficient' (Table 2).[20, 60, 61, 70] Values have been based on studies showing that serum PTH begins to plateau at serum 25(OH)D concentrations ≥30ng/mL, and intestinal calcium transport efficiency is maximized when serum 25(OH)D levels are above 32ng/mL.[68, 71–73] The upper limit for serum 25(OH)D is considered to be >60ng/mL and has been associated with hypercalcemia, hypercalciuria and often hyperphosphatemia, while values >100-150ng/mL have been associated with toxicity (Table 2).[61, 62, 74]

Conversely, vitamin D deficiency diseases like rickets (children) and osteomalacia (adults) have no recommended 'optimal' serum level as relatively low vitamin D levels may eliminate disease risk.[5, 13]

Toxicity

Hypervitaminosis D or vitamin D toxicity with excess 25(OH)D levels most commonly manifest as hypercalcemia. However in the literature, hypercalcemia directly resulting from large quantities of orally consumed supplemental vitamin D, has only been recorded in cases with 25(OH)D levels ranging from 128-648 ng/mL, achieved after prolonged oral supplementation with dosages from upwards of 10,000-40,000 IU/day.[15, 75] In general, serum 25(OH)D concentrations of >100-150ng/mL will produce a vitamin D toxicity in the human body, yet these values may be slightly lower for children.[76] There is no evidence of vitamin D toxicity after prolonged UV sun exposure as increases in pre-vitamin D3 concentrations lead to an increase in the formation of inactive vitamin D compounds, which acts as a natural protective mechanism.[28, 75] Therefore concerns about skin cancer risk may be the only contraindication for direct and prolonged exposure to the sun.

Immune regulation in Crohn's disease

Th1, Th2 and Th17 response

Immune regulation is driven by the functional differentiation of naïve CD4+ T cells into Th1, Th2, Th17 and CD4+ T-regulatory (T-reg) cell phenotypes.[77] Each of these governing immune cell responses, and the critical balance between them, are mediated by key regulatory cytokines produced by both macrophages and DCs.[13, 14, 77] In terms of the Th1 response, host defence against tumors or pathogens (viruses) results in a 'normal' up-regulation of the pro-inflammatory (Th1) helper T-cells, which leads to the secretion of the pro-inflammatory cytokines; interferon gamma (abbreviated either IFN-y or INF-y), interleukin-2 (IL-2), and tumor necrosis factor alpha (TNF-α), and ultimately defence against the foreign pathogen. Traditionally, IFN-y mediates the Th1 response, and IFN-y itself is directly induced by interleukin-12 (IL-12), a cytokine produced by antigen-presenting cells (APCs). [78, 79]

During CD however, chronic gastrointestinal inflammation and mucosal damage is propagated by an exaggerated Th1 T-cell immune response to the commensal ('normal') bacteria residing in the gut. The T-cell production of Th1 cytokines (IL-2, IFN-y and TNF-α) fuels the immune-mediated attack against intestinal cells during IBD, and has been associated with IBD symptoms in humans,[80] and the transfer of Crohn's like symptoms to naïve mice.[81, 82] In particular, pro-inflammatory cytokines TNF-α and IFN-y are thought to directly contribute to disease pathogenesis by damaging the epithelial mucosa and increasing its permeability to both bacterial and dietary antigens, the latter considered a hallmark characteristic of CD.[3, 83–87]

Conversely, the function of the Th2 cells is to secrete interleukin-4 (IL-4) and interleukin-5 (IL-5), both essential components of antibody-mediated immunity.[14]

The Th2 cells are produced in response to extracellular pathogens such as bacteria and parasites. When the Th2 cells are abnormally up-regulated in response to environmental antigens, conditions such as asthma and allergies develop, alternatively, in CD disease, there is a down-regulation of the Th2 response.

The fairly recent characterization of a third T-cell mechanism involved in IBD pathogenesis, distinct from the Th1 or Th2 cells of cellular immunity, comprises the Th17 immune response, a CD4+ T cell subset now believed to play an integral role in maintaining inflammation, which may lead to tissue damage.[88] The functional development Th17 cells is indirectly induced by the cytokine interleukin-23 (IL-23) and mediated by the cytokine interleukin-17 (IL-17).[89, 90] While IL-12 (direct inducer of IFN-y in Th1 response) and IL-23 are heterodimers and both associated with the pathogenesis of CD, unlike IL-12 (a heterodimer of p40 and p35 subunit), IL-23 (heterodimer of same p40 subunit as IL-12 with a unique p19 subunit) cannot directly regulate naïve CD4+ T cells in adopting the Th17 phenotype.[91] Instead, initial Th17 cells are generated by the collective activity of transforming growth factor-beta (TGFβ) and interleukin-6 (IL-6).[91] Only after initial Th17 cell generation, do the cells gain the ability to express the IL-23 receptor allowing their terminal differentiation, and the persistence of cytokines IL-17, interleukin-22 (IL-22) and interleukin-21 (IL-21).[92, 93] It is important to note that after the initial generation of Th17 cells via TGFβ and IL-6, in the absence of IL-23, Th17 cells lack the ability to induce inflammation, and instead produce the anti-inflammatory cytokine interleukin-10 (IL-10).[94]

The role of IL-23 and the Th17 immune response surrounding intestinal inflammation was initially demonstrated in immunodeficient (RAG-deficient or SCID mice) murine models with experimentally induced colitis, later reconstituted with naïve T-cells.[93] When the RAG-deficient mice were also made deficient in p35 (thus unable to produce IL-12 and abbreviated as IL-23p35), colitis developed. The IL-23p35-deficient mice exhibited a marked increase in colonic IL-17 with moderate reductions in TNF-α and IFN-y. Conversely, when the mice were instead made to be p19 deficient (thus unable to produce IL-23 and abbreviated as IL-23p19), intestinal inflammation did not develop, and marked reductions in colonic TNF-α and IFN-y, with mild increases in colonic IL-17, were observed. Both models did however develop splenomegaly and hepatomegaly, suggesting that underlying systemic inflammation is driven by both IL-12 and IL-23.[95] The difference in colonic IL-17 concentrations between the two models (IL-23p35-deficient mice with markedly increased colonic IL-17 *versus* IL-12p19-deficient with mildly increased colonic IL-17) also implies that the IL-17 cytokine may be down-regulated by the Th1 response, and not entirely controlled by IL-23.[93, 96] The contributory role of CD4+ Th17 cells was further underscored after the transfer of bacterial reactive CD4+ Th17 cells resulted in a more severe form of colitis in immunodeficient murine models, when compared to those transferred with bacterial reactive CD4+ Th1 cells. As in most experimental colitis, elevated concentrations of both IL-23 (intestine) and IL-17

(serum and mucosa) have been identified in CD patients, [97–100] and administration of anti-cytokine therapy (e.g., anti-IL-12p40) has proven beneficial.[98]

It is important to point out however, that after the transfer of T-cells to IL-23p19-deficient/RAG1-deficient mice, colitis still developed due to the presence of an intact IFN-y response, provided there was an absence of regulatory T-cell development caused by either an IL-10 or TGFβ deficiency. Moreover, IL-23p19-deficient mice exhibited an increase in regulatory T-cell concentrations, and IL-23p19-deficient/RAG1-deficient mice with an inability to develop regulatory T-cells (e.g., those lacking Foxp3), also developed colitis after the transfer of T-cells.[93, 101] Based on these findings, it is possible that the contributory effect of IL-23p19 may be through the down-regulation or 'neutralization' of T-regulatory responses such as Th1, rather than the failure of Th1 to moderate colitis, thus explaining why the immunodeficient (RAG-deficient or SCID mice) IL-23p19-deficient mice did not develop colitis.[93] Therefore, despite recent reports demonstrating the IL-12/IFN-y and IL-23/IL-17 pathways as mutually exclusive (i.e., IFN-y shown to suppress IL-17 and vice versa),[102, 103] it is possible that the Th1 and Th17 responses co-exist during CD pathogenesis, each governing different phases of the dynamic inflammatory process that occurs in human CD (in comparison to that of CD mouse models), however the extent of their relationship is not yet fully explicated.[93, 104]

Vitamin D and immune regulation

Th1, Th2 and Th17 Response

The bioactive form of vitamin D ($1,25[OH]_2D$) is now known to directly target T-cell function. For instance, in Th1 cells, $1,25(OH)_2D$ has been shown to suppress purified T-cell proliferation, reduce IFN-y and IL-2 production, and increase the production of IL-5 and IL-10, [105–108] while in the Th2 cells, there is an up-regulation of IL-4 production,[105] ultimately resulting in an overall shift away from a Th1 phenotype towards a more tolerogenic Th2 phenotype.[23, 109–116] In addition, it was shown that the activation of CD4+ T cells ensued a five-fold increase in VDR expression allowing the regulation of 102 identified $1,25(OH)_2D$-responsive genes.[110, 111]

Supporting evidence was first demonstrated in VDR knockout (KO) murine models (i.e., vitamin D signalling inhibited), which exhibited an up-regulated pro-inflammatory Th1 response (higher INF-y cytokine production), and down-regulated Th2 response (lower IL-4, IL-5 production) compared to wild-type (WT) mice.[117] Interestingly, the uncorrected vitamin D deficiency in these VDR KO mice resulted in an accelerated form of experimental IBD, that subsequently improved after $1,25(OH)_2D_3$ administration, as Th1 pro-inflammatory cytokine production decreased, and Th2 cell secretion of IL-4 increased. [13, 105, 118, 119] The administration of $1,25(OH)_2D_3$ was then evaluated in transgenic IL-10 KO murine models, as IL-10 plays a regulatory role based on its ability to suppress Th1 cytokine synthesis. Interleukin-10 KO mice are known to spontaneously develop IBD, exhibiting severe enterocolitis within 5-8 weeks of life, with a resultant 30%

mortality.[120] The uncontrolled immune response is directed towards commensal intestinal flora, as germ-free IL-10 KO mice did not develop the disease.[14] Upon the administration of $1,25(OH)_2D_3$ however, the IL-10 KO mice exhibited reduced inflammation, mortality and an improvement in IBD symptoms. While the KO mice models may not be representative of a "normal" immune reaction, the symptoms exhibited in the mice models are similar to symptoms in CD patients who present with decreased serum IL-10,[121] and in human cells, the administration of $1,25(OH)_2D_3$ and dexamethasone increased the production and function of T-reg CD4+ T cells, via IL-10 stimulation[122] and the T-reg-associated cytokine TGFβ1.[123] Notable suppression of IBD has also been demonstrated via dietary manipulation of vitamin D when combined with calcium, in IL-10 KO mice.[124, 125] Murine IL-10 KO models were divided into four groups, each receiving one of four diets namely; a diet deficient in $1,25(OH)_2D_3$ with no added calcium (0.2%), a diet deficient in $1,25(OH)_2D_3$ with high concentrations of calcium (2.2%), a diet treated with 20ng/day $1,25(OH)_2D_3$ with no added calcium, or a diet treated with 20ng/day $1,25(OH)_2D_3$ with high calcium. Serum calcium levels and intestinal weights were later measured. It was found that the group receiving the high $1,25(OH)_2D_3$ and high calcium diet maintained a similar intestinal composition compared to that of the WT control mice, while the $1,25(OH)_2D_3$ and calcium deficient groups developed symptomatic IBD.

Recently however, two independent studies reported that the administration of $1,25(OH)_2D_3$ in naïve murine precursers failed to inhibit[126]/resulted in negligible effects[113] on Th1 cell differentiation, and intriguingly, compared to Th17 cells, VDR mRNA in the Th1 cells was found to be approximately 30-fold lower. [113] Although the role of vitamin D and the T-cell phenotype Th17 has not yet been fully elucidated, *in vitro* studies have demonstrated a partial suppression of Th17 cell developmental programming, with increased IL-10 expression after $1,25(OH)_2D_3$ administration, [113] and in naïve CD4+ T cells, the metabolite inhibited IL-6 expression, an important cofactor for Th17 cell differentiation.[127, 128] Moreover, murine models with experimentally induced intestinal inflammation exhibited a reduction of IL-17 expression after $1,25(OH)_2D_3$ administration,[129] whilst the absence of $1,25(OH)_2D_3$ (secondary to CYP27B1 gene ablation) resulted in elevated IL-17 concentrations.[130]

Anti-microbial peptides

The innate immune system is the first barrier of defence against foreign organisms. The cellular response of the innate immune system involves a cascade of events largely triggered by two classes of pathogen recognition receptors, namely pathogen-associated molecular patterns or PAMPs (e.g., lipopolysaccharide, flagellin, viral proteins and single-and double stranded RNA) and toll-like receptors (TLRs), the latter being a sub-class of recognition receptor expressed on cell membranes or endosomes.[131] In humans, TLR signalling results in the production of anti-microbial peptides namely; cathelicidin and the α- and β-defensins.[132] Cathelicidin is produced when cells lining epithelial surfaces (e.g., skin, respiratory tract,

gastrointestinal tract),[133–135] neutrophils or macrophages are exposed to potential pathogens. Cathelicidin plays an integral role in innate immune function, and when deficient, whether in humans or cathelicidin KO mice, 'barrier site' (i.e., mucosal membranes and epithelial surfaces) infections are more prone to develop.[28] The effect of vitamin D on anti-microbial peptides was first demonstrated in cell lines and primary cultures (including ones derived from skin, macrophages, neutrophils, lung and colon) treated with $1,25(OH)_2D_3$, as it lead to the up-regulation of cathelicidin mRNA. Furthermore, it has been reported that the promotor region of genes coding for both cathelicidin and β-defensin possess the VDRE.[136–138] In human macrophages, TLR stimulation lead to an up-regulation of the VDR and cellular 1α-OHase production (CYP27B1) allowing cellular conversion of 25(OH)D into $1,25(OH)_2D$, and in the presence of TLR stimulation, cathelicidin mRNA in human macrophages is up-regulated by $1,25(OH)_2D_3$.[28, 139] Conversely, *in vitro,* when the VDR or CYP27B1 are inhibited, or when 25(OH)D substrate levels are low, cathelicidin is no longer up-regulated,[140] implying that the underlying pathway in terms of local $1,25(OH)_2D$ production (i.e., VDR and cellular 1α-OHase up-regulation) which enhances cathelicidin production, can only occur when vitamin D status is adequate.[28, 139]

Dendritic cells and the Epithelial Barrier

Dendritic cells are APCs which play a central role in antigen presentation to T-cells and have been associated with both the initiation and propagation of T-cell mediated gastrointestinal immune responses in IBD. Immature DCs function to promote T-cell tolerance whereas the function of mature DCs, is to activate naïve T-cells.[141, 142] DCs express the VDR,[143, 144] and their differentiation from monocytes,[145] maturation and survival are influenced by the availability of $1,25(OH)_2D$, which when present, results in an overall more tolerogenic DC phenotype.[28, 51, 57, 146–148] The immunosuppressive effect of $1,25(OH)_2D_3$ on DCs was shown *in vivo* [57, 58, 146–148] to reduce Th1 development (inhibiting IL-12 production),[130] increase Th2 development (promoting IL-10 production), [146–148] and compared to T-cells cultured with control DCs, T-cells cultured with $1,25(OH)_2D_3$-treated DCs secrete less pro-inflammatory cytokine IFN-y.[146] Likewise, administration of $1,25(OH)_2D_3$ to DCs suppressed the expression of p40 (IL-12 cytokine),[149] which may in turn reduce the faction between Th1 and Th17 cells.[150]

The epithelial lining of the gastrointestinal tract from the stomach to the anus acts as a physical barrier to pathogens and as an immune regulator through antigen presentation.[151, 152] Two types of intracellular junctions connect the epithelial cells and are referred to as either tight junctions or adherens junctions based on their functionality.[152, 153] These junctions are comprised of proteins called pore forming transmembranes. Defects in these proteins may lead to alterations in mucosal permeability to microbes thus increasing the risk for inflammation.[154] Claudin-2 is one pore forming transmembrane that has been implicated in the pathogenesis of IBD and its expression is ultimately induced by INF-y and inhibited by protein

tyrosine phosphatase N2 (abbreviated either PTPN2 or PTNP2).[15, 155] Interestingly, the gene bound to the VDR is in fact PTNP2 and the PTPN2 locus is considered as a high risk IBD locus associated with colonic CD and UC.[156] Consequently, the $1,25(OH)_2D$-VDR complex may act to impede the expression of PTNP2 thus inhibiting epithelial barrier pore formation and the altered intestinal permeability associated with disease development.[15, 17]

Summary

The immunoregulatory effect of vitamin D is an absolute result of the presence of VDR in cells involved in the human immune structure. The presence of $1,25(OH)_2D$ or $1,25(OH)_2D_3$ may both directly and indirectly regulate T-cell immune function, albeit more research is needed to better elucidate the broad relationship between the Th1 and Th17 responses, including the contributory roles of vitamin D, respectively. Nevertheless, vitamin D metabolites have shown to increase the number and function of regulatory T-cells and regulate antigen presentation in DCs, each well-established for their role in CD pathogenesis. For that reason, it appears that the ability for an immune system to properly develop and function optimally may be lost when vitamin D is deficient, potentially favouring the development of self-reactive T cells, and immune-mediated disease development.

Vitamin D and Crohn's disease

Epidemiological evidence

Crohn's disease is one of 80 known immune-mediated disorders, affecting an estimated 1.4 million individuals in the United States and 2.2 million in Europe.[2, 157] Disease activity usually follows a relapsing-remitting pattern, which may or may not be accompanied by a variety of extraintestinal manifestations (EIM).[1, 2] When present, EIMs can occur in any organ however the joints (e.g., arthritis; most frequently observed EIM occurring in about 25% of cases), skin, biliary tract and eyes are most commonly involved.[158–161] Disease activity is typically classified using either the Crohn's disease activity index (CDAI)[162] or the Harvey Bradshaw index (HBI).[163]

Similar to other immune-mediated inflammatory diseases, the actual development of disease reflects a complex interplay between genetics and an environmental trigger(s), and in the case of CD, the consequent abnormal gastrointestinal immune reaction to the intestinal microbiome.[164–166] Numerous gene mutations associated with; abnormal innate immune responses (NOD2/CARD15, TLR4, CARD9), differentiation of Th17 lymphocytes (IL-23R, JAK2, STAT3, CCR6, ICOSLG), autophagy (ATG16L1, IRGM, LRRK2), maintenance of epithelial barrier function (IBD5, DLG5, PTGER4, ITLN1, DMBT1, XBP1) and the initiation of secondary immune response (HLA-region, TNFSF15/TL1A, IRF5, PTPN2, PTPN22, NKX2-3, IL-12B, IL-18RAP, MST1), have been implicated in disease pathophysiology and susceptibility.[167–172] Disease incidence is known to cluster within families, as approximately 5-20% of patients with CD have a family history of IBD, making it a

known risk factor.[173, 174] Epidemiological studies show that in 75-80% of families with members suffering with IBD, affected members have similar disease type, with all members affected with either CD or UC.[173] In the remaining 20% of families, members are affected with 'mixed' forms, some with CD and some with UC, suggesting that CD and UC are to some extent genetically linked, sharing common IBD associated susceptibility genes.[173, 174] However genetic predisposition alone cannot account for disease onset. While the cooperation between genetic and environmental factors in CD development is observed throughout epidemiologic and genetic based research, it is first best described via twin studies. In monozygotic (MZ) twins (genetically identical individuals), concordance rate for CD disease reaches only 20-50%, significantly higher compared to dizygotic twins (DZ) (0-7%). Yet, the lack of a 100% concordance rate in MZ twins implies a low genetic penetrance, thus rendering one or more environmental factors(s) instrumental in the disease aetiology.[175–178] Cigarette smoking is a well-established risk factor for CD development.[179–181] Additional risk factors including appendectomy,[182] oral contraceptive use,[183] breastfeeding,[184] antibiotic use[185] and to some extent dietary habits have also been recognized in the literature, albeit with inconsistent findings.[186, 187] The role of environmental factors is further underlined by population studies, as families moving from low CD prevalence areas to high CD prevalence areas, subsequently developed an increased CD risk mirroring that to the overall population of that region, an effect particularly noted in first-generation children.[188–191]

An association between vitamin D status (as an environmental constituent) and CD incidence was initially hypothesized based on epidemiological evidence, yet like any retrospective observational evidence, unable to imply a causal association. For example, the long reported a 'north-south' gradient which shows a higher incidence and prevalence of CD in populations residing in northern Europe, North America, Australia and New Zealand compared to those in southern locales such as southern Europe, South America and Asia.[192–196] Theoretically the gradient may be explained because synthesis of VD3 primarily depends upon UV skin exposure; northern regions located at higher latitudes (i.e., North Europe, North America), with predictably lower yearly UV exposure, experience higher IBD rates, compared to southern regions that are closer to the equator experiencing greater yearly UV exposure, and thus the lower CD rates.[15, 196] Reinforcing evidence to support this link was further observed from a cohort analysis of the Nurses Health Study I and II, evaluating 72 719 women, where, compared to those residing in northern latitudes, women who resided in southern latitudes had a significantly lower risk of CD (Hazard ratio (HR) 0.48, 95% CI 0.03-0.77).[197] Similarly, Nerich et al[198] demonstrated a significant correlation between higher CD incidence rates and regions of lower sunlight exposure in France, ascertained using satellite data modelling surface UV radiation intensity, and a population wide health insurance database. Migration studies have also shown that immigrants who move from regions located closer to the equator, to regions of more northern latitudes, develop

an increased risk for IBD,[199] and in general, the frequency of diagnosis and disease relapse rates reportedly peak during winter months.[200–202] Recently, Ananthakrishnan et al[203] examined the association between predicted plasma 25(OH)D (determined by dietary and lifestyle variables) and CD risk, in a female cohort from the National Health Survey. Mean predicted 25(OH)D plasma levels were divided into quartiles ranging from 22ng/mL (lowest quartile) and 32 ng/mL (highest quartile), and evaluated using multivariate analysis. In keeping with the literature, the authors reported that women in the highest quartile had significantly half the incidence of CD (HR 0.54, 95% CI 0.30-0.99) compared to those in the lowest quartile.

Intriguingly, a recent hypothesis was proposed suggesting that the widely observed increase in CD incidence over the last two decades may too be correlated with the global trend towards decreased outdoor activity, obesity, increased pollution (industrialization) and low dietary vitamin D intake, resulting in suboptimal vitamin D serum concentrations and the rising CD incidence.[27, 204] Moreover, vitamin D deficiency is often reported in newly diagnosed CD patients further alluding to its contributory role in disease development, although larger prospective studies are needed to imply causality.[18, 200, 205]

Deficiency in Crohn's disease

Vitamin D deficiency is common in CD patients throughout the disease course, with proportions ranging between 22% and 70%.[7, 10, 18, 206–213] Inter-individual determinants of vitamin D status are influenced by 'modifiable' and/or 'non-modifiable' factors. Modifiable determinants which may result in suboptimal vitamin D in the CD patient include; malnutrition (reduced dietary intake), malabsorption secondary to surgical resection or diseased mucosa (predominantly in the ileum),[214] protein losing enteropathy, reduced physical activity, prolonged corticosteroid use,[215] anti-convulsant therapy,[216] smoking,[217] and reduced sunlight exposure (seasonal variation)[208] due to illness (Table 3).[16, 209, 213, 218–220]

Although somewhat counterintuitive, malnutrition in a CD patient can co-exist with overweight or obesity (Body mass index (BMI) >30), a scenario which is becoming increasingly prevalent in many CD patients.[221] Obesity has been associated with a higher risk of relapsing disease in the CD patient, and adipose tissue itself is now recognized for its pro-inflammatory actions and resultant low grade inflammatory state within the body.[222, 223] Interestingly, the increased adipose tissue present in overweight/obesity has also been associated with lower serum 25(OH)D, likely due to the increasing 25(OH)D storage pool with increasing adipose tissue.[224, 225] Thus overweight and obesity in the CD patient may contribute a compounding effect to any pre-existing vitamin D deficiency or those at risk for deficiency.

Non-modifiable determinants of vitamin D status include increasing age (lower cutaneous VD3 production),[226, 227] darker skin pigmentation,[51] longer disease

duration or polymorphisms in the Gc (DBP protein), CYP24A1 (24 hydroxylase), DHCR7 (7-dehydrocholesterol reductase), and CYP2R1 (25 hydroxylase) genes, although the latter polymorphisms are currently considered to be less influential compared to the environmentally-based, 'modifiable' determinants.[15, 228, 229]

Serum response to oral vitamin D supplementation is known to vary between individuals, likely due to the variable interactions between different combinations of the aforementioned determinants.[20] Likewise, due to the progressive nature of most of these determinants in CD, serum response to oral supplementation within one individual may be variable over time. Therefore regular monitoring of serum 25(OH)D is prudent, particularly during high dose oral or intramuscular supplementation, or when trying to reverse suboptimal serum levels.[20, 220, 230–232]

Bone health

Osteoporosis occurs in up to half of CD patients[233] and long term vitamin D deficiency in adult CD patients is associated with lower BMD and skeletal integrity.[7–10, 234–240] The majority of reports however have been cross-sectional and observational in nature, and future prospective interventional studies controlling for the individual VDR and DBP genetic polymorphisms are needed to further delineate the relationship between these variants, and their individual and/or combined effects on vitamin D metabolism. Nevertheless, an extensive series of reports have demonstrated significant improvements in BMD after vitamin D (dosages ranging between 400 to 1000IU/day of cholecalciferol) and calcium supplementation in CD patients, and overall it is well accepted that the preservation of skeletal integrity in CD remains a key function of vitamin D.[241–244]

Serum Vitamin D and disease activity

Low vitamin D status was first associated with disease activity in Cardiff, Wales during the summer months (May-July), based on a small cohort of 40 CD patients in 1985 (Table 4).[245] Plasma 25(OH)D_3 concentrations were significantly lower (mean± SD) in patients with active disease (10.12±1.4 ng/mL, n=11), compared to those with inactive disease (15.72±7.32 ng/mL, n=29). However many of the patients with normal concentrations of 1,25$(OH)_2D_3$ also had raised PTH concentrations, and since vitamin D2 was not measured, the validity of the data may be questionable. Since then, various patient cohorts have been examined via cross-sectional, retrospective studies. For instance, in 2004 a Japanese study performed by Tajika et al[207] during the winter months (December 2001-January 2002), found that lower 25(OH)D levels were significantly related to longer disease duration (>15 years; r=0.46, P=0.003) and increased disease activity (r=0.44, P=0.005), determined using the CDAI in 33 CD outpatients (Table 4). Of the 33 patients, 9 (27.3%) were considered to be vitamin D deficient (<10ng/mL), and compared to 15 age and sex matched controls, no significant difference was found between 25(OH)D concentrations or incidence of vitamin D deficiency. Smoking status was not recorded and normal serum 25(OH)D was broadly defined as 10-55ng/mL. Later,

Joseph et al[246] examined 34 consecutive CD patients, and 34 age and sex matched controls diagnosed with irritable bowel syndrome (IBS) between August 2004 and April 2007 from the gastrointestinal department of Christian Medical College Hospital in Vellore, India (Table 4). The authors reported that disease severity (determined using the HBI) was negatively correlated with serum 25(OH)D levels (correlation coefficient -0.327; P=0.007) while sunlight exposure was found to be positively correlated with 25(OH)D levels (correlation coefficient 0.484; P<0.004), and 25(OH)D concentrations were significantly lower in CD patients compared to controls (CD patients *versus* controls; 16.25±10.8 *versus* 22.78±11.9 ng/ml; P<0.05). Serum 25(OH)D cut off values were defined as deficiency <20ng/mL, 20-32ng/mL as insufficient and sufficiency as >32ng/mL. Among the CD patients 27 (79%) were vitamin D deficient, 4 (12%) were insufficient and 3 (9%) were vitamin D sufficient, smoking status was not recorded. In Denmark, another cross-sectional study by Jorgensen et al[247] conducted between June 2005- June 2006, of 182 CD patients and 62 controls, reported an inverse association between serum 25(OH)D, C-reactive protein (CRP) (P<0.05) and disease activity (determined using the CDAI, P<0.001), the latter maintaining significance following stratification by smoking habits, and insertion of smoking into a linear regression model (Table 4). The authors conservatively defined deficiency as <20ng/mL, with >20ng/mL being vitamin D replete, and a normal CRP being <74nmol/L. Crohn's disease patients receiving vitamin D supplementation (400-800 IU/day) had significantly lower CDAI (P<0.05) and CRP (P=0.07) concentrations, compared to non-users, and those receiving oral vitamin D supplementation, during the 'low-level' sunlight exposure season (November through April) had also significantly higher 25(OH)D levels (30.8ng/mL) compared to non-users (17.6 ng/mL) (P<0.001), a difference which failed to maintain significance in the 'high-level' season (May through October) between supplement users (32 ng/mL) and non-users (34.4 ng/mL). No difference in serum 25(OH)D levels was found between cases and controls, whether they were taking vitamin D supplementation or not. Similarly in a large retrospective observational study of 504 IBD (403 CD, 101 UC) patients followed from the Medical College of Wisconsins' Inflammatory Bowel Disease center since 1999, Ulitsky et al[18] found vitamin D deficiency to be independently associated with an increased disease activity (HBI≥3; OR 1.77, P=0.005) on multivariate analysis adjusting for current and past smoking and medication use, including 5-aminosalicylic acid compounds, immunomodulators and biologic agents (Table 4). A significant association between vitamin D deficiency, quality of life (assessed using a 10 point previously validated questionnaire[248]) and disease activity was also reported [regression coefficient 1.07 (95% CI: 0.43, 1.71)]. Of the CD patients, 205 (51%) of whom were identified as being vitamin D deficient, defined as a serum 25(OH)D <20ng/mL, with 20-30ng/mL as relatively insufficient and sufficiency as ≥31ng/mL. Interestingly, although the study was based in Wisconsin, a 'higher latitude' region, located in the north-central United States at 43°N, the authors noted a paradoxical seasonal variation, as vitamin D deficiency was significantly more prevalent in the summer months compared to the winter months.

While these previous reports appear to support the immunologic effect of vitamin D, they are not without limitations. First, the prevalence of vitamin D sufficiency or insufficiency (or the associated risk factors) described in these reports[18, 207, 245–247] are limited in their ability to prospectively examine the consequences of vitamin D deficiency due to the cross-sectional design. Second, the cumulative disease presentation was determined retrospectively, thus it is possible that vitamin D insufficiently (at the time of measurement) may have temporarily followed the outcome measured.[249] Limitations such as these may explain recent contradictory findings reported from a small cross-sectional study by Hassan et al[19] of sixty IBD patients (34 UC, 26 CD), conducted in Iran (36.20° latitude and 59.35° longitude), a four-season country with the maximum amount of sunshine in the summer (Table 4). Serum 25(OH)D concentrations were reportedly lower in patients with inactive disease (defined as a CDAI ≥150) compared to those with active disease (11.5±7.2 ng/mL *versus* 14.33±13.5 ng/mL), albeit the results failed to reach significance, even when UC and CD were considered separately. Interestingly, despite the geographical location of the study, 60 (95%) of the patients were found to be vitamin D deficient, when defined as ≤10 ng/mL, insufficiency as11-29 ng/mL and sufficiency as ≥30ng/mL. As such, the strongest evidence to date supporting the immunomodulatory role of vitamin D comes from a recent large North American (Boston, Massachusetts) cohort of IBD patients evaluating 2 tertiary medical centers (Table 4).[249] An extensive review of the electronic medical records of 3217 IBD patients (1763 CD patients) with ≥1 plasma 25(OH)D concentration measurements (modelled according to the lowest recorded measurement before the event of interest; surgery or hospitalization) was performed. Plasma measurements were stratified as; 'normal' (>30ng/mL), insufficient (20-29.9ng/mL) and deficient (<20ng/mL). An association was found between plasma 25(OH)D (<20ng/mL) the increased risk for surgery (OR, 1.76; 95% CI, 1.24,2.51) and disease related hospitalization (OR, 2.07; 95% CI, 1.59, 2.68) when compared to those with a 25(OH)D ≥30ng/mL after adjusting for age, gender, Charlson comorbidity index,[250] season, medication (immunomodulator and anti-TNF therapy) and follow-up duration. A dose-response effect with CD-related hospitalizations was also observed as 25(OH)D concentrations progressively decreased. However, the most convincing findings from this study were realized when the authors restricted the cohort to patients having a minimum of 2 plasma 25(OH)D measures (median interval of 294 days between each interval) previously recorded. Crohn's disease patients who achieved a 'normalization' of plasma 25(OH)D concentrations after a prior vitamin D insufficiency (i.e., from <29.9ng/mL to ≥30ng/mL), demonstrated a subsequent reduced risk for surgery on multivariate analysis (OR, 0.56; 95% CI, 0.32,0.98), and significantly lower median CRP concentrations (10.7mg/dL *versus* 16.2 mg/dL, adjusted regression coefficient [β], -5.2; 95% CI, -9.5, -1.02), compared to those who remained deficient, suggesting that vitamin D is a biologically relevant parameter. The effect on hospitalizations did not maintain significance on multivariate analysis.

Still, the intra-study comparison of vitamin D determination methods is one caveat that should always be considered. For instance, it is self-evident that differences in serum vitamin D cut off values defining 'sufficiency' and 'insufficiency' will influence data interpretation, as well as differences in disease activity measurement tools (CDAI *versus* HBI) and exclusion criteria variations, as seen between the previous reports (Table 4). Furthermore, variances in accuracy and precision between the measurement tools used to measure serum 25(OH)D concentration may influence findings. For example, the use of chemiluminescent assays or radioimmunoassays, which are more prone to interpretational and observational errors, have been the most readily available over the past two decades and therefore predominantly quoted in past literature.[75, 251, 252] Currently however, the most accurate assays are believed to be liquid chromatography mass spectrometry (LC-MS) as used by Jorgensen et al,[247] and high performance liquid chromatography (HPLC), both becoming increasingly available.[75] A new 'gold standard' recently implemented in over 700 laboratories worldwide now includes the use of a Vitamin D External Quality Assurance Scheme (DEQAS), which involves a quarterly 'all laboratory trimmed mean' evaluation to monitor and standardize the performance of 25(OH)D assays.[75, 253]

Regardless, it appears even when these variables are fairly consistent, disparities between the reports regarding the proportion of vitamin D-deficient patients is evident, [18, 19, 207, 245–247] a phenomenon likely attributable to region specific environmental or cultural determinants.[254] For instance, the UV availability in a certain region may not necessarily equate to an individuals' actual UV exposure, despite residing in that region. In countries with very hot climates spanning over the majority of the year, sun seeking may be an uncommon practice, especially in populations where beauty is associated with a fairer skin tone.[254–256] Clothing practices will also influence actual UV exposure on the skin, as seen in South Asia where clothing and wide brim hats usually cover most of the exposed skin, greatly minimizing or preventing sunlight exposure entirely.[254] In particular, religious orientated clothing practices particularly that of the veil (*hijab* or *niqab*), have been independently associated with vitamin D deficiency in both African and Middle Eastern populations.[257–260] Moreover, ethnic or regional variations in food consumption practices regarding high intakes of fatty fish, cod liver oil, or dietary dairy products, as well as variations in government food fortification policies may also influence the vitamin D status between populations.[259] A darker skin pigmentation will reduce cutaneous VD3 production compared to that of lighter skin individuals,[261, 262] then again, the use of high protection sunscreen is becoming increasingly more common in lighter skin individuals, which may reduce or block entirely UV irradiation of the skin.[263] Similarly, urbanization has been associated with lower vitamin D levels as individuals are more likely to work indoors[259, 264, 265] possibly in areas with higher air pollution, blocking, therefore reducing UV light exposure, compared to inhabitants of rural areas.[266] Overall, future research in the form of large prospective studies and randomized trials taking into consideration the

aforementioned variables are required to definitively establish the role of vitamin D modulation in the CD patient.

Vitamin D supplementation in Crohn's disease

Administration and formulation

Clinical trials with vitamin D supplementation in CD patients have demonstrated an immunomodulatory effect, however methodological variations regarding the dosage administered, formulation of vitamin D provided, and duration of supplementation remain. In a clinical trial by Jorgensen et al,[267] the authors reported a reduction in relapse rates of 94 CD patients in remission (defined as CDAI<150) of 13% versus 29% after 12 months, when administered 1200 IU/day cholecalciferol, however results failed to reach significance. Yet over a shorter 26 week longer period, a trial[268] of 15 CD patients reported that patients supplemented with larger doses of oral vitamin D provided as 10,000 IU/day cholecalciferol, had a significantly higher improvement of clinical scores compared to patients supplemented with only 1,000IU/day cholecalciferol, suggesting that dosing thresholds may need to be established to optimize effectiveness. In comparison, a recent pilot study of 18 CD patients with mild-to-moderate CD (defined as CDAI 150-400) evaluated the CDAI scores, quality of life scores and the oral vitamin D dosage required to achieve a serum concentration above 40ng/mL.[269] A daily dose of 1000IU was initiated and increased every two weeks by 1000IU until the goal serum of 40ng/mL, or until a maximum of 5000IU/day of cholecalciferol, was achieved. After 24 weeks, supplementation with up to the maximal dosage effectively raised serum 25(OH)D in patients. A significant improvement in both quality of life scores (P=0.0004) and a reduction in CDAI scores (P<0.0001) were reported in the patients. These findings suggest that restoration of normal serum vitamin D levels may be of most benefit in the management of CD patients with mild-to-moderate disease, regardless of the dosing prescription required to achieve this.

There is insufficient evidence demonstrating whether oral ergocalciferol (VD2) compared to oral cholecalciferol (VD3) supplementation provides more clinical value,[270–272] however it appears that cholecalciferol is preferred by many practitioners,[32] and is also more widely available compared to ergocalciferol.[15, 32] It must be noted however that in a short six week non-blinded trial of 37 CD patients in clinical remission (defined as CDAI<150), patients (n=18) who were supplemented daily with a bioactive form of vitamin D, 1,25$(OH)_2$D (provided as 0.5ug/day alfacalidol, a vitamin D analogue), experienced improved CDAI scores and CRP values compared to the remaining patients (n=17) receiving "plain" vitamin D3 (provided as 2000IU/day cholecalciferol).[15, 273] Thus, it is possible that oral supplementation with a bioactive oral analogue of vitamin D may be superior to choelcalciferol. Since the dosage of cholecalciferol provided in the trial was relatively small, larger prospective trials are needed.[268]

Nevertheless, dosing practices with oral cholecalciferol, administering smaller, daily dosages compared to larger intermittent dosages, remains inconclusive. In a trial of 26 vitamin D deficient patients,[274] no difference in serum 25(OH)D target levels were found between patients administered 50,000IU/day cholecalciferol for 10 days (500,000IU total dose), or those administered 3000IU/day cholecalciferol for one month followed by 1000IU/day for the following two months (total dose 150,000IU). Yet on a practical level, short term, intermittent high dose supplementation to reverse deficiency may be beneficial in the outpatient setting, in terms of convenience thus improved patient compliance, since shorter duration, large dose oral administration of vitamin D in deficient CD patients is deemed safe (Table 5).[20]

Supplementation

Current United States Department of Agriculture (USDA)[275] and the Institute of Medicine (IOM)[276] guidelines recommend a daily dietary allowance of 600IU, with an upper level of 4000IU starting from the age of 9 though >70 years, which includes pregnant and lactating females.[277] In the case of a low 25(OH)D, supplementation of 1500-2000IU/day may be required to achieve a serum of ≥30ng/mL.[20] These values need to be increased by a factor of 2-3 in obese individuals or those taking anti-convulsant therapy medication, the latter known for its disruptive effects on vitamin D metabolism.[20, 278]

Conversely, in the CD patient, evidence indicates that there may be upwards of a 30% attenuated or diminished response to oral vitamin D supplementation compared to healthy individuals,[220] and patients may require higher daily vitamin D intakes. In the case of suboptimal serum 25(OH)D concentrations of ≤3ng/mL, 4-≤9g/mL, 10-≤15ng/mL, 16-≤23ng/mL and 24-≤29ng/mL CD patients should be started on daily oral cholecalciferol of 5000IU, 4000IU, 3000IU, 2000IU and 1000IU, respectively (Table 5).[15] The dosage should be multiplied by a factor of 1.5-3 if small bowel involvement, malabsorption syndrome, or a BMI>30 is present,[15, 20] Large dose oral administration of vitamin D in vitamin D-deficient CD patients, following a regime of 50,000IU of cholecalciferol once a week for eight weeks, or until target serum 25(OH)D level is achieved, is deemed safe in the literature (Table 5).[20] In severe deficiency or where annual large vitamin D dosing may be appropriate, intramuscular doses of between 300,000-600,000IU of cholecalciferol have also shown to be safe and effective, however urinary calcium levels should be monitored.[270–272] It is important to note, that the above oral vitamin D supplementation guidelines are intended as a starting reference considering the high variability of serum 25(OH)D in response to vitamin D supplementation between individuals.[278] Therefore, baseline serum 25(OH)D levels should be re-checked at three months, then again every three to six months, adjusting the dosage until the target serum of 30-36ng/mL is achieved.[15] Thereafter a maintenance dosage of 1500-2000IU/day may be provided, however the upper threshold necessary for optimal outcome and maintenance, while avoiding any adverse consequences, has not yet been established (Table 5).[15]

Conclusion

Vitamin D supplementation in the CD patient is known to have an advantageous effect regarding the maintenance of skeletal integrity. Recent evidence now suggests that vitamin D also functions as an immunoregulator via its actions on the VDR, and in the CD patient, adequate vitamin D status may reduce both disease activity and inflammation. Serum 25(OH)D is the recommended index to measure vitamin D status, however the upper threshold necessary for optimal outcome, while avoiding any adverse consequences, has not yet been fully established. Nevertheless, current consensus suggests that a serum 25(OH)D of 30-36ng/mL is sufficient to maintain serum PTH and maximize intestinal calcium transport, and when necessary, oral supplementation can be provided as either ergocalciferol (VD2) or cholecalciferol (VD3), however the latter is more commonly used. Oral cholecalciferol can be administered either in smaller daily dosages, or in larger intermittent dosages which may improve patient compliance. Regular monitoring of serum 25(OH)D is recommended as individual serum response to vitamin D supplementation may vary. Considering the epidemiological and molecular evidence together, it seems that vitamin D is intrinsic to the development and function of the human immune system, however future research surrounding the vitamin should be actively pursued.

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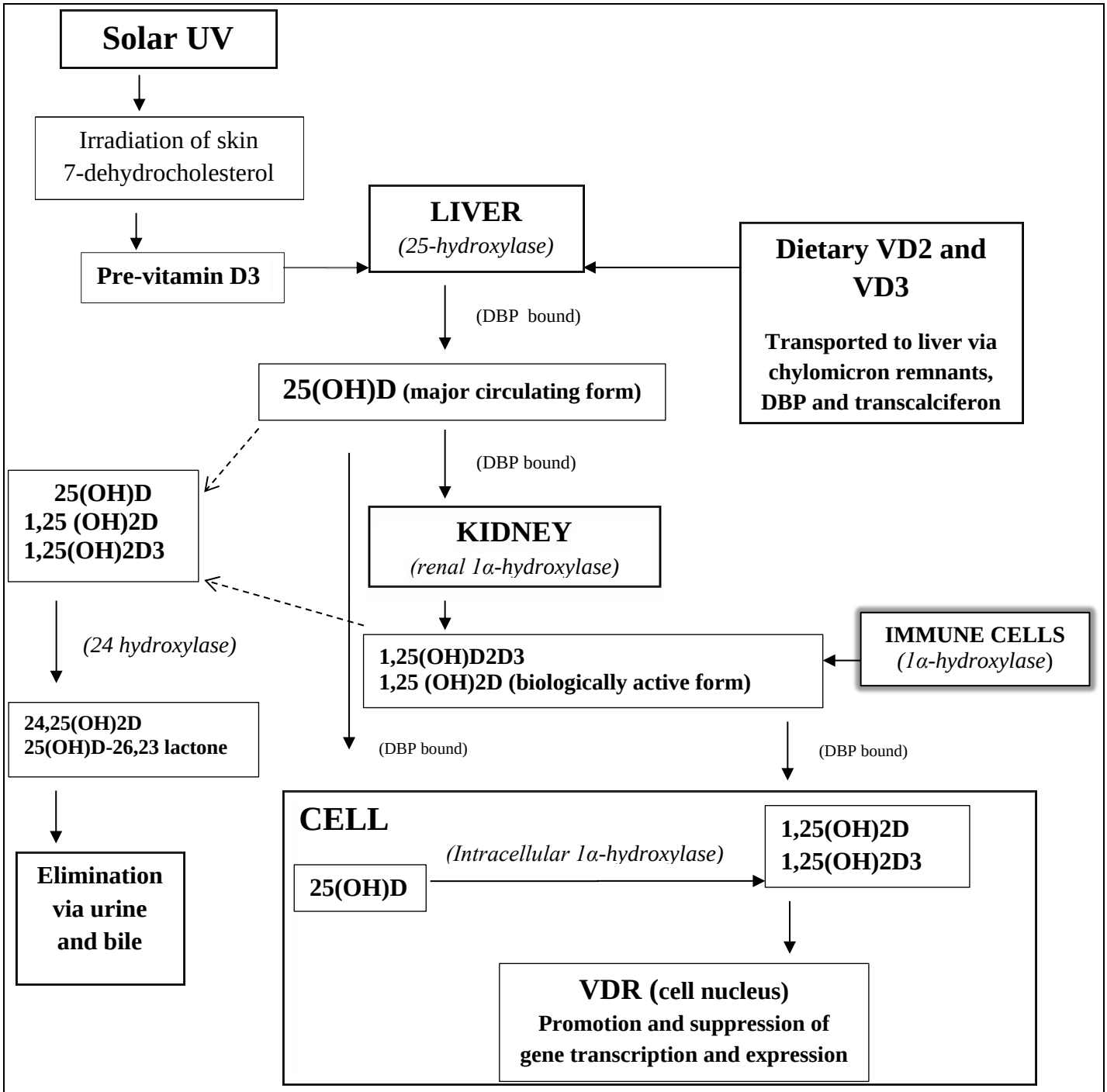

Figure 1. The synthesis, metabolism and cellular effects of vitamin D. Dietary and sunlight derived vitamin D are converted into the biologically active form $1,25(OH)_2D$ by either immune cells or sequential hydroxylations in the liver and kidney, respectively. Inside the cell the VDR complex functions as a ligand-activated transcription factor that will bind vitamin D response elements in the promoter region of target genes subsequently promoting or suppressing gene expression.

Table 1. Vitamin D: cholecalciferol (VD3) and ergocalciferol **(VD2)**

Natural and Food sources: Vitamin D3 (cholecalciferol)	
UVB exposure from sunshine, full body exposure †	~10,000-20,000IU
Cod liver oil (1tsp)	~400-1,000IU
Salmon (fresh, wild, 100g)	~600-1,000IU
Salmon (farmed, 100g)	~100-250 IU
Fortified milk (250ml)	100IU
Fortified orange juice (250ml)	100IU
Egg yolk ‡	~20IU/yolk
Food Sources; Vitamin D2 (ergocalciferol)	
Shiitake mushrooms (fresh, 100g)	~100IU
Shiitake mushrooms (sun dried, 100g)	~1600IU
IU, International Units. 1 IU=25ng (nanograms). † A dark skinned individual may need 5-16 times more exposure to achieve the same amount of vitamin D compared to a fair skinned individual. ‡ Contains both Vitamin D3 and Vitamin D2.	

Table 2. Interpretation of serum 25(OH)D concentration[20,62–64,70,74]

Serum 25(OH)D (ng/mL)[a]	**Interpretation of serum 25(OH) vitamin D concentration**
≤10	Severe deficiency
≤20	Deficiency
21-29	Insufficiency
≥30-36	Sufficiency
≥60	Upper limit; associated with hypercalcemia, hypercalciuria and hyperphosphatemia
>100-150	Toxicity
[a] Conversion factor for ng/mL to nmol/L is 2.496.	

Table 3. Contributing factors for vitamin D deficiency in the Crohn's disease patient [15, 16, 51, 208, 209, 213–218, 220, 224–229]

Malnutrition (reduced dietary intake)
Malabsorption
Protein losing enteropathy
Surgical resection (predominantly ileum)
Reduced physical activity
Lower bioavailability
Reduced sunlight exposure
Smoking
Prolonged corticosteroid therapy
Anti-convulsant therapy
Longer disease duration
Increasing age
Darker skin pigmentation (higher melanin content)
BMI ≥30
Genetic polymorphisms affecting vitamin D metabolism
BMI, body mass index.

Table 4: Vitamin D and disease activity in the adult Crohn's disease patient

Previous studies	**IBD Sample selection**	**Study location and seasonal variation**	**Serum 25(OH) vitamin D measurement (ng/mL)**	**Disease activity evaluation tool (CDAI, CRP,HBI)**	**Participant exclusion criteria**	**Overall findings**[a]
Harris 1985 [245]	40 CD patients	Cardiff, Wales Summer months: May-July	Plasma vitamin D3 (25[OH]D_3) measured[b] Radioimmunoassay (sheep antiserum)	HBI	Receiving oral vitamin D or cholestyramine supplementation	25(OH)D_3 concentration significantly lower in patients with active disease (defined as HBI >5).

Tajika 2004 [207]	33 CD patients 15 age-sex matched controls	Japan Winter months: December 2001-January 2002	Serum 25(OH)D Competitive protein binding assay <u>Cut off values:</u> 10-55ng/mL sufficient	CDAI	Receiving vitamin D, calcium, calcitonin, bisphosphonate, hormone replacement or fluoride Diagnosed with kidney, liver, cardiopulmonary, hypogonadism and inflammatory joint disease	Lower 25(OH)D concentration significantly correlated with longer disease duration and increased disease activity. No significant difference in 25(OH)D deficiency between cases and controls.
Joseph 2009 [246]	34 CD patients 34 age and sex matched out-patient controls with IBS	India Consecutive patients recruited during April 2004-August 2007	Serum 25(OH)D Radioimmunoassay <u>Cut off values:</u> <20ng/mL deficiency, 20-32ng/mL insufficiency, >32ng/ml sufficient	HBI, CRP	Receiving supplemental vitamin D or calcium over the past 6 months Diagnosed with renal, hepatic, thyroid disease and pregnant women	Significant negative correlation between disease severity, and significant positive correlation between duration of sunlight exposure with 25(OH)D concentration. 25(OH)D significantly lower in cases compared to controls.
Jorgensen	182 CD patie	Denmark	Serum 25(OH)D	CDAI, CRP	No exclusion criteria	Low 25(OH)D significantly associated with active disease,

2013 [247]	nts 63 healthy controls	Cross-sectional study between June 2005-June 2006	Isotope diluted chromatography-tandem mass spectrometry. <u>Cut off values:</u> <20ng/mL vitamin D deficiency, >20ng/mL vitamin D replete			after adjusting for smoking habits. Oral vitamin D supplementation significantly improved serum 25(OH)D during months with 'low-level' sunlight exposure. No significant difference in 25(OH)D between cases and controls.

Table 4: Vitamin D and disease activity in the adult Crohn's disease patient (cont.)

Ulitsky 2011 [18]	504 IBD patients (403 CD, 101 UC)	Wisonsin, USA Retrospective observational; patients followed since 1999 at Medical College of Wisconsin IBD center	Serum 25(OH)D Chemiluminescent immunoassay <u>Cut off values</u>: <20ng/mL deficiency, 20-30ng/mL relative insufficiency, >31ng/mL sufficiency	HBI and a validated quality of life questionnaire[2] [48]	No exclusion criteria Current and past smoking and medication use, including 5-aminosalicylic acid compounds, immunomodulators and biologic agents were recorded	25(OH)D deficiency independently associated with increased CD disease activity (defined as HBI≥3) on multivariate analysis adjusting for current and past smoking and medication use, including 5-aminosalicylic acid compounds, immunomodulators and biologic agents. Paradoxical seasonal variation, as deficiency significantly more prevalent in summer compared to winter months.

Hassan 2013[19]	60 IBD patients (26 CD, 34 UC)	Iran Summer months	Serum 25(OH)D Radioimmunoassay. <u>Cut off values:</u> <10ng/mL deficiency, 11-29ng/mL insufficiency, ≥30ng/mL sufficient	CDAI	Receiving anticonvulsants, vitamin D supplementation Diagnosed with renal failure, liver disease, malabsorption (defined as albumin <2mg/dl, cholesterol <100mg/dl, BMI ≤18.5kg/m²), and pregnancy or lactation	No significant association between 25(OH)D deficiency and IBD activity, even when CD and UC considered separately.
Ananthakrishnan 2013[249]	3217 IBD patients (1763 CD, 1454 UC)	Boston, Massachusetts USA Electronic medical record review of 2 tertiary medical centers	Plasma 25(OH)D Liquid chromatography with mass spectrometry since 2008, and radioimmunoassay prior 2008 <u>Cut off values:</u> <20ng/mL deficient, 20-29.9ng/mL	Examined IBD-related surgery or hospitalization	No exclusion criteria	25(OH)D (<20ng/mL) associated with increased risk for surgery and inflammatory bowel disease related hospitalization compared to patients with 25(OH)D (>30ng/mL). Normalization of 25(OH)D (from <29.9ng/mL to ≥30ng/mL) resulted in a reduced risk for subsequent IBD related surgery and lower median CRP levels. Results based on

			insufficient, >30ng/mL normal			multivariate analysis adjusting for age, gender, Charlson comorbidity index,[250] season, medication (immunomodulator and anti-TNF therapy) and follow-up duration.

CD, Crohn's disease, CDAI, Crohn's disease activity index; CRP, C-reactive protein; HBI, Harvey-Bradshaw index; IBD, Inflammatory bowel disease; consists of two subtypes, Crohn's disease (CD) and Ulcerative colitis (UC); IBS, Irritable bowel syndrome; ng/mL, nanogram per mililiter; UC, ulcerative colitis.

[a] Overall findings for each study presented for Crohn's disease (CD) patients.

[b] Three plasma vitamin D3 assays were measured: $25(OH)D_3$, $24,25(OH)_2D_3$ and $1,25(OH)_2D_3$. No cut off values were defined in the study. Serum vitamin D2 ($25[OH]D_2$) was not measured.

Table 5. Oral vitamin D supplementation guidelines in the Crohn's disease patient[15,20]

Serum 25(OH)D [a] **(ng/mL)**	**Supplementation in the Crohn's disease patient** [b,c] **Oral cholecalciferol (vitamin D3)**
≤3	5000 IU/day
4- ≤9	4000 IU/day
10- ≤15	3000IU/day
16- ≤23	2000 IU/day
24- ≤29	1000 IU/day
30- 36	1500-2000IU/day maintenance [a]

[a] Regular monitoring of serum levels should be performed at 3 months, then 3-6 monthly and adjust dosage until serum concentration of 30-36ng/mL is achieved.

[b] Multiply the dosage by a factor of 1.5-3 in patients with body mass index >30, patients on medications that affect vitamin D metabolism or those with small bowel involvement and/or other malabsorption syndromes.[15]

[c] Administration of 50,000IU oral cholecalciferol once a week for eight weeks, or until target serum 25(OH)D level is achieved may be provided for improved patient compliance.[20]